Handbook of Experimental Pharmacology 220

Gary R. Lewin
Bruce D. Carter *Editors*

Neurotrophic Factors

Springer

Handbook of Experimental Pharmacology

Volume 220

For further volumes:
http://www.springer.com/series/164

Gary R. Lewin • Bruce D. Carter
Editors

Neurotrophic Factors

Springer

Editors
Gary R. Lewin
Department of Neuroscience
Max-Delbrück-Center for
Molecular Medicine
Berlin
Germany

Bruce D. Carter
Department of Biochemistry
Vanderbilt University School of Medicine
Nashville
Tennessee
USA

ISSN 0171-2004 ISSN 1865-0325 (electronic)
ISBN 978-3-642-45105-8 ISBN 978-3-642-45106-5 (eBook)
DOI 10.1007/978-3-642-45106-5
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014933801

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Neurotrophic factors can be broadly understood as any secreted factor that has nourishing or sustaining effect on neurons. The archetypical neurotrophic factor is nerve growth factor (NGF) which was first discovered in series of elegant embryological and biochemical experiments carried out by Rita Levi-Montalcini and her colleagues. In the 1980s and 1990s, the work of Hans Thoenen, Yves-Alain Barde, and others paved the way for the discovery of new members of this family BDNF, NT-3, and NT-4. This family of neurotrophic factors became known as the neurotrophins. In parallel to these discoveries, other neurotrophic factors were discovered, notably the glial-derived neurotrophic factor (GDNF) which also belongs to a small sub-family of factors which includes neurtrin, artemin, and persephin. Our knowledge on the biology of neurotrophic factors has exploded in the last 15 years and it has become apparent that members of the neurotrophin family play important roles, not just in the development of the nervous system, but in the normal physiology and pathophysiology of the brain. For this reason we have chosen to largely restrict the focus of this new handbook of pharmacology volume on neurotrophic factors to the biology of the neurotrophins NGF, BDNF, NT-3, and NT-4. Research on the neurotrophins in the 1990s provided much hope that these factors would show therapeutic potential in a wide variety of neurodegenerative diseases from Alzheimer's to Parkinson's disease. It is probably fair to say that the research emphasis has moved away from pursuing a role for neurotrophins in neuroprotection. Nevertheless the last 15 years has witnessed outstanding progress in understanding the functional roles of these neurotrophic factors and their receptors in normal development and adult physiology, their mechanisms of action, as well as their role in the pathophysiology of disease. This book provides critical reviews of the role of neurotrophins and their receptors in a wide variety of diseases including neurodegenerative diseases like Huntington's, cognitive dysfunction, psychiatric disorders such as clinical depression, Rett syndrome, motor neurone disease, spinal cord injury, pain, metabolic disease, and cardiovascular disease. The book also contains contributions from leaders in the field dealing with the basic biology, transcriptional and post-translational regulation of the neurotrophins, and their receptors. The last decade has witnessed a radical change in the view of neurotrophins and their receptors, because of the discovery that the pro-peptide forms of NGF and BDNF, in particular, have distinct biological effects mediated by novel receptor constellations, including that of the VPS10p family transmembrane

receptor sortilin and the low-affinity neurotrophin receptor p75NTR. Thus there are more molecular targets for manipulating neurotrophins available and more validated disease processes in which neurotrophins play a relevant and powerful role. Pharmaceuticals tailored to interfere with neurotrophin function have not only been developed, but even show clinical efficacy in late stage clinical trials for the treatment of pain. This book will review all recent areas of progress in the study of neurotrophins and their biological roles. Importantly, world-renowned experts explain the detailed and complex biology of these factors in the context of disease, revealing future perspectives for new therapies based on neurotrophin signalling and their downstream targets.

We are very excited about this book as it contains contributions from the leading scientists in the field who bring a unique combination of expertise on the detailed molecular mechanisms by which neurotrophins signal as well as perspectives on their disease relevance. During the final stages of the production of this book, two pioneers in the field of neurotrophin research, Rita Levi-Montalcini and Hans Thoenen, sadly passed away. We both had the honour and the luck to benefit from close scientific contact with Hans Thoenen in the formative years of our research careers. We would like to dedicate this volume to the memory of these two wonderful scientists, Rita Levi-Montalcini and Hans Thoenen.

Berlin, Germany — Gary R. Lewin
Nashville, TN — Bruce D. Carter

Contents

Part I

The Neurotrophin Family

NGF, BDNF, NT3, and NT4

M. Bothwell

Abstract

The discovery of nerve growth factor (NGF) was a seminal event in history of research in developmental neurobiology. The further discovery that NGF was just one of a family of structurally similar growth factors, neurotrophins, provided important insights into the way nerve cells communicate, during development of the nervous system, and in neuroplasticity, memory, and learning in the adult nervous system. Four neurotrophins, NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and neurotrophin-4 (NT4), regulate a wide variety of neural functions, acting upon p75NTR, TrkA, TrkB, and TrkC receptors.

Keywords

Neurotrophin • NGF • BDNF • NT3 • NT4 • Evolution

1 Historical Background: Discovery of NGF and Other Neurotrophins

1.1 Discovery of NGF

The discovery and biochemical and functional characterization of nerve growth factor (NGF), by Rita Montalcini, Viktor Hamburger, and Stanley Cohen, was decades ahead of its time, in more ways than we can easily appreciate today. The discovery that the trophic effect of innervated tissues on sympathetic and sensory neuronal development was mediated by a diffusible factor (Levi-Montalcini and Hamburger 1953) was conceptually ground-breaking, and the successful isolation of this NGF from mouse salivary gland was remarkable given the primitive tools for

M. Bothwell (✉)
University of Washington, Seattle, WA, USA
e-mail: mab@uw.edu

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_1,

protein fractionation that were available at the time. It is also remarkable that the importance of NGF for neurons in vivo was established almost immediately by demonstrating that injection of NGF antibody caused death of sympathetic neurons (Cohen 1960). The precocious nature of these studies can only be understood by contrasting this to the history of experimentation with a variety of other growth factors that were discovered subsequently, since the loss-of-function experiments required to demonstrate the importance in vivo of other growth factors usually lagged the initial discovery of those growth factors by a decade or more.

It is hard to appreciate also, from a modern perspective, that the concept that a protein released by one cell could control the differentiation of neighboring cells was revolutionary at the time of discovery of NGF. Indeed, the possibility that NGF could be "instructive" for neuronal differentiation, rather than merely being "permissive" was still a hotly debated subject when the author of this chapter entered the field of NGF research in 1975.

The seed for the discovery of NGF was planted by the pioneering work of Viktor Hamburger in which it was shown that surgical removal of the wing buds of chick embryos reduced the ultimate number of motor neurons in the lateral motor column of the spinal cord and of sensory neurons in the dorsal root ganglia at segmental levels responsible for innervation of the missing target tissue, while transplantation of supernumerary limb buds had the opposite effect of allowing development of more motor neurons and sensory neurons (Hamburger 1934, 1939). Thus was born the so-called neurotrophic hypothesis, stating that "Each part of the peripheral field controls directly [development of] its own nervous center" along with the idea that some signal or substance must move from the axon terminus to the neuronal cell body to convey this signal.

The work of Rita Levi-Montalcini, initially independently (Levi-Montalcini and Levi 1943) and subsequently in collaboration with Viktor Hamburger (Hamburger and Levi-Montalcini 1949), demonstrated that these effects were not primarily an effect on neurogenesis, as initially supposed, but rather, largely reflected the ability of the innervated target to suppress developmental cell death of the innervating neurons. Attempts to model the limb bud effects with small pieces of sarcoma tumor, initially in vivo (Levi-Montalcini and Hamburger 1951), and subsequently in vitro (Levi-Montalcini and Hamburger 1953), using newly available tissue culture techniques demonstrated potent effects on development of sympathetic neurons as well as sensory neurons and importantly established that the effects were mediated by a diffusible factor. Subsequent studies revealed that a similar activity was present at much higher concentrations in cobra venom and mouse salivary gland, allowing the biochemical purification of the factors and importantly, demonstrating that an antiserum to the NGF protein caused degeneration of the nervous system of neonatal rodents (Cohen 1960; Cohen and Levi-Montalcini 1956). The later discovery that NGF was a member of a family of factors that control survival of sensory neurons was presaged by Levi-Montalcini's observation that NGF promoted the survival of small mediodorsally located sensory neurons in DRGs, while NGF had no effect on the larger ventrolaterally located sensory neurons.

1.2 Discovery of the Neurotrophin Gene Family

The sensory ganglia that are segmentally distributed along the trunk of vertebrates derive from the neural crest, but many cranial sensory ganglia derive instead from epithelial placodes. Target-derived trophic factors are required for developmental survival of both neural crest-derived and placode-derived peripheral sensory neurons, yet NGF only promotes survival of neural crest-derived sensory neurons. Such observations motivated experiments leading to the discovery and molecular cloning of brain-derived neurotrophic factor (BDNF) as a trophic factor for placode-derived sensory neurons (Barde et al. 1982). Nucleotide sequence analysis revealed that NGF and BDNF were structurally related (Leibrock et al. 1989). Several teams of investigators recognized independently that short highly conserved regions of NGF and BDNF transcripts permitted the design of primers for polymerase chain reaction that would jointly amplify both NGF and BDNF sequences and employing these primers discovered additional members of the NGF gene family—neurotrophin-3 (NT3) (Hohn et al. 1990; Jones and Reichardt 1990; Maisonpierre et al. 1990; Rosenthal et al. 1990) and neurotrophin-4 (NT4) (Berkemeier et al. 1991; Hallbook et al. 1991; Ip et al. 1992). The fourth mammalian neurotrophin identified was variously named NT4 or NT5, according to whether the discoverers included a previously described fish neurotrophin in their numbering scheme. As a compromise between the alternative nomenclatures, the fourth mammalian neurotrophin is frequently referred to as NT4/5. It will be called NT4 in this chapter. Some fish possess an additional neurotrophin family member, while birds lack NT4. The term neurotrophin was originally coined to describe members of the NGF gene family. A few present day investigators use the term "neurotrophin" as a synonym for "neurotrophic factor." Most investigators prefer to reserve the term "neurotrophin" for its original purpose, as a means to refer to NGF gene family members collectively.

2 Neurotrophin Structure

Mature neurotrophins exist as noncovalently associated dimers of ~13,500 Da protomers (Bothwell and Shooter 1977; Radziejewski et al. 1992). The affinity of the dimeric NGF association is sufficient to prevent dissociation even at the pM concentrations at which NGF acts physiologically (Bothwell and Shooter 1977) and this is probably also true for the other neurotrophins. High resolution structures have been determined for each of the neurotrophins (Butte et al. 1998; McDonald et al. 1991; Robinson et al. 1999). Each neurotrophin subunit has a backbone consisting of two pairs of antiparallel β-strands generating an elongated shape and stabilized by three disulfide bonds. The structure has been referred to as a cystine knot, and a similar folding organization has been observed in several other growth factors, including TGF-beta and PDGF family members (McDonald and Chao 1995). The highly conserved interaction interface of the neurotrophin dimers permits the formation of heterodimers between different neurotrophins in vitro but

evidence is lacking for the existence of such heterodimers in vivo (Jungbluth et al. 1994; Philo et al. 1994; Robinson et al. 1995).

Early studies of the biochemistry of NGF placed particular attention on the high molecular weight complex in which NGF could be isolated from mouse submaxillary salivary gland (Greene et al. 1969; Nichols and Shooter 1985). The sedimentation velocity of this complex was 7 svedbergs—accordingly the complex was known as 7S NGF. Curiously, this complex was ultimately found to represent NGF (known at the time as low molecular weight NGF, 2.5S NGF or beta-NGF) in association with alpha and gamma subunits which represent two different members of the glandular kallikrein family of proteases (Bothwell et al. 1979; McDonald and Blundell 1991). The physiological relevance of the high molecular weight complex of NGF is unclear, as this form of NGF appears to exist only in mouse, and in mouse, only in salivary glands. The manner in which this peculiar biological adaptation of NGF may have evolved in mice is discussed below.

3 Neurotrophin Receptors

The four mammalian neurotrophins interact with four receptors; $p75^{NTR}$, TrkA, TrkB, and TrkC. The function of these multiple receptors is complex, as $p75^{NTR}$ and Trk receptors can function independently, but in neurons that express both $p75^{NTR}$ and Trk receptors, the receptors interact physically and functionally in ways that may alter the signaling properties of each. The structure and signaling functions of these receptors are discussed in detail elsewhere in this book. Briefly, all four neurotrophins, both as proneurotrophins and as mature fully processed neurotrophins, can bind and activate signaling by $p75^{NTR}$, whereas the Trk receptors prefer to bind mature neurotrophins and are selective for particular neurotrophins. NGF preferentially binds and activates TrkA, NT3 preferentially binds and activates TrkC, and BDNF and NT4 preferentially bind and activate TrkB. For this reason, BDNF and NT4 are typically functionally redundant in mammals, and reflecting this redundancy, the NT4 gene has apparently been lost during evolution of birds. NT3 is the most promiscuous of the neurotrophins as alternative splicing of the TrkA transcript can generate forms of TrkA that are effectively activated by NT3 (Clary and Reichardt 1994). Importantly, however, NGF and NT3 are not functionally equivalent with respect to TrkA activation, as they influence TrkA signaling differently (Harrington et al. 2011).

One or more of the four neurotrophin receptors are expressed in a wide variety of types of neurons and glia in both the central and peripheral nervous system and also in a variety of non-neural cell types. Thus, neurotrophins have an extraordinary range of biological functions, with the neurotrophin preference of various cell populations being determined by the particular neurotrophin receptor or receptors they express.

4 Neurotrophin Processing and Secretion

Like most other secreted biologically active polypeptides, protein synthesis of neurotrophins occurs in the rough endoplasmic reticulum, where the proneurotrophins are packaged into secretory vesicles. Proneurotrophins, which range from about 210 to 270 amino acid residues in length, are processed within these vesicles by proteases of the proprotein convertase family (Seidah et al. 1996), producing the mature neurotrophins which are about 120 residues in length. In the case of BDNF, the cleaved prodomain is stored with and cosecreted with mature BDNF (Dieni et al. 2012). Whether this pro-peptide has any biological function, and whether the pro-domains of other neurotrophins are secreted, is unknown. In some cases, vesicular processing of neurotrophins is incomplete, leading to secretion of unprocessed pro-neurotrophins from which the mature neurotrophin may be released by plasmin and matrix metalloproteinases following secretion (Lee et al. 2001).

The seminal experiments that lead to the proposal of the "neurotrophic hypothesis" and the discovery of neurotrophins examined neuronal populations that innervated non-neural peripheral target tissues. However, in many cases, and particularly in the central nervous system, the neurotrophin-producing innervated target cell may also be a neuron. Neurotrophin secretion by neuronal and non-neuronal cells differs in several important ways that were not immediately appreciated by investigators. Firstly, neurons (and neuroendocrine cells) have distinct regulated and constitutive secretory pathways, whereas non-neuronal cell types typically have only the constitutive secretory pathway (Kelly 1985). Thus, while the manner of secretion of the four neurotrophin is similar in non-neural cell types, in neurons, this is not the case, as NGF, NT3, and NT4 traffic mainly through the constitutive secretory pathway in neurons and neuroendocrine cells, whereas BDNF selectively traffics through the regulated secretory pathway (Farhadi et al. 2000; Griesbeck et al. 1999; Hibbert et al. 2003; Mowla et al. 1999). This distinction is particularly important in the context of BDNF functions in learning and memory, where control of BDNF secretion by neural activity is likely to be essential, as discussed elsewhere in this book. The second important distinction between neurons and many non-neural cell types is that neurons are highly polarized cells. Initially, no doubt with a mindset influenced by early studies of neurotrophic functions with non-neural neurotrophin-producing cells, the expectation was that neurons would secrete neurotrophins principally at the somatodendritic membrane domains, as these are normally the site of axonal synaptic contacts. However, in neurons, much of BDNF secretion follows the same secretory pathway as neuropeptides, being packaged in dense core vesicles, which are transported anterogradely down axons and secreted at the axon terminus (Conner et al. 1997; Dieni et al. 2012; von Bartheld et al. 1996; Zhou and Rush 1996).

Two proBDNF-binding proteins have been implicated in directing proBDNF to the regulated secretory pathway, carboxypeptidase E (Lou et al. 2005), and sortilin (Chen et al. 2005). Importantly, a common allelic variant of the human *BDNF* gene

encodes a Val/Met substitution within a region of the BDNF pro-domain that binds sortilin. Consequently, the Met-containing proBDNF variant is poorly sorted into the activity-regulated secretory pathway, resulting in poor performance in some memory tasks (Chen et al. 2004, 2005; Egan et al. 2003).

5 Differential Activity of Neurotrophins and Proneurotrophins

It is beyond the scope of this chapter to provide a detailed discussion of the various functions of neurotrophins or of the structure and function of neurotrophin receptors. For these topics, the reader may consult other chapters in this volume. For the purposes of this chapter, it is sufficient to say that all four neurotrophins interact effectively with the $p75^{NTR}$ neurotrophin receptor, whereas TrkA functions primarily as an NGF receptor, TrkB as a receptor for BDNF and NT4, and TrkC as a receptor for NT3 (Bothwell 1991). Additional complexity is provided by the ability of NT3 to interact weakly with TrkA and TrkB receptors and by the ability of $p75^{NTR}$ to influence neurotrophin/Trk receptor interactions (Huang and Reichardt 2003).

The structural basis governing the selectivity of neurotrophin/receptor interactions has been extensively characterized by X-ray crystallographic analysis of neurotrophin/receptor complexes and by mutagenic structure/function studies. The extracellular domain of Trk receptors contains leucine-rich repeat domains and two C2-immunoglobulin-like domains, and the second immunoglobulin-like domain represents the main neurotrophin-binding region (Holden et al. 1997; Urfer et al. 1998), although leucine-rich repeat domains may also contribute to binding (Windisch et al. 1995). The interaction of neurotrophins with Trk receptors is mediated largely by two neurotrophin interaction surfaces. One important interaction involves amino acid residues that are highly conserved among neurotrophins, whereas a second neurotrophin/Trk interaction surface employs neurotrophin amino acid residues that are not conserved among neurotrophins. The former surface (known as the conserved patch) contributes importantly to the affinity of binding, whereas the latter surface (known as the specificity patch) clearly is largely responsible for determining the selectivity of binding of particular neurotrophins to particular Trk isoforms (Hirata-Fukae et al. 2008). A similarly detailed comparison of the binding interactions of all the neurotrophins with $p75^{NTR}$ has not been published, although high resolution structures of both NGF and NT3 with $p75^{NTR}$ have been reported (Aurikko et al. 2005; Gong et al. 2008; He and Garcia 2004).

Initially it appeared that it might be possible to generalize neurotrophin function by stating that the different neurotrophins have a similar range of functions, but directed against different cell populations, depending on the receptors expressed. It has become clear, however, that the truth is much more complicated than this, as the four different neurotrophin receptors can mediate very different and in some cases even opposite cellular responses.

Although the function of neurotrophins in the peripheral nervous system contributes to the neurotrophic support of various neuronal populations, in complete accord with the "neurotrophic hypothesis" originally developed by Victor Hamburger and Rita Levi-Montalcini, the neurotrophins mediate an extraordinary range of other functions, which are not amenable to simplified generalizations. For example, neurotrophins can either promote neuronal survival or promote neuronal cell death, depending on the circumstance, the receptor employed, and whether mature or proneurotrophins forms are present. In some cases neurotrophin/receptor association promotes pro-survival signaling, in other cases, neurotrophin/receptor association promotes pro-death signaling (Rajagopal et al. 2004), and in still other cases, neurotrophin/receptor association terminates activity of a receptor that constitutively signaling pro-death (Huang and McNamara 2010; Lee et al. 2002). Further, while neurotrophin action sometimes conforms to the original neurotrophic hypothesis, which predicts that neurotrophins should convey trophic signals from the axon terminus retrogradely back to the neuronal soma, in some cases, and particularly for BDNF, neurotrophins may be transported anterogradely down axons to convey signals to postsynaptic cells (Altar et al. 1997; Conner et al. 1997; Smith et al. 1997; von Bartheld et al. 1996; Zhou and Rush 1996). Other neurotrophin functions, such as control of neuronal dendritic branching (McAllister et al. 1997) and control of synaptic function (Kang and Schuman 1995; Lohof et al. 1993; Patterson et al. 1992), differ radically from the original concept of the neurotrophic hypothesis. Other chapters in this volume provide a more comprehensive discussion of the range of neurotrophin functions.

6 Evolution of Neurotrophins

6.1 Invertebrate Origins of Neurotrophins

The neurotrophin-signaling system, including neurotrophin-like ligands and $p75^{NTR}$ and Trk-like receptors, evolved before the evolution of vertebrates. The genome of the arthropod Daphnia pulex encodes clearly recognizable neurotrophin, $p75^{NTR}$, and Trk orthologs (Wilson 2009). Bilaterian organisms fall within two major branches, protostomes, including arthropods such as Daphnia, and deuterostomes, from which vertebrates evolved. The genomes of prechordate deuterostomes *Strongylocentrotus purpuratus* (sea urchin) and *Saccoglossus kowalevskii* (acorn worm) also encode neurotrophin, $p75^{NTR}$, and Trk orthologs (Bothwell 2006). Thus, the shared ancestor of protostomes and deuterostomes must have possessed neurotrophin, $p75^{NTR}$, and Trk orthologs.

It remains to be determined whether the invertebrate neurotrophin orthologs bind and activate the invertebrate neurotrophin receptor orthologs. Interestingly, however, the sea urchin neurotrophin has a pro-domain, a mature neurotrophin domain, and a predicted site for proteolytic processing that are closely similar to these domains in vertebrate neurotrophins, suggesting that proteolytic processing might control selective association of proneurotrophin and mature neurotrophin

with $p75^{NTR}$ and Trk receptors, in invertebrates as in vertebrates. Indeed, the sea urchin proneurotrophin is processed by the proprotein convertase furin, releasing mature 13 kDa neurotrophin, when expressed in human HEK293 cells (Mark Bothwell and Mark Hudson, unpublished results).

Curiously, the neurotrophin-signaling system has apparently been lost during evolution of several bilaterian classes. Clear orthologs of neurotrophin, $p75^{NTR}$, and Trk genes cannot be identified in protostome species such as *Drosophila melanogaster* or *Caenorhabditis elegans* or in deuterostome species such as *Ciona intestinalis* (Bothwell 2006). Lack of a neurotrophin-signaling system in *Ciona* is especially curious, as tunicates such as *Ciona* are commonly believed to the closest living prechordate relatives of vertebrates.

The lack of a neurotrophin-signaling system in *Drosophila* is a subject of mild controversy, as a family of *Drosophila* neurotrophic factors have been named as neurotrophins (Zhu et al. 2008). These secreted proteins, like neurotrophins, do contain a cystine knot structural fold, but they are only marginally more similar in sequence to neurotrophins than they are to a variety of non-neurotrophin proteins that contain cystine knot structures, and rather than signaling via $p75^{NTR}$-like or Trk-like receptors, they apparently employ receptors of the toll-like receptor family.

The chordate ancestor of modern vertebrates probably had only one neurotrophin and one Trk gene. The multiple neurotrophin and Trk paralogs in modern vertebrates (four neurotrophins in mammals, three neurotrophins in birds, three Trks in both mammals and birds) apparently arose as a result of the two genome duplications that occurred as an early event in vertebrate evolution (Hallbook et al. 2006).

6.2 Evolutionary Adaptations of NGF

6.2.1 Mouse Saliva NGF

The abundance of NGF in mouse salivary glands and in the venoms of various snakes (Cohen and Levimontalcini 1956), which greatly aided the original isolation of NGF, somewhat distorted understanding of the biochemistry of NGF in the early years after its discovery, since these sites of NGF synthesis represent peculiar species-specific adaptations with associated specializations of NGF synthesis and storage. Mouse salivary gland NGF is stored in secretory vesicles as a zinc ion-stabilized heterotrimeric complex, 7S NGF, consisting of alpha, beta, and gamma subunits. The beta subunits contain all of the neurotrophic activity (hence the name beta-NGF, which is still in common use), whereas the alpha and gamma subunits are members of the tissue kallikrein family of proteases (Bothwell et al. 1979). The tissue kallikrein gene family has undergone substantial expansion in rodents—orthologs of alpha and gamma NGF subunits do not exist in most other mammals, including humans. Thus, the 7S complex of NGF is only of interest as a peculiar mouse-specific specialization, and even in mice, there is little evidence for this heterotrimeric complex in tissues other than salivary glands.

6.2.2 NGF as a Noxious Component of Venoms

The question inevitably arises—why is NGF so highly enriched in the submaxillary salivary glands of mice and in the venom glands of snakes? The question is particularly poignant since NGF is abundant in the venoms of rattle snakes, vipers, and Australian elapid snakes, and the venom glands of these snake families are believed to have evolved independently (Jackson 2007), indicating that the highly enriched production of NGF in salivary or venom glands has evolved on at least four separate occasions, and implying that there is a strong selective pressure for this adaptation. It is reasonable to speculate that the selective pressure derives from the ability of NGF elicit pain. Ironically, the ability of NGF to elicit profound pain was not fully appreciated until it emerged as a serious side effect of human clinical trials of NGF for treatment of peripheral neuropathy (Petty et al. 1994). Although venomous snakes use their venom to immobilize prey, this effect is too slow to be a reliable defense mechanism against predators of snakes. The ability of snake venoms to cause immediate pain, mediated by NGF, may be a specialization to discourage predators. NGF is secreted at much higher concentrations in the saliva of male mice than in female mice and is released specifically in a specialized nondigestive saliva produced in response to epinephrine (Wallace and Partlow 1976), which is presumably elevated during the aggressive encounters that typify the behavior of male mice. Thus, salivary NGF in mice may also function as a pain-producing weapon.

6.2.3 Fowlpox NGF

One other evolutionary adaptation of NGF deserves comment. The genome of the fowlpox virus contains genes encoding two NGF orthologs (Afonso et al. 2000). Although the functional properties of the encoded NGF proteins have not been characterized, viruses commonly capture host genes that enhance the ability of the virus to evade host defenses. It would be interesting to learn what feature of avian skin biology is modified by the virally encoded NGFs. As conventional forms of NGF act through both $p75^{NTR}$ and TrkA receptors, it would also be interesting to learn whether the viral NGF has become specialized for activation of one or the other of these two receptors.

References

Afonso CL, Tulman ER, Lu Z, Zsak L, Kutish GF, Rock DL (2000) The genome of fowlpox virus. J Virol 74:3815–3831

Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, Lindsay RM, Wiegand SJ (1997) Anterograde transport of brain-derived neurotrophic factor and its role in the brain. Nature 389:856–860. doi:10.1038/39885

Aurikko JP, Ruotolo BT, Grossmann JG, Moncrieffe MC, Stephens E, Leppanen VM, Robinson CV, Saarma M, Bradshaw RA, Blundell TL (2005) Characterization of symmetric complexes of nerve growth factor and the ectodomain of the pan-neurotrophin receptor, p75NTR. J Biol Chem 280:33453–33460. doi:10.1074/jbc.M503189200

Barde YA, Edgar D, Thoenen H (1982) Purification of a new neurotrophic factor from mammalian brain. EMBO J 1:549–553

Berkemeier LR, Winslow JW, Kaplan DR, Nikolics K, Goeddel DV, Rosenthal A (1991) Neurotrophin-5: a novel neurotrophic factor that activates trk and trkB. Neuron 7:857–866

Bothwell M (1991) Keeping track of neurotrophin receptors. Cell 65:915–918

Bothwell M (2006) Evolution of the neurotrophin signaling system in invertebrates. Brain Behav Evol 68:124–132

Bothwell MA, Shooter EM (1977) Dissociation equilibrium constant of beta nerve growth factor. J Biol Chem 252:8532–8536

Bothwell MA, Wilson WH, Shooter EM (1979) The relationship between glandular kallikrein and growth factor-processing proteases of mouse submaxillary gland. J Biol Chem 254:7287–7294

Butte MJ, Hwang PK, Mobley WC, Fletterick RJ (1998) Crystal structure of neurotrophin-3 homodimer shows distinct regions are used to bind its receptors. Biochemistry 37:16846–16852. doi:10.1021/bi981254o

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J Neurosci 24:4401–4411. doi:10.1523/JNEUROSCI.0348-04.2004

Chen ZY, Ieraci A, Teng H, Dall H, Meng CX, Herrera DG, Nykjaer A, Hempstead BL, Lee FS (2005) Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. J Neurosci 25:6156–6166. doi:10.1523/JNEUROSCI.1017-05.2005

Clary DO, Reichardt LF (1994) An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. Proc Natl Acad Sci U S A 91:11133–11137

Cohen S (1960) Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum. Proc Natl Acad Sci U S A 46:302–311

Cohen S, Levi-Montalcini R (1956) A nerve growth-stimulating factor isolated from snake venom. Proc Natl Acad Sci U S A 42:571–574

Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S (1997) Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. J Neurosci 17:2295–2313

Dieni S, Matsumoto T, Dekkers M, Rauskolb S, Ionescu MS, Deogracias R, Gundelfinger ED, Kojima M, Nestel S, Frotscher M, Barde YA (2012) BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. J Cell Biol 196:775–788. doi:10.1083/jcb.201201038

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112:257–269

Farhadi HF, Mowla SJ, Petrecca K, Morris SJ, Seidah NG, Murphy RA (2000) Neurotrophin-3 sorts to the constitutive secretory pathway of hippocampal neurons and is diverted to the regulated secretory pathway by coexpression with brain-derived neurotrophic factor. J Neurosci 20:4059–4068

Gong Y, Cao P, Yu HJ, Jiang T (2008) Crystal structure of the neurotrophin-3 and p75NTR symmetrical complex. Nature 454:789–793. doi:10.1038/nature07089

Greene LA, Shooter EM, Varon S (1969) Subunit interaction and enzymatic activity of mouse 7S nerve growth factor. Biochemistry 8:3735–3741

Griesbeck O, Canossa M, Campana G, Gartner A, Hoener MC, Nawa H, Kolbeck R, Thoenen H (1999) Are there differences between the secretion characteristics of NGF and BDNF? Implications for the modulatory role of neurotrophins in activity-dependent neuronal plasticity. Microsc Res Tech 45:262–275. doi:10.1002/(SICI)1097-0029(19990515/01)45:4/5<262::AID-JEMT10>3.0.CO;2-K

Hallbook F, Ibanez CF, Persson H (1991) Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. Neuron 6:845–858
Hallbook F, Wilson K, Thorndyke M, Olinski RP (2006) Formation and evolution of the chordate neurotrophin and Trk receptor genes. Brain Behav Evol 68:133–144. doi:10.1159/000094083
Hamburger V (1934) The effects of wing bud extirpation on the development of the central nervous system in chick embryos. J Exp Zool 68:449–494
Hamburger V (1939) Motor and sensory hyperplasia following limb-bud transplantations in chick embryos. Physiol Zool 12:268–284
Hamburger V, Levi-Montalcini R (1949) Proliferation, differentiation and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions. J Exp Zool 111:457–502
Harrington AW, St Hillaire C, Zweifel LS, Glebova NO, Philippidou P, Halegoua S, Ginty DD (2011) Recruitment of actin modifiers to TrkA endosomes governs retrograde NGF signaling and survival. Cell 146:421–434. doi:10.1016/j.cell.2011.07.008
He XL, Garcia KC (2004) Structure of nerve growth factor complexed with the shared neurotrophin receptor p75. Science 304:870–875. doi:10.1126/science.1095190
Hibbert AP, Morris SJ, Seidah NG, Murphy RA (2003) Neurotrophin-4, alone or heterodimerized with brain-derived neurotrophic factor, is sorted to the constitutive secretory pathway. J Biol Chem 278:48129–48136. doi:10.1074/jbc.M300961200
Hirata-Fukae C, Li HF, Hoe HS, Gray AJ, Minami SS, Hamada K, Niikura T, Hua F, Tsukagoshi-Nagai H, Horikoshi-Sakuraba Y, Mughal M, Rebeck GW, LaFerla FM, Mattson MP, Iwata N, Saido TC, Klein WL, Duff KE, Aisen PS, Matsuoka Y (2008) Females exhibit more extensive amyloid, but not tau, pathology in an Alzheimer transgenic model. Brain Res 1216:92–103. doi:10.1016/j.brainres.2008.03.079
Hohn A, Leibrock J, Bailey K, Barde YA (1990) Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. Nature 344:339–341. doi:10.1038/344339a0
Holden PH, Asopa V, Robertson AG, Clarke AR, Tyler S, Bennett GS, Brain SD, Wilcock GK, Allen SJ, Smith SK, Dawbarn D (1997) Immunoglobulin-like domains define the nerve growth factor binding site of the TrkA receptor. Nat Biotechnol 15:668–672. doi:10.1038/nbt0797-668
Huang YZ, McNamara JO (2010) Mutual regulation of Src family kinases and the neurotrophin receptor TrkB. J Biol Chem 285:8207–8217. doi:10.1074/jbc.M109.091041
Huang EJ, Reichardt LF (2003) Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 72:609–642. doi:10.1146/annurev.biochem.72.121801.161629
Ip NY, Ibanez CF, Nye SH, McClain J, Jones PF, Gies DR, Belluscio L, Le Beau MM, Espinosa R 3rd, Squinto SP et al (1992) Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity. Proc Natl Acad Sci U S A 89:3060–3064
Jackson K (2007) The evolution of venom-conducting fangs: insights from developmental biology. Toxicon 49:975–981. doi:10.1016/j.toxicon.2007.01.007
Jones KR, Reichardt LF (1990) Molecular cloning of a human gene that is a member of the nerve growth factor family. Proc Natl Acad Sci U S A 87:8060–8064
Jungbluth S, Bailey K, Barde YA (1994) Purification and characterisation of a brain-derived neurotrophic factor/neurotrophin-3 (BDNF/NT-3) heterodimer. Eur J Biochem 221:677–685
Kang HJ, Schuman EM (1995) Neurotrophin-induced modulation of synaptic transmission in the adult hippocampus. J Physiol (Paris) 89:11–22
Kelly RB (1985) Pathways of protein secretion in eukaryotes. Science 230:25–32
Lee R, Kermani P, Teng KK, Hempstead BL (2001) Regulation of cell survival by secreted proneurotrophins. Science 294:1945–1948. doi:10.1126/science.1065057
Lee FS, Rajagopal R, Chao MV (2002) Distinctive features of Trk neurotrophin receptor transactivation by G protein-coupled receptors. Cytokine Growth Factor Rev 13:11–17
Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengerer B, Masiakowski P, Thoenen H, Barde YA (1989) Molecular cloning and expression of brain-derived neurotrophic factor. Nature 341:149–152. doi:10.1038/341149a0

Levi-Montalcini R, Hamburger V (1951) Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. J Exp Zool 116:321–361
Levi-Montalcini R, Hamburger V (1953) A diffusible agent of mouse sarcoma producing hyperplasia of sympathetic ganglia and hyperneurotization of viscera in the chick embyro. J Exp Zool 123:233–287
Levi-Montalcini R, Levi G (1943) Recherches quantitatives sur la marche du processus de différenciation des neurons dans les ganglions spinaux de l'embryon de poulet. Arch Biol Liège 54:189–200
Lohof AM, Ip NY, Poo MM (1993) Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. Nature 363:350–353. doi:10.1038/363350a0
Lou H, Kim SK, Zaitsev E, Snell CR, Lu B, Loh YP (2005) Sorting and activity-dependent secretion of BDNF require interaction of a specific motif with the sorting receptor carboxypeptidase e. Neuron 45:245–255. doi:10.1016/j.neuron.2004.12.037
Maisonpierre PC, Belluscio L, Squinto S, Ip NY, Furth ME, Lindsay RM, Yancopoulos GD (1990) Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. Science 247:1446–1451
McAllister AK, Katz LC, Lo DC (1997) Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. Neuron 18:767–778
McDonald NQ, Blundell TL (1991) Crystallization and characterization of the high molecular weight form of nerve growth factor (7 S NGF). J Mol Biol 219:595–601
McDonald NQ, Chao MV (1995) Structural determinants of neurotrophin action. J Biol Chem 270:19669–19672
McDonald NQ, Lapatto R, Murray-Rust J, Gunning J, Wlodawer A, Blundell TL (1991) New protein fold revealed by a 2.3-A resolution crystal structure of nerve growth factor. Nature 354:411–414. doi:10.1038/354411a0
Mowla SJ, Pareek S, Farhadi HF, Petrecca K, Fawcett JP, Seidah NG, Morris SJ, Sossin WS, Murphy RA (1999) Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. J Neurosci 19:2069–2080
Nichols RA, Shooter EM (1985) Subunit interactions of the nerve and epidermal growth factor complexes: protection of the biological subunit from proteolytic modification. Dev Neurosci 7:216–229
Patterson SL, Grover LM, Schwartzkroin PA, Bothwell M (1992) Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. Neuron 9:1081–1088
Petty BG, Cornblath DR, Adornato BT, Chaudhry V, Flexner C, Wachsman M, Sinicropi D, Burton LE, Peroutka SJ (1994) The effect of systemically administered recombinant human nerve growth factor in healthy human subjects. Ann Neurol 36:244–246. doi:10.1002/ana.410360221
Philo J, Talvenheimo J, Wen J, Rosenfeld R, Welcher A, Arakawa T (1994) Interactions of neurotrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF), and the NT-3.BDNF heterodimer with the extracellular domains of the TrkB and TrkC receptors. J Biol Chem 269:27840–27846
Radziejewski C, Robinson RC, DiStefano PS, Taylor JW (1992) Dimeric structure and conformational stability of brain-derived neurotrophic factor and neurotrophin-3. Biochemistry 31:4431–4436
Rajagopal R, Chen ZY, Lee FS, Chao MV (2004) Transactivation of Trk neurotrophin receptors by G-protein-coupled receptor ligands occurs on intracellular membranes. J Neurosci 24:6650–6658. doi:10.1523/JNEUROSCI.0010-04.2004
Robinson RC, Radziejewski C, Stuart DI, Jones EY (1995) Structure of the brain-derived neurotrophic factor/neurotrophin 3 heterodimer. Biochemistry 34:4139–4146
Robinson RC, Radziejewski C, Spraggon G, Greenwald J, Kostura MR, Burtnick LD, Stuart DI, Choe S, Jones EY (1999) The structures of the neurotrophin 4 homodimer and the brain-derived neurotrophic factor/neurotrophin 4 heterodimer reveal a common Trk-binding site. Protein Sci 8:2589–2597. doi:10.1110/ps.8.12.2589

Rosenthal A, Goeddel DV, Nguyen T, Lewis M, Shih A, Laramee GR, Nikolics K, Winslow JW (1990) Primary structure and biological activity of a novel human neurotrophic factor. Neuron 4:767–773

Seidah NG, Benjannet S, Pareek S, Chretien M, Murphy RA (1996) Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. FEBS Lett 379:247–250

Smith MA, Zhang LX, Lyons WE, Mamounas LA (1997) Anterograde transport of endogenous brain-derived neurotrophic factor in hippocampal mossy fibers. Neuroreport 8:1829–1834

Urfer R, Tsoulfas P, O'Connell L, Hongo JA, Zhao W, Presta LG (1998) High resolution mapping of the binding site of TrkA for nerve growth factor and TrkC for neurotrophin-3 on the second immunoglobulin-like domain of the Trk receptors. J Biol Chem 273:5829–5840

von Bartheld CS, Byers MR, Williams R, Bothwell M (1996) Anterograde transport of neurotrophins and axodendritic transfer in the developing visual system. Nature 379:830–833. doi:10.1038/379830a0

Wallace LJ, Partlow LM (1976) alpha-Adrenergic regulation of secretion of mouse saliva rich in nerve growth factor. Proc Natl Acad Sci U S A 73:4210–4214

Wilson KH (2009) The genome sequence of the protostome Daphnia pulex encodes respective orthologues of a neurotrophin, a Trk and a p75NTR: evolution of neurotrophin signaling components and related proteins in the bilateria. BMC Evol Biol 9:243. doi:10.1186/1471-2148-9-243

Windisch JM, Auer B, Marksteiner R, Lang ME, Schneider R (1995) Specific neurotrophin binding to leucine-rich motif peptides of TrkA and TrkB. FEBS Lett 374:125–129

Zhou XF, Rush RA (1996) Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons. Neuroscience 74:945–953

Zhu B, Pennack JA, McQuilton P, Forero MG, Mizuguchi K, Sutcliffe B, Gu CJ, Fenton JC, Hidalgo A (2008) Drosophila neurotrophins reveal a common mechanism for nervous system formation. PLoS Biol 6:e284. doi:10.1371/journal.pbio.0060284

Deciphering Proneurotrophin Actions

B.L. Hempstead

Abstract

Like most growth factors, neurotrophins are initially synthesized as precursors that are cleaved to release C-terminal mature forms. The well-characterized mature neurotrophins bind to Trk receptors to initiate survival and differentiative responses. More recently, the precursor forms or proneurotrophins have been found to act as distinct ligands by binding to an unrelated receptor complex consisting of the p75 neurotrophin receptor (p75) and sortilin to initiate cell death. Induction of proNGF and p75 has been observed in preclinical injury models and in pathological states in the central nervous system, and strategies that block the proNGF/p75 interaction are effective in limiting neuronal apoptosis. In contrast, the mechanisms that regulate expression of other proneurotrophins, including proBDNF and proNT-3, are less well understood. Here, recent findings on the biological actions, regulation of expression, and pathophysiological effects of proneurotrophins will be reviewed.

Keywords

ProNGF • ProBDNF • ProNT3 • p75NTR • Synaptic plasticity • Neurodegeneration • Long term depression • Long term potentiation • Sortilin • Cell death • Apoptosis

Neurotrophins are a family of proteins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4 (NT-4/5). They exhibit well-characterized activities to promote neuronal survival and differentiation, to modulate synaptic plasticity and to play important roles in both the developing and adult nervous system (Chao 2003). While only four

B.L. Hempstead (✉)
Department of Medicine, Weill Cornell Medical College, Room C610, 1300 York Ave, New York, NY 10065, USA
e-mail: blhempst@med.cornell.edu

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_2,

neurotrophin genes are found in mammals, they are sufficient to modulate a diverse repertoire of functions in the peripheral and central nervous systems and in non-neuronal organs. Indeed, several conceptual advances have been made during the last decade that reveal how this might be accomplished. Here we will focus on proneurotrophins as unique ligands that complement and oppose the actions of mature neurotrophins. Given the breadth of this rapidly moving field, it is difficult to acknowledge all contributions, and oversights are unintended. Related areas including mechanisms of p75 activation and signaling, mature neurotrophin actions, and functions of sortilin family members are reviewed in other chapters of this publication.

Like most growth factors, neurotrophins are initially synthesized as precursors or proneurotrophins consisting of a N-terminal prodomain and a C-terminal mature domain. Following translation, proneurotrophins form noncovalent dimers via interactions of the mature domain which forms a cysteine knot-like structure (Bradshaw et al. 1993). Dimeric proneurotrophins can be cleaved by intracellular proteases, including furin and proconvertase, in the Golgi or in secretory vesicles to generate mature neurotrophins, which are dimers consisting of the mature domains (Seidah et al. 1996). Mature neurotrophins selectively bind to members of the family of Trk receptor tyrosine kinases, as well as to the p75 neurotrophin receptor, a TNFR superfamily member (Huang and Reichardt 2001; Dechant and Barde 2002; Hempstead 2002). The interaction of mature neurotrophins with Trk receptors initiates the differentiative and synaptic activities of mature neurotrophins. Mature neurotrophins also bind to the p75 receptor, although the biological outcomes depend upon whether p75 is expressed independently or as a receptor complex with Trk receptors. When p75 is co-expressed with TrkA, mature NGF binds to the complex with higher affinity than is observed when TrkA is expressed in the absence of p75 (Hempstead et al. 1991). Although these results have been interpreted as evidence that p75 can bind and then pass mature NGF to TrkA to facilitate binding (Barker 2007), other studies suggested that p75 was exerting an allosteric action on TrkA and that binding of mature NGF to p75 was not required for this effect (Esposito et al. 2001). Application of mature neurotrophins to p75 expressing cells has also been found to induce apoptosis, and genetic deletion of p75 in mice results in impaired sympathetic neuron or retinal ganglion cell death (Bamji et al. 1998; Frade and Barde 1999). However, high concentrations of mature neurotrophins were required to initiate cell death by p75 in vitro (Casaccia-Bonnefil et al. 1996; Yoon et al. 1998; Kenchappa et al. 2006), suggesting that an alternate form of neurotrophins might selectively activate p75 at more physiologic concentrations. Indeed, the precursor form of NGF, proNGF, can be released intact from cells and has been found to selectively activate p75 to induce apoptosis at subnanomolar concentrations (Lee et al. 2001). This finding suggests that the precursor is a distinct, biologically active ligand and that mature and proNGF can induce opposing actions. Subsequent studies have further defined the receptor complex to which proNGF binds: proNGF interacts with high affinity to complex consisting of p75 and the type I transmembrane protein sortilin, wherein the NGF mature domain binds to p75, and the prodomain

binds to sortilin (Nykjaer et al. 2004). Sortilin specifically recognizes the prodomains of the three proneurotrophins (proNGF, proBDNF, and proNT-3) and forms a co-receptor complex with $p75^{NTR}$ to convey proneurotrophin-induced apoptotic signaling at subnanomolar ligand concentrations (Nykjaer et al. 2004; Teng et al. 2005; Jansen et al. 2007; Willnow et al. 2008; Yano et al. 2009). Thus, the specificity of neurotrophin action is regulated by the form of ligand that is released from cells (proneurotrophin or mature), as well as by the interaction with distinct receptor complexes, with proneurotrophins preferentially activating p75 and sortilin, whereas mature neurotrophins activate Trk receptors.

More recent studies have determined that proNGF can also interact with another sortilin family member, SorCS2, when it is co-expressed with p75 (Deinhardt 2011; Siao 2012). SorCS2 is a transmembrane protein that is closely related to sortilin and is highly expressed in the developing and adult nervous system (Willnow et al. 2008). Like sortilin, SorCS2 interacts with the prodomain of proNGF.

1 Actions in ProNGF in Development

The ability of proNGF to induce apoptosis during development has been studied using several strategies. Although it would be tempting to generate a gene-targeted mouse that lacks the prodomain of NGF as a means to discriminate proNGF from mature NGF function, this is not an effective strategy as the prodomains of neurotrophins are required for efficient protein folding and intracellular trafficking (Suter et al. 1991; Chen et al. 2005). Also, the results obtained upon deletion of p75 must be interpreted carefully, as p75 interacts with all forms of neurotrophins, and with multiple co-receptors including TrkA, TrkB, and TrkC to modulate mature neurotrophin responsiveness, and with the Nogo receptor, Lingo-1 and ephrin A, to regulate axonal guidance (Schecterson and Bothwell 2008). Thus, genetic deletion of p75 can yield multiple and complex phenotypes based on potential interactions with other receptor components and thus attributing a specific phenotype to proNGF requires careful analysis of the expression patterns of other ligands and co-receptors.

However, utilization of neurons from *p75*-deficient mice has been a valuable tool to assess proNGF actions, as this imparts a proNGF-resistant phenotype in vitro (Lee et al. 2001). Mice deficient in *sortilin* have also been generated and characterized regarding proNGF-induced apoptosis during development (Jansen et al. 2007). Prior studies have documented impaired apoptosis of developing retinal ganglion cells in E15.5 embryos that were deficient in *p75* or *ngf* (48 % or 56 % reduction, respectively) (Frade and Barde 1998, 1999). Similarly, embryos deficient in *sortilin* exhibit reduced retinal ganglion cell death (63 % reduction) (Jansen et al. 2007). The immunodetection of proNGF but not mature NGF at this developmental window, together with the protection of these neurons in *sortilin*-deficient and *p75*-deficient mice, suggests that elimination of post-mitotic retinal ganglion cells is mediated by proNGF in late development. Surprisingly, neonatal mice deficient in *sortilin* exhibit no reduction in the numbers of sympathetic

ganglion neurons (Jansen et al. 2007), suggesting that other co-receptors may regulate sympathetic neuron elimination in vivo. An additional challenge in attributing the phenotypes of *sortilin*-deficient mice to proNGF activities is that sortilin binds to numerous other ligands, including proBDNF, proNT-3, TGF-beta family members, and apolipoprotein B (Strong et al. 2012; Kjolby et al. 2010; Kwon and Christian 2011); thus, documenting expression of proNGF and p75 is required to confirm that the effects of *sortilin* deficiency reflect the specific actions of proNGF.

2 ProNGF in Aging

ProNGF levels are very low in the central and peripheral nervous systems of uninjured young adult rodents (Harrington et al. 2004; Jansen et al. 2007). However, several studies indicate that proNGF levels are upregulated in adults of advanced age. For example, proNGF levels are elevated in the peripheral nerves of 60-week-old mice, and this expression correlates with age-dependent death of sympathetic neurons (Jansen et al. 2007). A more extensive analysis of ligand and receptor levels in aged or young adult rats documented increased levels of proNGF and p75 and decreased levels of mature NGF and phosphoTrkA in the prefrontal cortex and hippocampus of aged rats, as compared to younger adults (Terry et al. 2011; Allard et al. 2012). Although these effects correlated with impaired performance in spatial learning and recognition memory, a causal role was not investigated. New studies using transgenic overexpression of proNGF suggests it induces memory deficits (Tiveron et al. 2013). A systematic and quantitative analysis of proNGF levels in postmortem human brains from aged but cognitively normal individuals has not been performed. However, a report in aged rodents suggests that proNGF is an apoptotic ligand in basal forebrain cholinergic neurons (Al-Shawi et al. 2008). Indeed, proNGF levels have been found to be elevated in Alzheimer's disease patients (Fahnestock et al. 2001; Pedraza et al. 2005) and in animal models of Alzheimer's disease (3xTg-AD mice; Perez et al. 2011). Future studies will be required to determine whether impairment of the interaction of proNGF with sortilin may prevent age-associated neuronal loss, as has been proposed for proNGF:p75 antagonists (Massa et al. 2006). However, a transgenic line approach has been used to selectively deplete mature NGF, but not proNGF (line AD-11) (Capsoni et al. 2010). This strategy results in an imbalance, with impaired TrkA signaling, but sustained proNGF:p75 signaling. These studies have suggested that imbalance of the proNGF:mature NGF ratio in the CNS can trigger cholinergic neuron loss similar to that observed in Alzheimer's disease (Capsoni et al. 2010). Consistent with this hypothesis, the activation of p75 by proNGF was observed to suppress survival signaling by TrkA, specifically by impairing PTEN induction that blunts PI3-kinase-Akt activation (Song et al. 2010). Collectively, these studies suggest that selectively shifting the balance between pro-apoptotic and pro-survival pathways, triggered by proNGF or mature NGF, respectively, one can potentially prevent neuronal loss.

3 ProNGF Actions Following Injury

Because apoptosis was the first identified action of proNGF in vitro, many studies have examined the roles of proNGF following acute injury in the peripheral and central nervous systems. Following spinal cord injury in rodents, proNGF and p75 expression are induced within a few days and maintained for at least 1 week; in a related model of corticospinal neuron axotomy, p75, sortilin and proNGF are all coordinately up regulated, and overexpression is maintained for 2 weeks (Brunello et al. 1990; Beattie et al. 2002; Harrington et al. 2004; Arnett et al. 2007; Jansen et al. 2007). To examine a causative role for proNGF in promoting neuronal apoptosis following corticospinal axotomy, two approaches have been used. First, genetic deletion of *p75* or *sortilin*, or haploinsufficiency of *ngf*, rescues most of the death of corticospinal neurons after axotomy (Harrington et al. 2004; Jansen et al. 2007). In addition, infusion of function-blocking antibodies specific for the prodomain of proNGF markedly reduces apoptosis, strongly suggesting that proNGF is an inducible, proapoptotic cytokine (Harrington et al. 2004). More recently, administration of a ProNGF/p75 antagonist has been shown to promote functional recovery following spinal cord injury (Tep et al. 2013)

ProNGF has also demonstrated pro-apoptotic actions in cultured spinal motor neurons that express p75 and sortilin (Domeniconi et al. 2007). Using peroxynitrite as an oxidant and to generate of free radicals, reactive astrocytes were found to upregulate proNGF production, suggesting that proNGF may be a potential therapeutic target for the treatment of motor neuron disease. Astrocytes are also a significant source of the proNGF that is induced following seizures in rodents (Volosin et al. 2008). In a pilocarpine model of seizure induction, proNGF and proBDNF are upregulated by astrocytes, but not by microglia. Following seizures, infusion of function-blocking antibodies specific for the prodomain of NGF impairs hippocampal neuron apoptosis in vivo, suggesting that proNGF is the relevant ligand that mediates the apoptotic effects (Volosin et al. 2008).

Increased proNGF expression has been observed in spongiform encephalomyelopathy (Stoica et al. 2008) and Parkinson's disease models (Wang et al. 2008); however, a mechanistic role for proNGF in these slow onset neurodegenerative diseases has not been demonstrated. However, recent studies have examined a potential role for progranulin, as progranulin loss of function has been associated with frontotemporal lobar degeneration (FTLD), and modulates sortilin function (Hu et al. 2010). Although the precise mechanisms by which progranulin deficiency contributes to neuronal dysfunction in aging, the enhanced expression of proNGF in aging rodent animal models (Terry 2011) raises the possibility that proNGF might compete with the binding of progranulin to sortilin to augment the progression or onset of cognitive degeneration in a $p75^{NTR}$-indpendent mechanism. This potential action, however, will require experimental validation.

Studies of models of retinal injury suggest that proNGF is induced in microglia in a model of retinal dystrophy (Srinivasan et al. 2004) and that sortilin and p75 are induced in retinal ganglion cells following elevation in intraocular pressure, suggesting that proNGF may play a role in the retinal neuron death that occurs in this ischemic setting (Wei et al. 2007). More recent studies have examined a

potential role for proNGF in promoting retinal neurodegeneration in the retina of diabetic rodents (Al-Gayyar et al. 2013). In the peripheral nervous system, injured sciatic neurons express proNGF and this may result in the loss of p75-expressing neurons following transection (Arnett et al. 2007). Collectively, these diverse models of injury or aging suggest that proNGF may be a pathophysiologically relevant proapoptotic ligand.

3.1 ProNGF Actions in Non-neuronal Organ Systems

As NGF is normally synthesized by many organs to promote innervation during development, misregulation of NGF expression, or impaired conversion of proNGF to mature NGF in disease could contribute to pathology. Several recent reports have established that proNGF is misregulated in breast cancer, following myocardial infarction, and in psoriasis. In human breast cancer specimens, proNGF is upregulated and appears to mediate cell invasion, an effect requiring TrkA and sortilin, rather than p75 (Demont et al. 2012). These studies suggest that NGF and TrkA may be relevant preclinical targets for further examination (Hondermarck 2012). ProNGF and p75 have also been studied in dermatologic diseases, including psoriasis where a failure to induce apoptosis of transit amplifying cells in the dermis, in a p75-dependent manner, may contribute to this disease (Truzzi et al. 2011). Although initial studies focused on proNGF as the relevant p75 ligand, due to its expression in keratinocytes, additional p75 ligands such as BDNF may contribute. Lastly, prior studies have documented induction of the *ngf* gene by cardiac myocytes following ischemic injury using rodent models of myocardial infarction (Hiltunen et al. 2001; Meloni et al. 2010). More recently, the *ngf* isoform that is induced has been shown to be proNGF, which is upregulated by cardiac myocytes following ischemia reperfusion injury in rodents and in humans following fatal myocardial infarction. Coordinate upregulation of p75 and SorCS2 is observed by the pericytes and smooth muscle cells in cardiac vessels in the ischemic zone. Furthermore, deletion of *p75* limits the infarct size, suggesting that proNGF represents a new target to limit microvascular dysfunction (Siao et al. 2012). Additional studies will be required to determine whether proNGF plays a role in the vasculature of other organ systems.

4 Acute Proneurotrophin Actions on Neuronal Morphology

Recent studies have examined relatively acute, non-apoptotic functions for proNGF and proBDNF. These studies build upon older reports that document that p75 interacts with RhoA (Yamashita et al. 1999) and fascin (Shonukan et al. 2003), signaling intermediates that are coupled to cytoskeletal reorganization. Two seminal studies have also indicated that p75 regulates neuronal morphology: (a) loss of p75 in gene-targeted mice leads to enhanced dendritic arborization (Zagrebelsky et al. 2005) and (b) p75 activation by BDNF leads to axonal pruning (Singh et al. 2008). However, these studies did not directly compare the effects

proneurotrophins vs. mature neutrophins in eliciting morphological actions. In more recent experiments using live imaging of neuronal growth cones, proNGF was found to induce rapid growth cone collapse in neurons expressing p75 and the sortilin family member, SorCS2 (Deinhardt et al. 2011). These effects were dependent upon two coordinated signaling pathways. First proNGF induced a dissociation of the Trio GEF from the p75/SorCS2 receptor complex, which resulted in local Rac inactivation. This was coupled with PKC activation and fascin phosphorylation, leading to a reduction in actin bundling and neurite retraction.

ProBDNF has also been demonstrated to exert rapid morphological effects on neurons, specifically at the neuromuscular junction, where proBDNF secreted from myocytes induces retraction of motors neuron axons, as well as synaptic depression (Yang et al. 2009a; Je et al. 2012). A similar effect of recombinant proBDNF has been observed using dorsal root ganglion neurons, where proBDNF led to acute neurite collapse in a Rho A-dependent fashion (Sun et al. 2012). This retraction requires expression of p75. Lastly, using cultured retinal ganglion cells, Marler and colleagues have provided evidence that proBDNF is secreted from these cells and acts locally to enable repellant axon guidance in a p75-ephrin A-dependent fashion (Marler et al. 2010). Further studies will be required to identify whether proBDNF interactions with p75 can be mediated by a range of co-receptors, including sortilin family members and/or ephrins, in a cell type-specific manner.

5 ProBDNF Effects on Synaptic Plasticity

The effects of mature BDNF on hippocampal structure and synaptic plasticity are well described (Korte et al. 1998; Keng et al. 1997; Lu et al. 2008, and recently reviewed by Park and Poo 2013); however, the effects of proBDNF are less clear. Several studies suggest that endogenous proBDNF can be released from neurons. One study has used hippocampal neurons from a knock-in mouse expressing HA-epitope-tagged BDNF (Yang et al. 2009b) to quantitatively detect secreted proBDNF and mature BDNF using antibodies to the HA-tag, rather than relying on antibodies that recognize either proBDNF or mature BDNF. With this approach, both proBDNF and mature BDNF were secreted following depolarization. In contrast, mature BDNF was the predominant secreted form secreted from hippocampal neurons cultured with astrocytes and the $GABA_A$ receptor antagonist bicuculline (Matsumoto et al. 2008). However, using electrical stimulation of cultured hippocampal neurons, proBDNF was the predominant form secreted after low-frequency stimulation (LFS; used to induce LTD), whereas mature BDNF was released following high frequency stimulation (HFS; used to induce LTP; Nagappan et al. 2009). It is well established that tPA is secreted following depolarization (Lochner et al. 2006, 2008) and thus proBDNF may be locally converted to mature BDNF by the coordinated release of proBDNF and tPA from axons.

The actions of proBDNF have been best described using recombinant proBDNF protein. Treatment of cultured neurons with recombinant proBDNF promotes

neuronal death and process retraction, mediated by $p75^{NTR}$ (Teng et al. 2005; Sun et al. 2012). Recombinant proBDNF also influences synaptic plasticity in area CA1 of the hippocampus; following perfusion of slices with cleavage-resistant proBDNF, LTD was significantly enhanced, an effect that requires expression of p75 (Woo et al. 2005). In contrast, mature BDNF is required for the maintenance of LTP induced by stimuli that simulates theta rhythm (TBS) (Korte et al. 1998; Chen et al. 2010; Keng et al. 1997; and recently reviewed, Park and Poo 2013). Together, these results suggest that proBDNF and mature BDNF have opposing effects in vivo, with proBDNF supporting LTD and mature BDNF important to LTP. The effects of exogenous proBDNF have been extended to other classes of neurons, as proBDNF negatively regulates neuromuscular synaptic activity via $p75^{NTR}$ (Yang et al. 2009a; Je et al. 2012).

One aspect of proBDNF and mature BDNF action in the hippocampus that is unresolved is the relative levels of the two isoforms that are expressed during postnatal development and in adulthood. In one study, hippocampal proBDNF expression was found to be highest in the second postnatal week, when axonal projections are being established and synapses are forming, as quantitated using a tagged *bdnf* allele to measure proBDNF and mature BDNF levels (Yang et al. 2009b). In the adult mouse, however, mature BDNF was found to be the predominant form (Yang et al. 2009b). Other studies suggest that mature BDNF is the predominant isoform from postnatal day 4 until 12 weeks of age (Rauskolb et al. 2010). However, the levels of $p75^{NTR}$ are highest in early postnatal life and diminish in adulthood (Yang et al. 2009b). Thus the effects of endogenous proBDNF may be most prominent in early postnatal development and further studies to document the levels of endogenous proBDNF and its effects in vivo are warranted.

6 ProBDNF in Disease States

ProNGF has been most intensively studied regarding its expression in disease states in humans and in preclinical rodent models, as noted above. However, several studies have documented elevated levels of proBDNF in postmortem brain sections from subjects with cognitive impairment from HIV neurotoxicity (Bachis et al. 2012). The levels of proBDNF and mature BDNF have also been studied in a small number of postmortem sections from subjects with autism or unaffected controls. In the fusiform gyrus, increased levels of proBDNF and decreased levels of mature BDNF were observed in subjects with autism, suggesting local defects in proteolytic processing (Garcia et al. 2012). These results contrast with those obtained using brain tissue from Alzheimer's disease patients, where reduction in both proBDNF and mature BDNF was observed, as compared to control (Peng et al. 2005). Further studies with larger sample sizes, as well as in vivo models that identify actions of endogenous proBDNF will be helpful in clarifying the potential roles of this isoform in disease.

7 Other Proneurotrophins: ProNT-3 and ProNT-4

The majority of studies to date have focused on the biological actions of proNGF and proBDNF. However, the NT-3 and NT-4 mature products are derived from precursor proteins, raising the possibility that proNT-3 and proNT-4 may exhibit biological actions distinct from their mature neurotrophin counterparts. ProNT-3 has been biochemically generated, and it mediates apoptotic actions on SCG neurons, utilizing p75 and sortilin as co-receptors (Yano et al. 2009). In addition, proNT-3 has been detected in the developing inner ear, and sortilin and p75 receptors are also present on spiral ganglion neurons. Although no changes in spiral ganglion numbers have been detected in *sortilin* null mice during development, proNT-3 may play a role following barotrauma injury (Tauris et al. 2011). Currently, there are no reports as to whether proNT-4 exhibits pro-apoptotic activity or other biological actions. However, its prodomain is substantially smaller than those of the other three proneurotrophins and the NT-4 prodomain does not bind sortilin (Chen et al. 2005); suggesting that NT-4 might exist strictly as a TrkB ligand.

8 Regulation of Conversion of Proneurotrophins to Mature Neurotrophins

In adult tissues, mature NGF and mature BDNF are the predominant isoforms, present at very low, subnanomolar levels (Shetty et al. 2003; Rauskolb et al. 2010). Thus, it is not clear how proNGF, secreted in injury response states, escapes the mechanisms that normally ensure efficient intracellular conversion to mature NGF. In neuroendocrine cells and hippocampal neurons, proNGF is cleaved efficiently by furin and the mature domain is trafficked to secretory vesicles in the constitutive pathway, whereas the prodomain remains in the cell body and sorted to lysosomes for degradation (Mowla et al. 1999). Indeed, secretion of a soluble proNGF prodomain has been difficult to detect, although Dicou and colleagues have observed peptides of the prodomain in inflammatory states (Dicou 2008). These studies suggest that there is efficient conversion of proNGF to mature NGF in uninjured organs, and constitutive secretion of mature NGF is the norm. However, the intracellular chaperones that traffic proNGF to the trans-Golgi network where furin cleavage occurs have not been characterized. One candidate is sortilin, a VpS10p protein that has been well characterized, as described above, as a cell surface co-receptor with p75 for proNGF; however, direct experimental evidence to support this is currently lacking. ProBDNF has been demonstrated to bind to sortilin to direct intracellular trafficking of to regulated secretory vesicles (Chen et al. 2005), where proBDNF can be cleaved by proconvertase (Seidah et al. 1996). However, sortilin can also traffic proBDNF and other cargo, including sphingomyelinase, to the lysosome (Evans et al. 2011; Ni and Morales 2006), and it is not clear how these targeting decisions are regulated. Other chaperones including carboxypeptidase E bind to the mature domain of BDNF, but not mature NGF (Lou et al. 2005). Therefore, many questions still remain regarding the intracellular

proteins that regulate proNGF intracellular trafficking, and release, as well as the mechanisms that regulate the efficiency of proBDNF cleavage within secretory vesicles. Recent reports, however, have documented that the prodomain of BDNF is detectable in vivo (Dieni et al. 2012; Anastasia et al. 2013).

9 Extracellular Cleavage of Proneurotrophins

Recombinant proNGF and proBDNF are susceptible to cleavage by numerous proteases, including plasmin, tryptase, and specific matrix metalloproteinases (MMPs) (Lee et al. 2001; Bruno and Cuello 2006; Althaus and Kloppner 2006; Spinnler et al. 2011). Nonetheless, intact proNGF is detectable for several days to weeks following central nervous system injury, with little evidence of conversion to mature NGF in these vivo settings (Beattie et al. 2002; Harrington et al. 2004; Jansen et al. 2007). These observations suggest that proteolysis of extracellular proNGF is impaired following in vivo injury and may result from the coordinate induction of inhibitors of MMPs and plasmin, such as tissue inhibitors of metalloproteinase (TIMPs), neuroserpin, and alpha-2 macroglobulin. This is in agreement with prior studies documenting that these proteins are induced in neurodegenerative diseases such as Parkinson's and Huntington's diseases and following neuronal excitotoxicity (Bruno and Cuello 2006; Dzwonek et al. 2004; Jaworski et al. 1999; Lorenzi et al. 2003). Indeed, recent studies using a seizure model of CNS injury demonstrates that MMP-7 is downregulated, whereas its inhibitor, TIMP-1, is induced, leading to stabilization of proNGF. Furthermore, exogenous delivery of MMP-7 following seizures enhances proNGF cleavage and reduced neuronal apoptosis (Le and Friedman 2012), suggesting that the efficiency of proNGF to NGF conversion can be experimentally manipulated to provide neuroprotection.

10 Molecular Strategies to Alter ProNGF Effects

Given the induction in proNGF and p75 in numerous pathophysiologically relevant preclinical models that result in cellular apoptosis or acute morphological remodeling, there has been broad interest in targeting proNGF/p75 signaling. The low levels of p75 and proNGF in the uninjured central nervous system and induction of both ligand and receptor within several hours to days of acute injury suggest that there is a window of opportunity for administration of agents to block the induction of ligand or receptors or their interaction. By silico modeling, small molecules have been identified that interact with a p75 structural domain important for mature NGF binding; in addition, these molecules block proNGF actions in cultured neurons (Massa et al. 2006). These molecules are now being tested in rodent models of spinal cord injury (Tep et al. 2013). Additional modeling approaches to impair proNGF/p75/sortilin interactions may provide additional reagents to block proNGF actions. The crystallographic structure of p75 with

mature NGF, p75 with proNGF, and the structure of sortilin are all available (He and Garcia 2004; Quistgaard et al. 2009; Feng et al. 2010). Although the structure of the proNGF/p75/sortilin complex has remained elusive, its eventual solution may provide information for the development of antagonists in the future.

Lastly, the activation of intracellular or extracellular proteases to specifically cleave proneurotrophins to mature neurotrophins is another attractive target. To this end, a more detailed understanding of the regulation of intracellular trafficking of proNGF in injured cells, and mechanisms that permit inefficient intracellular cleavage are needed. In addition, the stability of proNGF in the injured central nervous system suggests that specific protease inhibitors in the local inflammatory environment may prevent efficient extracellular cleavage of proNGF, and strategies to locally manipulate the proteolytic landscape following acute injury are being studied (Le and Friedman 2012). A quantitative assessment of locally produced proteases and their specific inhibitors in the injured central nervous system will provide specific candidate molecules to promote proneurotrophin to mature neurotrophin conversion.

Acknowledgements The author is supported in part by funding from the NIH/NINDS and NS30687 and NS064114.

References

Al-Gayyar MM, Mysona BA, Matragoon S, Abdelsaid MA, El-Azab MF, Sanab AY, Ha Y, Smith SB, Bollinger KE, El-Remessy AB (2013) Diabetes and overexpression of proNGF cause retinal neurodegeneration via activation of RhoA Pathway. PLoS One 8:e54692

Allard S, Leon WC, Pakavathkumar P, Bruno MA, Ribeiro-da-Silva A, Cuello AC (2012) Impact of the NGF maturation and degradation pathway on the cortical cholinergic system phenotype. J Neurosci 32:2002–2012

Al-Shawi R, Hafner A, Olsen J, Chun S, Raxa S, Thrasivoulou C, Lovestone S, Killick R, Simons P, Cowen T (2008) Neurotoxic and neurotrophic roles of proNGF and the receptor sortilin in the adult and ageing nervous system. Eur J Neurosci 28:1940

Althaus HH, Kloppner S (2006) Mature pig oligodendrocytes rapidly process human recombinant pro-nerve growth factor and do not undergo cell death. J Neurochem 98:506–517

Anastasia A, Deinhardt K, Chao MV, Will NE, Irmady K, Lee FS, Hempstead BL, Bracken C (2013) Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. Nat Commun 4:2490

Arnett MG, Ryals JM, Wright DE (2007) Pro-NGF, sortilin and p75NTR: potential mediator of injury induced apoptosis in the mouse dorsal root ganglion. Brain Res 1183:32–42

Bachis A, Avdoshina V, Zecca L, Parsadanian M, Mocchetti I (2012) Human immunodeficiency virus type 1 alters brain-derived neurotrophic factor processing in neurons. J Neurosci 32: 9477–9484

Bamji SX, Majdan M, Pozniak CD, Belliveau DJ, Aloyz R, Causing CG, Miller FD (1998) The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. J Cell Biol 140:911–923

Barker PA (2007) High affinity not in the vicinity? Neuron 53:1–4

Beattie MS, Harrington AW, Lee R, Kim JY, Boyce SL, Longo FM, Bresnahan JC, Hempstead BL, Yoon SO (2002) ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. Neuron 36:375–386

Bradshaw RA, Blundell TL, Lapetto R, McDonald NQ, Murray-Rust J (1993) Nerve growth factor revisited. Trends Biochem Sci 18:48–52

Brunello N, Reynolds M, Wrathall JR, Mocchetti I (1990) Increased nerve growth factor receptor mRNA in contused rat spinal cord. Neurosci Lett 118:238–240

Bruno MA, Cuello AC (2006) Activity-dependent release of precursor nerve growth factor, conversion to mature nerve growth factor and its degradation by a protease cascade. Proc Natl Acad Sci U S A 103:6735–6740

Capsoni S, Tiveron C, Vignone D, Amato G, Cattaneo A (2010) Dissecting the involvement of tropomysin-related kinase A and p75 neurotrophin receptor signaling in NGF deficit-induced neurodegeneration. Proc Natl Acad Sci U S A 107:12299–12304

Casaccia-Bonnefil P, Carter BD, Dobrowsky RT, Chao MV (1996) Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. Nature 383:716–719

Chao MV (2003) Neurotrophins and their receptor: a convergence point for many signaling pathways. Nat Rev Neurosci 4:299–309

Chen ZY, Ieraci A, Teng H, Dall H, Meng CX, Herrera DG, Nykjaer A, Hempstead BL, Lee FS (2005) Sortilin controls the intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. J Neurosci 25:6156–6166

Chen LY, Rex CS, Sanaiha Y, Lynch G, Gall CM (2010) Learning induces neurotrophin signaling at hippocampal synapses. Proc Natl Acad Sci U S A 107:7030–7035

Dechant G, Barde YA (2002) The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. Nat Neurosci 5:1131–1136

Deinhardt K, Kim T, Spellman DS, Mains RE, Eipper BA, Neubert TA, Chao MV, Hempstead BL (2011) Neuronal growth cone retraction relies on proneurotrophin receptor signaling through Rac. Sci Signal 4(202):ra82

Demont Y, Corbet C, Page A, Ataman-Őnal Y, Choquet-Kastylevsky G, Fliniaux I, Le Bourhis X, Toillon RA, Bradshaw RA, Hondermarck H (2012) Pro-nerve growth factor induces autocrine stimulation of breast cancer cell invasion through tropomyosin-related kinase A (TrkA) and sortilin protein. J Biol Chem 287:1923–1931

Dicou E (2008) High levels of the proNGF peptides LIP1 and LIP2 in the serum and synovial fluid of rheumatoid arthritis patients: evidence for two new cytokines. J Neuroimmunol 194: 143–146

Dieni S, Matsumoto T, Dekkers M, Rauskolb S, Ionescu MS, Deogracias R, Gundelfinger ED, Kojima M, Nestel S, Frotscher M, Barde YA (2012) BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. J Cell Biol 196(6):775–788

Domeniconi M, Hempstead BL, Chao MV (2007) Pro-NGF secreted by astrocytes promotes motor neuron cell death. Mol Cell Neurosci 34:271–279

Dzwonek J, Rylski M, Kaczmarek L (2004) Matrix metalloproteinases and their endogenous inhibitors in neuronal physiology of the adult brain. FEBS Lett 567:129–135

Esposito D, Patel P, Stephens RM, Perez P, Chao MV, Kaplan DR, Hempstead BL (2001) The cytoplasmic and transmembrane domains of the p75 and Trk A receptors regulate high affinity binding to nerve growth factor. J Biol Chem 276:32687–32695

Evans SF, Irmady K, Ostrow K, Lim T, Nykjaer A, Saftig P, Blobel C, Hempstead BL (2011) Neuronal brain-derived neurotrophic factor is synthesized in excess, with levels regulated by sortilin mediated trafficking and lysosomal degradation. J Biol Chem 286:29556–29567

Fahnestock M, Michalski B, Xu B, Coughlin MD (2001) The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. Mol Cell Neurosci 18:210–220

Feng D, Kim T, Ozkan E, Light M, Torkin R, Teng KK, Hempstead BL, Garcia KC (2010) Molecular and structural insight into proNGF engagement of p75NTR and sortilin. J Mol Biol 396:967–984

Frade JM, Barde YA (1998) Microglial-derived nerve growth factor causes cell death in the developing retina. Neuron 20:35–41

Frade JM, Barde YA (1999) Genetic evidence for cell death mediated by nerve growth factor and the neurotrophin receptor p75 in the developing mouse retina and spinal cord. Development 126:683–690

Garcia KL, Yu G, Nicolini C, Michalski B, Garzon DJ, Chiu VS, Tongiorgi E, Szatmari P, Fahnestock M (2012) Altered balance of proteolytic isoforms of pro-brain-derived neurotrophic factor in autism. J Neuropathol Exp Neurol 71:289–297

Harrington AW, Leiner B, Blechschmitt C, Arevalo JC, Lee R, Morl K, Meyer M, Hempstead BL, Yoon SY, Giehl KM (2004) Secreted proNGF is a pathophysiological death-inducing ligand after CNS injury. Proc Natl Acad Sci U S A 101:6226–6230

He XL, Garcia KC (2004) Structure of nerve growth factor complexed with the shared neurotrophin receptor p75. Science 304:870–875

Hempstead BL (2002) The many faces of p75NTR. Curr Opin Neurobiol 12:260–267

Hempstead BL, Martin-Zanca D, Kaplan DR, Parada LF, Chao MV (1991) High-affinity NGF-binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. Nature 350:678–683

Hiltunen JO, Laurikainen A, Väkevä A, Meri S, Saarma M (2001) Nerve growth factor and brain-derived neurotrophic factor mRNAs are regulated in distinct cell populations of rat heart after ischaemia and reperfusion. J Pathol 194:347–353

Hondermarck H (2012) Neurotrophins and their receptors in breast cancer. Cytokine Growth Factor Rev 23:357–365

Hu F, Padukkavidana T, Vaegter CB, Brady OA, Zheng Y, Mackenzie IR, Feldman HH, Nykjaer A, Strittmatter SM (2010) Sortilin-mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. Neuron 68:654–667

Huang EJ, Reichardt LF (2001) Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 72:609–642

Jansen P, Giehl K, Nyengaard JR, Teng K, Lioubinski O, Sjoegaard SS, Breiderhoff T, Gotthardt M, Lin F, Eilers A, Petersen CM, Lewin GR, Hempstead BL, Willnow TE, Nykjaer A (2007) Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. Nat Neurosci 10:1449–1457

Jaworski J, Biedermann IW, Lapinska J, Szkiarczyk A, Figiel I, Konopka D, Nowicka D, Filipkowski RK, Hetman M, Kowalczyk A, Kaczmarek L (1999) Neuronal excitation-driven and AP-1 dependent activation of tissue inhibitor of metalloproteinase-1 gene expression in rodent hippocampus. J Biol Chem 274:28106–28112

Je HS, Yang F, Ji Y, Nagappan G, Hempstead GL, Lu B (2012) Role of pro-brain-derived neurotrophic factor (proBDNF) to mature BDNF conversion in activity-dependent competition at developing neuromuscular synapses. Proc Natl Acad Sci U S A 109:15924–15929

Kenchappa RS, Zampieri N, Chao MV, Barker PA, Teng HK, Hempstead BL, Carter BD (2006) Ligand-dependent cleavage of the p75 receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. Neuron 50:219–232

Keng H, Welcher AA, Shelton D, Schuman EM (1997) Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. Neuron 19:653–664

Kjolby M, Andersen OM, Breiderhoff T, Fjorback AW, Pedersen KM, Madsen P, Jansen P, Heeren J, Willnow TE, Nykjaer A (2010) Sort1, encoded by the cardiovascular risk locus 1p13.3, is a regulator of hepatic lipoprotein export. Cell Metab 12:213–223

Korte M, Kang H, Bonhoeffer T, Schuman E (1998) A role for BDNF in the late-phase of hippocampal long-term potentiation. Neuropharmacology 37:553–559

Kwon S, Christian JL (2011) Sortilin associates with transforming growth factor-beta family proteins to enhance lysosome-mediated degradation. J Biol Chem 286:21876–21885

Le AP, Friedman WJ (2012) Matrix metalloproteinase-7 regulates cleavage of pro-nerve growth factor and is neuroprotective following kainic acid-induced seizures. J Neurosci 32:703–712

Lee R, Kermani P, Teng KK, Hempstead BL (2001) Regulation of cell survival by secreted pro-neurotrophins. Science 294:1945–1948

Lochner JE, Honigman LS, Grant WF, Gessford SK, Hansen AB, Silverman MA, Scalettar BA (2006) Activity-dependent release of tissue plasminogen activator from the dendritic spines of hippocampal neurons revealed by live-cell imaging. J Neurobiol 66:564–577

Lochner JE, Spangler E, Chavarha M, Jacobs C, McAllister K, Schuttner LC, Scalettar BA (2008) Efficient copackaging and cotransport yields postsynaptic colocalization of neuromodulators associated with synaptic plasticity. Dev Neurobiol 68:1243–1256

Lorenzi S, Albers DS, LeWitt PA, Chirichigno JW, Hilgenberg SL, Cudkowicz ME, Beal MF (2003) Tissue inhibitors of matrix metalloproteinases are elevated in cerebrospinal fluid of neurodegenerative diseases. J Neurol Sci 207:71–76

Lou H, Kim SK, Zaitsev E, Snell CR, Lu B, Loh YP (2005) Sorting and activity-dependent secretion of BDNF require interaction of a specific motif with the sorting receptor carboxypeptidase e. Neuron 45:245–255

Lu Y, Christian K, Lu B (2008) BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? Neurobiol Learn Mem 89:312–323

Marler KJ, Poopalasundaram S, Broom ER, Wentzel C, Drescher U (2010) Pro-neurotrophins secreted from retinal ganglion cell axons are necessary for ephrinA-p75NTR-mediated axon guidance. Neural Dev 5:1749–8104

Massa SM, Xie Y, Yang T, Harrington AW, Kim ML, Yoon SO, Kraemer R, Moore LA, Hempstead BL, Longo FM (2006) Small, nonpeptide p75NTR ligands induce survival signaling and inhibit proNGF-induced death. J Neurosci 26:5288–5300

Matsumoto T, Rauskolb S, Polack M, Klose J, Kolbeck R, Korte M, Barde YA (2008) Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat Neurosci 11:131–133

Meloni M, Caporali A, Graiani G, Lagrasta C, Katare R, Van Linthout S, Spillmann F, Campesi I, Madeddu P, Quaini F, Emanueli C (2010) Nerve growth factor promotes cardiac repair following myocardial infarction. Circ Res 106:1275–1284

Mowla SJ, Pareek S, Farhadi HF, Petrecca K, Fawcett JP, Seidah NG, Morris SJ, Sossin WS, Murphy RA (1999) Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. J Neurosci 19:2069–2080

Nagappan G, Zaitsev E, Senatorov VV Jr, Yang J, Hempstead BL, Lu B (2009) Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. Proc Natl Acad Sci U S A 106:1267–1272

Ni X, Morales CR (2006) The lysosomal trafficking of acid sphingomyelinase is mediated by sortilin and mannose 6-phosphate receptor. Traffic 7:889–902

Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, Jacobsen C, Kliemannel M, Schwarz E, Willnow TE, Hempstead BL, Petersen CM (2004) Sortilin is essential for proNGF-induced neuronal cell death. Nature 427:843–848

Park H, Poo MM (2013) Neurotrophin regulation of neural circuit development and function. Nat Rev Neurosci 14:7–23

Pedraza CE, Podlesniy P, Vidal N, Arevalo JC, Lee R, Hempstead B, Ferrer I, Iglesias M, Espinet C (2005) Pro-NGF isolated from the human brain affected by Alzheimer's disease induces neuronal apoptosis mediated by p75NTR. Am J Pathol 166:533–543

Peng S, Wuu J, Mufson EJ, Fahnestock M (2005) Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. J Neurochem 93:1412–1421

Perez SE, He B, Muhammad N, Oh KJ, Fahnestock M, Ikonomovic MD, Mufson EJ (2011) Cholinotrophic basal forebrain system alterations in 3xTg-AD transgenic mice. Neurobiol Dis 41:338–352

Quistgaard EM, Madsen P, Groftehauge MK, Nissen P, Petersen CM, Thirup SS (2009) Ligands bind to sortilin in the tunnel of a ten-bladed beta-propeller domain. Nat Struct Mol Biol 16:96–98

Rauskolb S, Zagrebelsky M, Dreznjak A, Deogracias R, Matsumoto T, Wiese S, Erne B, Sendtner M, Schaeren-Wiemers N, Korte M, Barde YA (2010) Global deprivation of brain-

derived neurotrophic factor in the CNS reveals an area-specific requirement for dendritic growth. J Neurosci 30:1739–1749
Schecterson LC, Bothwell M (2008) An all-purpose tool for axon guidance. Sci Signal 1:pe50
Seidah NG, Benjannet S, Pareek S, Chrétien M, Murphy RA (1996) Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. FEBS Lett 379:247–250
Shetty AK, Zaman V, Shetty GA (2003) Hippocampal neurotrophin levels in a kainate model of temporal lobe epilepsy: a lack of correlation between brain-derived neurotrophic factor content and progression of aberrant dentate mossy fiber sprouting. J Neurochem 87:147–159
Shonukan O, Bagayogo I, McCrea P, Chao M, Hempstead B (2003) Neurotrophin-induced melanoma cell migration is mediated through the actin-bundling protein fascin. Oncogene 22:3616–3623
Siao CJ, Lorentz CU, Kermani P, Marinic T, Carter J, McGrath K, Padow VA, Mark W, Falcone DJ, Cohen-Gould L, Parrish DC, Habecker BA, Nykjaer A, Ellenson LH, Tessarollo L, Hempstead BL (2012) ProNGF, a cytokine induced after myocardial infarction in humans, targets pericytes to promote microvascular damage and activation. J Exp Med 209(12):2291–2305
Singh KK, Park KJ, Hong EJ, Kramer BM, Greenberg ME, Kaplan DR, Miller FD (2008) Developmental axon pruning mediated by BDNF-p75NTR-dependent axon degeneration. Nat Neurosci 11:649–658
Song W, Volosin M, Cragnolini AB, Hempstead BL, Friedman WJ (2010) ProNGF induces PTEN via p75NTR to suppress Trk-mediated survival signaling in brain neurons. J Neurosci 30:15608–15615
Spinnler K, Fröhlich T, Arnold GJ, Kunz L, Mayerhofer A (2011) Human tryptase cleaves pro-nerve growth factor (pro-NGF): hints of local, mast cell-dependent regulation of NGF/pro-NGF action. J Biol Chem 286:31707–31713
Srinivasan B, Roque CH, Hempstead BL, Al-Ubaidi MR, Roque RS (2004) Microglia-derived pronerve growth factor promotes photoreceptor cell death via p75 neurotrophin receptor. J Biol Chem 279:41839–41943
Stoica G, Lungu G, Kim HT, Wong PK (2008) Up-regulation of pro-nerve growth factor, neurotrophin receptor p75 and sortilin is associated with retrovirus-induced spongiform encephalomyelopathy. Brain Res 1208:204–216
Strong A, Ding Q, Edmondson AC, Millar JS, Sachs KV, Li X, Kumaravel A, Wang MY, Ai D, Guo L, Alexander ET, Nguyen D, Lund-Katz S, Phillips MC, Morales CR, Tall AR, Kathiresan S, Fisher EA, Musunuru K, Rader DJ (2012) Hepatic sortilin regulates both apolipoprotein B secretion and LDL catabolism. J Clin Invest 8:2807–2816
Sun Y, Lim Y, Li F, Liu S, Lu JJ, Haberberger R, Zhong JH, Zhou XF (2012) ProBDNF collapses neurite outgrowth of primary neurons by activating RhoA. PLoS One 7:e35883
Suter U, Heymach JV Jr, Shooter EM (1991) Two conserved domains in the NGF propeptide are necessary and sufficient for the biosynthesis of correctly processed and biologically active NGF. EMBO J 10:2395–2400
Tauris J, Gustafsen C, Christensen EI, Jansen P, Nykjaer A, Nyengaard JR, Teng KK, Schwarz E, Ovesen T, Madsen P, Petersen CM (2011) Proneurotrophin-3 may induce Sortilin-dependent death in inner ear neurons. Eur J Neurosci 33:622–631
Teng HK, Heng KK, Lee R, Wright S, Tevar S, Almeida RD, Kermani P, Torkin R, Chen ZY, Lee FS, Kraemer RT, Nykjaer A, Hempstead BL (2005) ProBDNF induces neuronal apoptosis via activation of a receptor complex of p57NTR and sortilin. J Neurosci 25:5455–5463
Tep C, Lim TH, Ko PO, Getahun S, Ryu JC, Goettl VM, Massa SM, Basso M, Longo FM, Yoon SO (2013) Oral administration of a small molecule targeted to block proNGF binding to p75 promotes myelin sparing and functional recovery after spinal cord injury. J Neurosci 33:397–410
Terry AV Jr, Kutivanawalla A, Pillai A (2011) Age-dependent alternations in nerve growth factor (NGF)-related proteins, sortilin, and learning and memory in rats. Physiol Behav 102(2):149–157
Tiveron C, Fasulol, Capsoni S, Malerbaf, Marinelli S, Paoletti F, Piccinin S, Scardigli R, Amato G, Brandi R, Capelli P, D'Aguanno S, Florenzano F, LaRegina F, Lecci A, Meli G, Pistillo L,

Berretta N, Nistico R, Pavone F, Cattaheot A (2013) Pro NGF/NGF imbalance triggers learning and memory deficits, neurodegeneration and spontaneous epileptic-like discharges in transgenic mice. Cell Death Differ 20(8):1017–1030

Truzzi F, Marconi A, Atzei P, Panza MC, Lotti R, Dallaglio K, Tiberio R, Palazzo E, Vaschieri C, Pincelli C (2011) p75 neurotrophin receptor mediates apoptosis in transit-amplifying cells and its overexpression restores cell death in psoriatic keratinocytes. Cell Death Differ 18:948–958

Volosin M, Trotter C, Cragnolini A, Kenchappa RS, Light M, Hempstead BL, Carter BD, Friedman WJ (2008) Induction of proneurotrophins and activation of p75NTR-mediated apoptosis via neurotrophin receptor-interacting factor in hippocampal neurons after seizures. J Neurosci 28:9870–9879

Wang YQ, Bian GL, Bai Y, Cao R, Chen LW (2008) Identification and kainic acid-induced up-regulation of low affinity p75 neurotrophin receptor (p75NTR) in the nigral dopamine neurons of adult rats. Neurochem Int 53:56–62

Wei Y, Wang N, Lu Q, Zhang N, Zheng D, Li J (2007) Enhanced protein expressions of sortilin and p75NTR in retina of rat following elevated intraocular pressure-induced retinal ischemia. Neurosci Lett 429(2–3):169–174

Willnow TE, Petersen CM, Nykjaer A (2008) VPS10P-domain receptors – regulators of neuronal viability and function. Nat Rev Neurosci 9:899–909

Woo NH, Teng HK, Siao CJ, Chiarutti C, Pang TP, Milner TA, Hempstead BL, Lu B (2005) Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat Neurosci 8:1069–1077

Yamashita T, Tucker KL, Barde YA (1999) Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. Neuron 24:585–593

Yang F, Je HS, Ji Y, Nagappan G, Hempstead B, Lu B (2009a) Pro-BDNF-induced synaptic depression and retraction at developing neuromuscular synapses. J Cell Biol 185:727–741

Yang J, Siao CJ, Nagappan G, Marinic T, Jing D, McGrath K, Chen ZY, Mark W, Tessarollo L, Lee FS, Lu B, Hempstead BL (2009b) Neuronal release of proBDNF. Nat Neurosci 12: 113–115

Yano H, Torkin R, Martin LA, Chao MV, Teng KK (2009) Proneurotrophin-3 is a neuronal apoptotic ligand: evidence for retrograde-directed cell killing. J Neurosci 29(47):14790–14802

Yoon SO, Casaccia-Bonefil P, Carter BD, Chao MV (1998) Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival. J Neurosci 18:3273–3281

Zagrebelsky M, Holz A, Dechant G, Barde YA, Bonhoeffer T, Korte M (2005) The p75 neurotrophin receptor negatively modulates dendrite complexity and spine density in hippocampal neurons. J Neurosci 25:9989–9999

Spatiotemporal Intracellular Dynamics of Neurotrophin and Its Receptors. Implications for Neurotrophin Signaling and Neuronal Function

F.C. Bronfman, O.M. Lazo, C. Flores, and C.A. Escudero

Abstract

Neurons possess a polarized morphology specialized to contribute to neuronal networks, and this morphology imposes an important challenge for neuronal signaling and communication. The physiology of the network is regulated by neurotrophic factors that are secreted in an activity-dependent manner modulating neuronal connectivity. Neurotrophins are a well-known family of neurotrophic factors that, together with their cognate receptors, the Trks and the p75 neurotrophin receptor, regulate neuronal plasticity and survival and determine the neuronal phenotype in healthy and regenerating neurons. Is it now becoming clear that neurotrophin signaling and vesicular transport are coordinated to modify neuronal function because disturbances of vesicular transport mechanisms lead to disturbed neurotrophin signaling and to diseases of the nervous system. This chapter summarizes our current understanding of how the regulated secretion of neurotrophin, the distribution of neurotrophin receptors in different locations of neurons, and the intracellular transport of neurotrophin-induced signaling in distal processes are achieved to allow coordinated neurotrophin signaling in the cell body and axons.

Keywords

Neurotrophins • Trks • p75 • Endosomes • Rab GTPases • Molecular motors • Retrograde signaling

The nervous system is a highly wired structure formed by neurons and glial cells, which together sculpt neuronal networks. The physiology of the network is

F.C. Bronfman (✉) • O.M. Lazo • C. Flores • C.A. Escudero
Physiology Department, Pontificia Universidad Católica de Chile, Santiago, Chile

Millennium Nucleus in Regenerative Biology (MINREB), Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile
e-mail: fbronfman@bio.puc.cl

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_3,

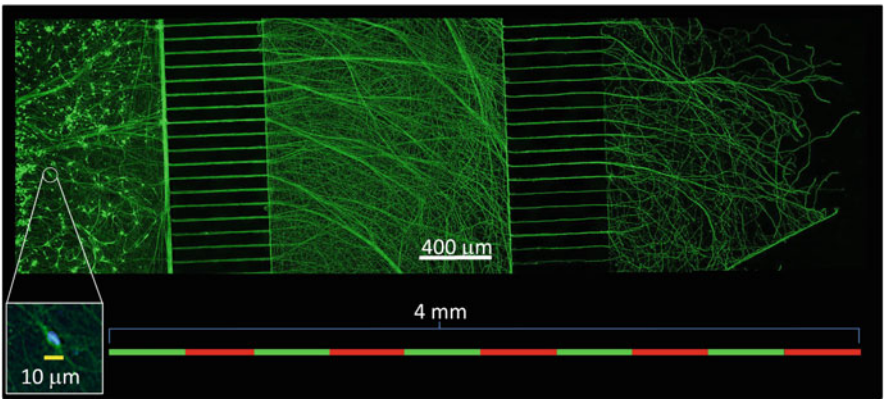

Fig. 1 Hippocampal axons can be as long as 400 times the diameter of the cell body. Hippocampal neurons were cultured in microfluidic chambers for 10 days in the presence of BDNF in the axonal compartment. The neurons were loaded with the fluorescent probe Calcien-AM (shown in *green*) to label the neuronal morphology. Nucleus was labeled with Hoechst staining (shown in *blue*)

regulated by neurotrophic factors that are secreted in an activity-dependent manner, modulating neuronal connectivity. Neurons exhibit a polarized morphology with two different compartments: the somatodendritic arbor and the axon, which are functionally differentiated to form and participate in neuronal networks. The neuronal soma acquires a particular morphology with branched prolongations specialized to form and receive synaptic contacts. The axon is a single prolongation that is specialized to transmit information from and back to the cell body, and it can be as long as 400 times the diameter of the neuronal soma in the case of rat hippocampal neurons (Fig. 1) or human lumbar motor neurons that can have axons longer than 10,000 times the diameter of the cell body. This special morphology imposes an important challenge for neuronal signaling and communication (Horton and Ehlers 2003b; Ibanez 2007).

Neurotrophins (NGF, BDNF, NT3, and NT4) are a well-known family of neurotrophic factors that, together with their cognate receptors, the Trks (TrkA, TrkB, and TrkC) and the p75 neurotrophin receptor (p75), regulate the development of neuronal networks by participating in the growth of neuronal processes, synaptic development and plasticity, neuronal survival, differentiation, and myelination. In the mature NS, neurotrophins determine the neuronal phenotype participate in neuronal plasticity and survival and in healthy and regenerating neurons. While each neurotrophin has a preferred Trk (NGF/NT3 binds TrkA; BDNF/NT4 binds TrkB; and NT3 binds TrkC), all neurotrophins bind p75 with a similar affinity. Additionally, two co-receptors for p75 have been described as participating in p75 signaling events: the neurotensin-3 receptor sortilin and the Nogo receptor (NogoR) for myelin-associated glycoproteins (Barker 2004; Greenberg et al. 2009; Huang and Reichardt 2001; Lu et al. 2005). In contrast to the Trks, p75 is capable of inducing opposing biological outcomes depending on its expression level,

association with different co-receptors at the plasma membrane, and the type of ligand (Bronfman and Fainzilber 2004; Gentry et al. 2004). p75 alone potentiates TrkA survival pathways and, in association with sortilin, induces cell death. Furthermore, it potentiates neurite outgrowth when acting alone or induced growth cone collapse in the presence of NogoR (Barker 2004; Higuchi et al. 2003).

Considering the particular morphology of the neuron, it is important to understand how the distribution of neurotrophin receptors in different locations of neurons, the regulated secretion of neurotrophin, and the intracellular transport of neurotrophin-induced signaling in distal processes are achieved to allow coordinated neurotrophin signaling in the cell body and axons. It is it now becoming clear that neurotrophin signaling and vesicular transport are coordinated to modify neuronal functioning because disturbances of vesicular transport mechanisms lead to disturbed neurotrophin signaling and to diseases of the nervous system (Bronfman et al. 2007; Perlson et al. 2010; Salinas et al. 2010). In this chapter, we will emphasize the role of key proteins that regulate vesicle transport and, thus, signaling, including Rab GTPases and molecular motors. Rab GTPases comprise a large family of small GTPases that control membrane identity and vesicle dynamics through the recruitment of different effector proteins (Stenmark 2009). There are two main classes of molecular motors that coordinate the transport of cargoes to the minus and plus ends of microtubules. The kinesins are a large gene family (KIFs, for kinesin superfamily proteins) that coordinates the transport of vesicles, macromolecular complexes, and organelles to the plus end of microtubules, thereby moving materials in an anterograde manner to the distal process of neurons, whereas cytoplasmic dynein is a protein complex that moves cargoes to the minus end of microtubules, thus moving materials in a retrograde fashion from the neuronal distal process to the cell body (Hirokawa et al. 2009; Kardon and Vale 2009).

1 Secretion and Anterograde Transport of Neurotrophins and Their Receptors

1.1 Neurotrophin Discovery and Biological Sources

Neurotrophins were first described as target-derived growth factors regulating the survival and differentiation of neurons from the peripheral nervous system (PNS, sensory and sympathetic neurons). The neurotrophic hypothesis, postulated by Rita Levi-Montalcini and Viktor Hamburger, stated that factors secreted in limiting amounts by tissues and target organs would ensure the correct number of neurons and their target fields, explaining the massive cell death of neurons during development in the PNS (Huang and Reichardt 2001; Korsching 1993; Levi-Montalcini 1966, 1987). It was later shown that neurotrophins have multiple functions in the central nervous system (CNS), including the regulation of synaptic plasticity and neuronal morphology. Most target tissues in the CNS also secrete neurotrophins that exert their effects by signaling back to the cell body (Bibel and Barde 2000;

Bilsland et al. 2010; Holzbaur 2004; Huang and Reichardt 2001; Lu et al. 2005; Mufson et al. 1999). Over the years, it has been shown that neurotrophins can be secreted not only by target tissues, which can be postsynaptic neurons or other types of cells, such as muscle, but also by presynaptic neurons, astrocytes, microglia, and glial cells, such as Schwann cells and oligodendrocytes, having paracrine and autocrine actions on neurons and other cell types (Bagayogo and Dreyfus 2009; Bessis et al. 2007; Cao et al. 2007; Dai et al. 2001; Lessmann et al. 2003; Matsuoka et al. 1991; Ohta et al. 2010; Schinder and Poo 2000; Verderio et al. 2006; Yune et al. 2007).

1.2 Coordination of Neurotrophin Processing, Local Translation, and Postsynaptic Secretion

Neurotrophins are homodimeric proteins synthesized as precursors (proneurotrophins) and are secreted to the extracellular space in a constitutive and regulated manner. As for many secreted proteins, after cleavage of the signal peptide in the endoplasmic reticulum, the homodimer transits through the Golgi, where it is subjected to glycosylation in its prodomain. The homodimers accumulate in vesicles in the *trans*-Golgi network (TGN), where the prodomains are cleaved by Furin and pro-convertases (PCs) to be secreted as non-glycosylated mature neurotrophins. The efficiency of cleavage varies according to neuronal and cell type. In hippocampal neurons, in the case of BDNF, proBDNF is secreted in an activity-dependent manner to the extracellular space, where it can be cleaved by the tissue plasminogen activator to be converted to mature BDNF (Lessmann et al. 2003; Nagappan et al. 2009; Yang et al. 2009). The regulation of neurotrophin secretion has been best studied in the case of BDNF. Although no studies have been reported that address how neurotrophins are sorted to either constitutive or regulated pathways, it is known that a polymorphism in the prodomain region (BDNF val to met) reduces the activity-dependent secretion of BDNF (Chen et al. 2004). This region of the BDNF prodomain has been shown to bind sortilin, a Vps10p domain protein that is known to bind the prodomain of proNGF to induce neuronal cell death by forming a complex with p75 (Nykjaer et al. 2004). In the TGN, sortilin has been associated with the proper intracellular trafficking of proteins in and out of the Golgi. The majority of sortilin resides in intracellular membranes that correspond to the TGN, endosomes, and secretory granules and vesicles in dendrites and axons (Willnow et al. 2008). Therefore, it is likely that this transmembrane protein plays a major role in targeting other soluble proteins and receptors out of the TGN to other cellular compartments. Thus, through interaction with sortilin, the prodomain of BDNF (not proNT4) regulates the sorting of BDNF to the regulated secretory pathway, and truncated versions of sortilin cause missorting of BDNF to the constitutive pathway, without affecting NT4-regulated secretion in hippocampal neurons, pointing to a neurotrophin-specific sorting mechanism to the regulated secretory pathway (Chen et al. 2005b).

The postsynaptic secretion of neurotrophins, particularly of BDNF, has been well documented (Cohen-Cory et al. 2010; Lessmann et al. 2003). Evidence has shown that BDNF and NT3 are more efficiently targeted to dendritic secretory granules in hippocampal neurons than are NT4 and NGF. Although secretion of neurotrophin is slower than neurotransmitter release, neurotrophin secretion co-localizes with PDZ-95, a postsynaptic marker of glutamatergic synapses. Regulated secretion of BDNF in glutamatergic synapses is tightly regulated, such that it will occur only in active synapses. For these phenomena to take place, there must be coordination of the local translation of BDNF messages and the secretion of vesicles in dendrites (Brigadski et al. 2005; Cohen-Cory et al. 2010).

The *BDNF* gene is characterized by complex transcriptional regulation; it can be transcribed from at least eight different promoters and can be polyadenylated on at least at two different sites, leading to the production of mRNA with a short 3′ untranslated region (UTR) or a long 3′UTR (Aid et al. 2007). The functional significance of the more than 16 transcripts that can be produced is unknown, but it has been suggested that this serves as a mechanism to add different layers of complexity to the regulation of the transcription and local translation of the *BDNF* gene (Greenberg et al. 2009). Dendritic transport of mRNA depends on specific dendritic targeting elements (DTEs) or cis-acting elements that are usually located in the 3′UTR; these sequences then target a specific mRNA for microtubular transport toward distal dendrites. BDNF mRNAs appear to share a common mechanism of transport with the mRNAs for calcium/calmodulin-dependent protein kinase II (alpha-CaMKII) and arc protein (Falley et al. 2009; Hirokawa 2006; Raju et al. 2011). These messages are transported in RNA granules in machinery that includes the trans-activating elements staufen and Pur-α and the kinesin-5 (KIF5) subfamily of molecular motors, in addition to the RNA-associated protein CArG box binding factor A (CBF-A). For *BDNF* specifically, an mRNA with a long 3′UTR seems to be more efficiently transported to dendrites compared to an mRNA with a short 3′UTR. A knockout mouse specific for this particular transcript exhibits abnormal pruning and enlargement of dendritic spines, as well as selective impairment of long-term potentiation in dendrites, but not the soma of hippocampal neurons (An et al. 2008).

In the cerebral cortex, promoter IV-dependent transcription of *BDNF* accounts for the majority of activity-dependent BDNF transcription (Hong et al. 2008). This transcript is apparently related to translation and secretion in the cell body, as it has been reported that the transcript mainly localizes to the cell soma, and exon II and VI *BDNF*-containing transcripts are more efficiently targeted to neurites (Chiaruttini et al. 2009). This phenomenon is presumed to be independent of DTEs in the 3′UTR of *BDNF* transcripts. The study performed by Chiaruttini and collaborators also indicated that there is a sequence coding for the prodomain of the *BDNF* transcript that binds translin, an RNA-binding protein involved in RNA transport. This is the same sequence used by sortilin to bind proBDNF. Interestingly, the val66met polymorphism in the BDNF gene causes translin to lose its ability to bind to *BDNF* transcripts and impairs its transport to dendrites. Thus, the reduced hippocampal dendritic complexity, memory deficits, and susceptibility to

mood disorders caused by this BDNF polymorphism may be due to the effects of deficits in the regulated secretion of BDNF mediated by the interaction of the BDNF prodomain with sortilin and to the inhibition of *BDNF* transcript transport to dendrites that is mediated in part by Translin (Bath and Lee 2006; Chen et al. 2006; Pezawas et al. 2004). Additional studies are needed to clarify the molecular mechanisms implicated in the activity-dependent targeting of specific *BDNF* mRNAs to dendrites. However, it can be proposed that the local synthesis and secretion of BDNF in active synapses is carried out through a mechanism that involves the transport of specific *BDNF* transcripts in a KIF5-dependent fashion and the coordination of BDNF synthesis in endoplasmic reticulum membranes and secretion from Golgi outposts localized to distal dendrites. Thus, postsynaptically secreted BDNF activates postsynaptic TrkB receptors, resulting in autocrine regulation of synaptic potentiation, or presynaptic TrkB potentiation of neurotransmitter release and regulation of target innervation (see below) (Fig. 2) (Cohen-Cory et al. 2010; Horton and Ehlers 2003a).

1.3 Anterograde Transport of Neurotrophin and Its Receptors

There is good evidence that BDNF-regulated secretion can occur in the presynaptic terminal. For this phenomenon to happen, dense core vesicles (DCV) derived from the Golgi apparatus in the neuronal soma have to undergo anterograde travel in a kinesin-dependent fashion to the synaptic terminal. It has been shown that BDNF and NT3 undergo anterograde transport and accumulate in DCV in synapses. Additionally, it has recently been found that BDNF anterograde transport in DCV is dependent on KIF1A (a member of a subfamily of kinesin 3) (Lo et al. 2011), suggesting that targeting to anterograde transport is regulated in part during the targeting of BDNF-DCV to a specific kinesin subfamily. In the presynaptic terminal BDNF is secreted in an activity-dependent manner similarly to other neuropeptides, exerting postsynaptic effects that regulate the development and maintenance of neuronal networks (Altar and DiStefano 1998; Lessmann et al. 2003; Matsumoto et al. 2008; Shinoda et al. 2011) (Fig. 2). In sensory neurons, the anterograde transport and release of BDNF in the axon enhance myelination, pointing to a potential role for anterograde-transported BDNF during development and regeneration (Ng et al. 2007). An unexpected finding reported by Butowt and von Bartheld (2001) was that in chick retinal ganglion cells, endocytosed NT-3 is sorted to the Golgi in a kinase-dependent manner, after which it undergoes anterograde transport to the presynaptic terminal in a process that depends on p75 anterograde transport (Butowt and von Bartheld 2001). This result implies that the anterograde transport of neurotrophins can be receptor mediated in a cellular pathway including ligand/receptor endocytosis in the cell body and posterior sorting to the anterograde transport pathway. Thus, there are at least two different forms of neurotrophins sorted to the anterograde pathway: one is non-receptor mediated and the other is receptor mediated.

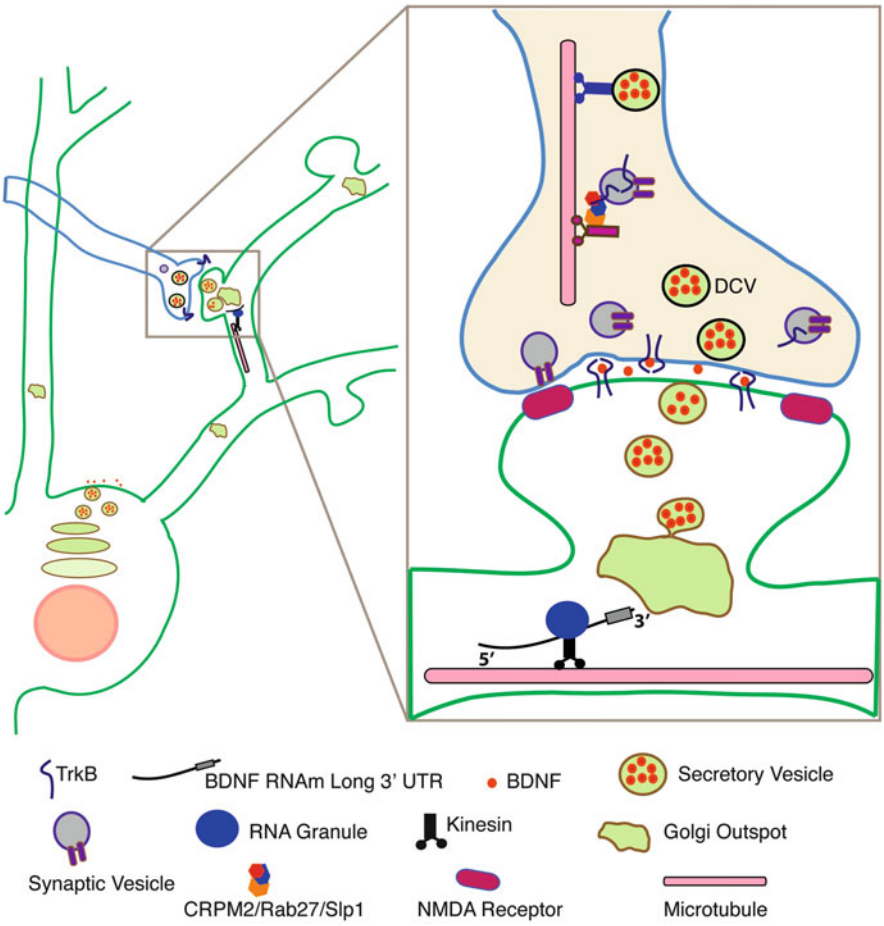

Fig. 2 Schematic diagram of a glutamatergic synapse in the central nervous system. In the presynaptic terminal, delineated in *blue*, it is illustrated how dense core vesicles (DCV) and synaptic vesicle precursors are transported to the terminal by different KIF members. DCV are filled with BDNF and they fuse with the plasma membrane in response to increase calcium concentration. The anterograde transport of synaptic vesicle precursors carrying TrkB receptors is regulated by the monomeric GTPase Rab27. There is a coordination of local translation and secretion of BDNF in the postsynaptic neuron that is delineated in *green*. The mRNA for BDNF is transported to the dendritic spine also by a KIF member. TrkB receptors are located at both pre- and postsynaptic membranes

As indicated above, sortilin is involved in regulating the secretion of BDNF in neurons. It was recently reported that in sensory neurons, sortilin facilitates the anterograde transport of Trks from the cell body along the axon (Vaegter et al. 2011). Determining whether the anterograde transport of BDNF and other neurotrophins also depends on sortilin, as has been shown for the regulated

secretion of BDNF and the anterograde transport of Trks, is a matter that will require further research.

Of note, in hippocampal neurons, anterograde-transported TrkB-positive vesicles are co-transported with VAMP2, a synaptic vesicle-associated protein, indicating that the final destination of these receptors is the presynaptic terminal (Gomes et al. 2006). The anterograde transport of TrkB is specifically mediated by conventional kinesin (kinesin-1) and the complex CRPM2/Slp1/Rab27a (Arimura et al. 2009). The CRPM2 protein associates with kinesin to bind microtubules, and through the Rab27a effector Slp1, which binds the TrkB cytosolic tail, Rab27a and CRPM2 engage TrkB in the anterograde axonal pathway (Fig. 2). Thus, upstream signaling pathways regulating CRPM2 activity will increase the transport of TrkB to the presynaptic terminal. Rab27a has been shown to be involved in the regulated secretion of TGN-derived secretory granules in many cellular models and may, therefore, play a key role in the targeting and insertion of TGN-derived TrkB in the presynaptic terminal (Fukuda 2008). Another monomeric GTPase of the Rab family involved in the anterograde transport of Trks is Rab11. Rab11 regulates the dynamics of the recycling endosome in many cells, and in sympathetic neurons, after TrkA endocytosis, Rab11 regulates the transcytosis and anterograde transport of TrkA to the sympathetic growth cone, where it enhances NGF sensitivity (Ascano et al. 2009). The kinesin associated with Rab11 vesicular trafficking is kinesin 2, through interaction with the Rab11 effector FIP5 (Schonteich et al. 2008). Although it has not been demonstrated that this complex regulates Rab11-dependent TrkA transcytosis, it is likely that different molecular motors and associated complexes regulate TGN-derived anterograde Trk vesicles *versus* transcytosis from the cell body of endocytosed Trks to the presynaptic terminal.

2 Internalization and Retrograde Signaling of Neurotrophin Receptors

After secretion, neurotrophins bind their cognate receptors, which can be located along the axon, in the neuronal cell body, or at the synapse (pre- or postsynaptic). After ligation, the neurotrophin/receptor complex rapidly activates signaling pathways in the plasma membrane and undergoes internalization. For quite some time, endocytosis of the neurotrophin/receptor complex was considered to be a mechanism solely involved in the downregulation of signal transduction. However, it is now well established that the internalization and post-endocytic trafficking of receptors are essential for signaling and neuronal function. After internalization, growth factor receptors continue signaling from endosomes, where they are associated with different signaling adaptors than in the plasma membrane. Additionally, the efficiency of endocytosis and the recycling of the receptors back to the cell surface is a mechanism that regulates the availability of the receptors for initiating signaling. Finally, the efficiency of ligand/receptor degradation in late endocytic pathways determines the duration of signaling inside the cell, thus having an important impact on cellular function (Fig. 3) (Bronfman et al. 2007;

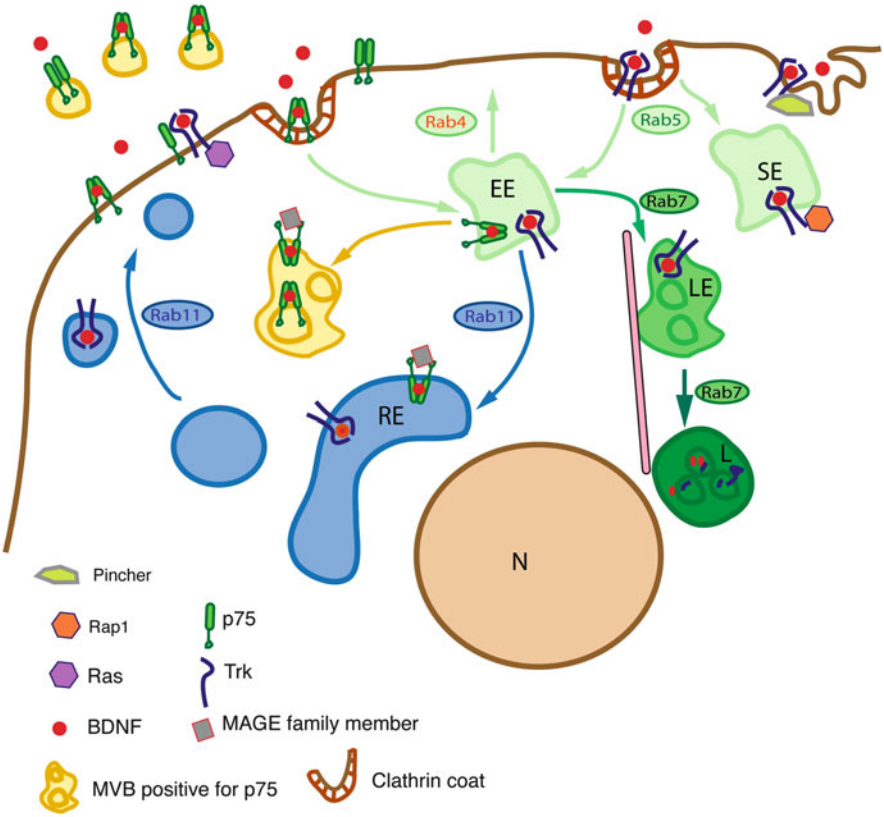

Fig. 3 Schematic diagram of the intracellular trafficking dynamic of the p75 and Trks receptors. In the plasma membrane p75 is found as a dimmer and the neurotrophin (Nt) binding triggers a conformational change that induces signaling adaptors binding (Vilar et al. 2009). p75 receptor is internalized through clathrin-coated pits and is found in early endosomes (EE) positive for Rab5 and recycling endosomes (RE) positive for Rab11. However, the majority of the receptor is accumulated in a multivesicular body (MVB) that is negative for late endocytic markers such as Rab7. The Trks are internalized through clathrin-coated pits and also by a mechanism that involves the formation of membrane ruffles, the actin cytoskeleton and the chaperone pincher. After internalization, Trks associates with signaling endosomes (SE) where activation of the GTPase Rap1 triggers the long-lasting activation of ERK1/2 and cellular differentiation. Trks are also found in the recycling pathways regulated by Rab11 and Rab4. Finally, downregulation of Trks signaling is initiated in its transit thought the late endosome (LE) and is achieved, in part, by degradation of the receptor in the lysosomes (L), process that is regulated by Rab7. Maturation from early endosomes to lysosomes needs the transport of endosomes from the cell periphery to the perinuclear region using microtubules and the molecular motor dynein

Miaczynska et al. 2004b; Platta and Stenmark 2011; Sorkin and Von Zastrow 2002).

The first evidence that neurotrophin receptors continue signaling inside the cell came from the groups of Mobley for the Trks and Fainzilber for p75, using PC12

cells as a model. Regarding TrkA, it was found that the internalized and activated TrkA receptor, together with NGF and PLC-γ1, was associated with intracellular vesicles. Later, it was reported that p75 internalizes more slowly than TrkA, accumulating in different vesicles, where the receptor is associated with NGF and signaling adaptors of the MAGE family (Bronfman et al. 2003; Grimes et al. 1996, 1997; Tcherpakov et al. 2002). The first description of the intracellular signaling of neurotrophin receptors gathered a great deal of interest related to understanding the mechanism of the internalization and trafficking of neurotrophin receptors because it was clear that it would shed new light on how neurons and neuronal networks interpret neurotrophin signaling. A summary of the more compelling findings on this topic will be presented below.

2.1 Trks Internalization and Intracellular Trafficking

In general terms, activated receptors in the plasma membrane can be internalized via clathrin-mediated or clathrin-independent routes. The clathrin-independent routes include at least eight different mechanisms, including caveolar-type endocytosis, macropinocytosis, Arf6-dependent endocytosis, and cholesterol-dependent and caveolin- and clathrin-independent pathways. All clathrin-mediated or clathrin-independent pathways of internalization are thought to converge on peripheral early endosomes (also referred to as sorting endosomes) (Doherty and McMahon 2009; Mayor and Pagano 2007). From there, some components are either rapidly recycled back to the plasma membrane or more slowly recycled through the recycling endosome (or pericentriolar endosome). From the early endosome, receptors are also sorted to late endosomes and lysosomes, where proteins are degraded (Di Fiore and De Camilli 2001; Miaczynska et al. 2004b; Sorkin and Von Zastrow 2002; Stenmark 2009) (Fig. 3).

The dynamics of intracellular trafficking, including through the endo-lysosomal system, are coordinated by Rab GTPases, which are a large family of small GTPases that control membrane identity and vesicle budding, uncoating, motility, and fusion through the recruitment of different and diverse effector proteins (Stenmark 2009). For example, Rab5 is a key regulator of early endosomal trafficking (Sonnichsen et al. 2000); Rab11 and Rab4 regulate transport through the recycling pathway; and Rab7 regulates transit from early endosomes to late endosomes and from late endosomes to lysosomes (Fig. 3) (Bucci et al. 1992, 2000; Cavalli et al. 2001; Somsel Rodman and Wandinger-Ness 2000).

Two different major routes of Trk internalization have been suggested in the literature: one is clathrin and dynamin dependent, and the other involves a macropinocytic process that depends on the new chaperone Pincher and is Rac and actin dependent, but dynamin independent. Dynamin is a GTPase that causes the pinching and scission of vesicles from the plasma membrane, and Rac is a small GTPase from the Rho family that regulates the dynamics of the actin cytoskeleton. In PC12 cells, NGF increases the association of clathrin with membranes, and TrkA is recovered in fractions containing clathrin-coated vesicles, together with signaling

components of the ERK1/2 pathway. Additionally, TrkA and TrkB internalization is inhibited by monodansylcadaverine, a drug that inhibits clathrin-mediated internalization. In support of a role for clathrin-coated pits in TrkA internalization, TrkA mutants with a truncated carboxyl-terminal domain that therefore lack potential clathrin-mediated internalization motifs exhibit dramatically decreased NGF internalization (Fig. 3) (Beattie et al. 2000; Doherty and McMahon 2009; Howe et al. 2001; Joset et al. 2010; Jullien et al. 2003; Mayor and Pagano 2007; Shao et al. 2002; Zhang et al. 2000; Zheng et al. 2008). Both types of internalization appear to take place in the cell body, as well as in axons because both dynamin and Pincher dominant negative mutants inhibit the internalization and retrograde transport of activated Trk receptors in sympathetic neurons (Valdez et al. 2005; Ye et al. 2003). Although Trks have been found in caveolae-like domains and lipid rafts (cholesterol and sphingolipid-rich membrane domains) upon ligand stimulation, there is no evidence that Trks are internalized through a clathrin-independent but cholesterol-dependent pathway. Similar to what happens to other receptor tyrosine kinases (RTKs), such as the epidermal growth factor (EGF) receptor (EGFR), localization to lipid rafts is necessary for signaling. However, in contrast to what is seen for EGFR, the Trks exhibit increased localization to lipid rafts after ligand binding. It is of note that for TrkB, translocation to lipid rafts in cortical and hippocampal neurons is necessary for synaptic modulation and requires TrkB receptor phosphorylation and internalization, suggesting that association with lipid rafts occurs on intracellular membranes (Assaife-Lopes et al. 2010; Huang et al. 1999; Limpert et al. 2007; Nishio et al. 2004; Pereira and Chao 2007; Zwang and Yarden 2009).

In general, the most important pathway for RTK internalization is clathrin-mediated internalization. Macropinocytosis is associated with areas where plasma membrane spreading and ruffling take place, which is a process, regulated by actin dynamics (Cavalli et al. 2001; Kirkham and Parton 2005) (Fig. 3). Although EGFR has also been shown to use this internalization mechanism, it has been suggested that this route is utilized only when there is an excess of ligand (Zwang and Yarden 2009). Most studies related to the Pincher-mediated macropinocytosis of Trks have been performed in cells overexpressing the Trk receptor or a chimeric version of it, and different concentrations of ligands have not been tested; therefore, the physiological relevance of this process has to be viewed with caution (Philippidou et al. 2011; Shao et al. 2002; Valdez et al. 2005).

Cargo recognition during clathrin-mediated endocytosis is mediated by different adaptors that possess a phospholipid-interacting motif and may also interact with transmembrane receptors. These adaptors interact with clathrin, increasing its affinity for the plasma membrane and for the cytosolic motif present in the cytoplasmic tails of transmembrane receptors. The best studied adaptor protein functioning at the plasma membrane is the AP-2 complex, which recognizes the YXXϕ sequence (where ϕ is a hydrophobic residue, and X is a variable residue) and dileucine sequences [DE]XXXL[LI] (where the second leucine can be isoleucine, and X is a variable residue that can be followed by an asparagine and lysine). These sequences are found in classic endocytic receptors, such as the transferrin receptor.

There are also other adaptors such as Epsin and EPS15 that can associate directly with clathrin, recognizing mono- or polyubiquitins in the cytoplasmic tail of receptors (Bonifacino and Traub 2003; Hawryluk et al. 2006; Marmor and Yarden 2004). EGFR activation induces the binding of the c-Cbl ubiquitin ligase, which adds multiple monoubiquitins to the receptor, inducing its internalization through the EPS15 and Epsin adaptors and clathrin (de Melker et al. 2001; Marmor and Yarden 2004). Other clathrin adaptors that work differently than AP2 are AP180 and Dab2. AP180 is specifically expressed in the nervous system and induces the internalization of synaptic vesicle-associated proteins, and DAB2 binds to NPXY sequences (whereas X is a variable residue) found in the lipoprotein receptors LDLR and ApoER2, inducing their internalization from the apical domain of epithelial cells (which is proposed to be equivalent to the presynaptic terminal of neurons) (Cuitino et al. 2005; Morris et al. 2002; Rodriguez-Boulan and Powell 1992; Slepnev and De Camilli 2000; Sorkin 2004).

There is little information about which clathrin adaptors mediate the clathrin-dependent internalization of Trks. There is one report that AP-2 mediates the internalization of TrkB in hippocampal neurons. Both TrkA and TrkB possess AP-2 and DAB2 consensus sequences; however, it is not known whether they serve as binding sequences for AP2 or DAB2 (Fig. 4). It would be of interest to evaluate whether DAB2 and AP180 participate in the clathrin-dependent endocytosis of Trks in the synaptic terminal to mediate Trk presynaptic local effects or retrograde signaling (see below). Additionally, similar to EGFR, the Trks are multimonoubiquitinated, and TrkA, in particular, is multimonoubiquitinated or polyubiquitinated in a Nedd4-2-dependent or TRAF6-dependent manner, respectively. However, through reducing TRAF6-mediated ubiquitination alone, the internalization of the receptor is diminished, and it is possible that Nedd4 ubiquitination mediates sorting to lysosomes and not internalization (see below). To date, there have been no studies reported indicating whether the Trks are recognized by clathrin adaptors such as EPS15 and Epsin that specifically recognize ubiquitinated cargoes (Arevalo et al. 2006; Geetha et al. 2005).

The neuroendocrine PC12 cell line is a frequently used model of NGF signaling expressing both p75 and TrkA receptors. When treated with NGF, PC12 cells differentiate to a sympathetic neuron-like phenotype, extending neurites and increasing the expression of different neurotransmitters (Greene and Tischler 1976). Numerous studies on neurotrophin signaling and trafficking have been performed in this neuronal model system. Many lines of research support the idea that intracellular trafficking of neurotrophin receptors regulates neurotrophin-signaling outcomes. In PC12 cells, inhibition of dynamin and TRAF6-dependent internalization inhibits the neurite extension induced by NGF (Geetha et al. 2005; Zhang et al. 2000). This is consistent with the fact that internalization of TrkA is necessary for sustained activation of the ERK1/2 pathways that are required for PC12 cell differentiation. Different publications indicate that this is achieved by increasing the activation of Rap1 that is mainly associated with endosomes (Fig. 3) (Kao et al. 2001; Mochizuki et al. 2001; Nomura et al. 2004; Wu et al. 2001; York et al. 2000). Interestingly, endosomal signaling mediated by TrkA and Rap1 and

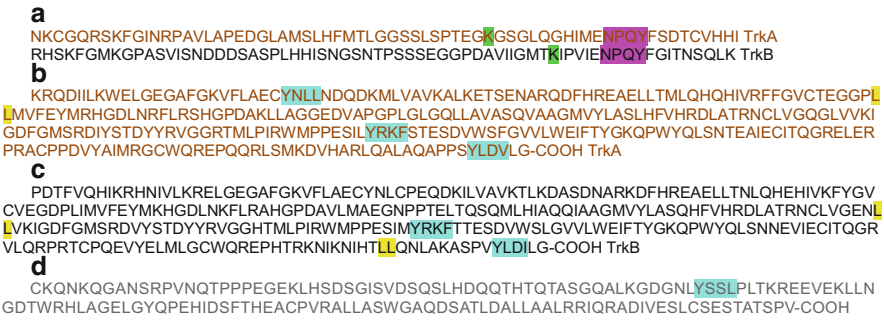

Fig. 4 Potential sequence of neurotrophin receptors regulating its internalization. The juxtamembrane portion of the TrkA and TrkB receptor is shown in **a**, the sequence NPXY is labeled in *pink*, and it is a potential binding site for the clathrin adaptor Dab2. NPXY is also the binding site for signaling adaptors such as Shc and Grb2 leading to activation of ERK1/2 and IP3K (Huang and Reichardt 2003). The lysine (K) labeled in *green* is ubiquitinated by TRAF6 in TrkA and regulates its internalization (Geetha et al. 2005). It is not known whether this lysine is also ubiquitinated in TrkB. In **b** and **c** is shown the rest of the intracellular domain of TrkA (**b**) or TrkB (**c**) and the intracellular domain of p75 (**d**). In *blue* are labeled the YXXϕ, which is a potential binding site for the clathrin adaptor AP2. In *yellow* are shown the dileucine motifs in the context of [DE]XXXL[LI], which are also a potential binding site for the clathrin adaptor AP2 (Bonifacino and Traub 2003)

sustained ERK1/2 activation require dynein retrograde transport of endosomes from the cell periphery to the perinuclear region of PC12 cells and sensory neurons. This indicates that during microtubular and dynein-dependent transport, TrkA-positive endosomes mature and acquire the adaptors and signaling molecules necessary for TrkA signaling to Rap1 and ERK1/2 (Wu et al. 2007).

Although TrkA and the EGFR are both targeted to the degradative pathway, EGFR is more efficiently targeted to the lysosomal pathway, whereas the TrkA receptor continues signaling in Rab5-positive endosomes for a longer period of time. Which molecular interaction might account for this difference in trafficking kinetics and signaling? There are at least two different reports in the literature regarding this point. The first is related to the association of Trks with the ankyrin-rich transmembrane protein ARMS, which does not associate with EGFR. ARMS is rapidly tyrosine phosphorylated after the binding of neurotrophins to Trk receptors and provides a docking site for the CrkL–C3G complex, resulting in sustained Rap1-dependent ERK activation (Arevalo et al. 2004). The other report is related to the association of endocytosed TrkA with RabGAP5, a protein that downregulates Rab5 activity to facilitate neurite outgrowth and differentiation. Downregulation of Rab5 activity delays the maturation of early endosomes into late endosomes and lysosomes, precluding TrkA degradation. Consistently, overexpression of a dominant negative form of Rab7 induced endosomal accumulation of TrkA and potentiated Erk1/2 phosphorylation and neurite outgrowth (Liu et al. 2007; Saxena et al. 2005a). Another factor contributing to different signaling outcomes between EGFR and TrkA is the efficiency of lysosomal targeting by monoubiquitination.

Compared to TrkA, the EGFR rapidly targets to the degradative pathway through monoubiquitination by the ubiquitin ligase c-Cbl, whereas TrkA is monoubiquitinated by the E3 ubiquitin ligase Nedd4-2, which targets the receptor for endosomal degradation. However, TrkA monoubiquitination does not impair signaling but rather appears to potentiate sustained ERK1/2 activation, suggesting that the TrkA receptor may continue signaling even in the late endosomal pathway (Arevalo et al. 2004; Georgieva et al. 2011; Haglund et al. 2002; Marmor and Yarden 2004; Saxena et al. 2005b).

Another molecular adaptor that might function downstream of the TrkA internalization to achieve sustained signaling and differentiation is the APPL1 cytosolic protein. Under certain conditions, APPL1 associates with Rab5 and defines a subpopulation of Rab5-positive endosomes. With respect to EGF signaling, it has been shown that after downregulation of Rab5 activity in early endosomes, APPL1 is released from the endosomal membrane and translocates to the nucleus, where it associates with components of the nucleosome remodeling and histone deacetylation machinery. In the case of TrkA, APPL1 associates with TrkA through two different means: indirectly via GIPC1 (a PDZ protein) and directly through the APPL1 phosphotyrosine-binding domain. Cell fractionation studies have demonstrated that APPL1, GIPC1, and phosphorylated TrkA are present in the same endosomal fractions and that both GIPC1 and APPL1 are recruited to TrkA-positive endosomes upon ligand stimulation. Additionally, both the APPL1 and GIPC1 proteins are required for NGF-induced ERK1/2 and Akt activation and neurite outgrowth. Although it has not been demonstrated that APPL1 is released from endosomes and translocates to the nucleus after binding TrkA in endosomes, these results are consistent with a potential role for APPL1 in NGF-dependent transcription to induce neuronal differentiation (Lin et al. 2006; Miaczynska et al. 2004a; Varsano et al. 2006).

Another means of increasing the sustained activation of signaling molecules is through the recycling of activated receptors. Chen and collaborators found that TrkA is more efficiently recycled to the plasma membrane compared to TrkB in PC12 cells because it possesses a post-endocytic recycling signal in the juxtamembrane domain. Accordingly, in PC12 cells, TrkA causes sustained signaling of phosphatidylinositol 3-kinase/Akt, resulting in increased cell survival, whereas TrkB does not have this effect. Targeting of TrkA to the recycling pathways does not require the receptor to exhibit kinase activity, while targeting of the receptor to the degradative pathway does, suggesting that kinase activity modulates targeting of the receptor to the degradative or recycling pathway (Chen et al. 2005a; Saxena et al. 2005b). Although the TrkB receptor does not possess the recycling signal present in TrkA, it has been described (in hippocampal neurons) that there is a regulated recycling of TrkB that depends on its kinase activity and the adaptor Hrs. This regulated recycling pathway appears to be different than the constitutive recycling pathways used by transferrin and is required for sustained ERK1/2 signaling induced by TrkB (Huang et al. 2009). Highlighting the functional role of the recycling pathway in hippocampal neurons, we have found that TrkB recycling in dendrites is mainly mediated by Rab11, a RabGTPase regulating the

recycling of receptors back to the plasma membrane, and that inhibition of Rab11 activity reduces the dendritic ramifications induced by BDNF. Inhibition of Rab11 activity also reduced targeting of TrkB to dendrites. Additionally, we showed that TrkB activation increases Rab11 activity and changed Rab11 dynamics in dendrites. This is one of the first examples indicating that regulation of Rab11 activity by TrkB is necessary for structural plasticity induced by BDNF (Lazo et al. 2013). Consistent with the idea that post-endocytic trafficking of the TrkB receptor is necessary for signaling, inhibition of TrkB internalization reduces phosphatidylinositol 3-kinase/Akt signaling and neurite outgrowth of hippocampal neurons (Huang et al. 2009; Zheng et al. 2008).

2.2 p75 Internalization and Intracellular Trafficking

Regarding p75 internalization, we have previously shown that pharmacological inhibition of clathrin-mediated internalization completely blocks ligand-dependent p75 internalization in PC12 cells. Additionally, p75 is internalized with slower kinetics compared to transferrin and TrkA (Bronfman et al. 2003; Saxena et al. 2004). These different kinetics of internalization result in targeting p75 and TrkA to different types of endosomes (McCaffrey et al. 2009). In contrast to what is seen for the Trks, p75 is not targeted to the degradative pathway (i.e., late endosomes and lysosomes) within the time frame of these experiments. Initially, observations of partial colocalization with transferrin suggested that endocytosed p75 accumulates in recycling endosomes, where it continues signaling (Bronfman et al. 2003; Saxena et al. 2005b). However, recent studies by our group using quantitative confocal microscopy and deconvolution have indicated that after internalization, p75 evades Rab5-positive early endosomes and accumulates in two different organelles. One organelle is positive for Rab11, and another one positive for the tetraspanin CD63. CD63 labels multivesicular bodies for exosomal release and we found p75 in exosomes derived from PC12 cells and sympathetic neurons (Fig. 3) (Escudero et al. unpublished work). Additionally studies are needed to understand the particular trafficking features of p75 in different types of neurons. These studies are important because p75 continues signaling inside the cells and we have shown that endocyted p75 is proteolytically processed. Several lines of evidences have indicated that after proteolytic processing, p75-derived COOH-terminal fragments are important for signaling; therefore, internalized p75 may interact with signaling adaptors in endosomes or to be proteolytically processed to generate signaling fragments (Bronfman 2007; Bronfman et al. 2003; Kanning et al. 2003; Kenchappa et al. 2006; Urra et al. 2007).

Other mechanisms of internalization are apparent in neurons. For example, in motor neurons, p75 internalization is ligand independent and is inhibited by the expression of dominant negative forms of dynamin, but not of AP-2 or AP180 clathrin adaptors, suggesting that in the motor neuron cell body, p75 internalization is clathrin independent. However, in motor neuron axons, clathrin-dependent and independent pathways coexist. The clathrin-dependent route targets p75 for

retrograde transport in the axon (Deinhardt et al. 2007). Similar to what is seen in motor neurons, in sympathetic neurons that express TrkA and p75, BDNF internalization is partially inhibited by sucrose (pharmacological inhibition of clathrin-dependent pathways) and nystatin (an independent drug that disrupts lipid rafts), suggesting that there are also clathrin-dependent and clathrin-independent mechanisms for the internalization of p75 in sympathetic neurons (Hibbert et al. 2006). Similar to the Trks, p75 also associates with lipid rafts to carry out signaling; however, additional studies are needed to understand the role of lipid rafts in the regulation of internalization, signaling, and p75 stability in the plasma membrane (Fujitani et al. 2005; Huang et al. 1999; Nishio et al. 2004). We have analyzed p75 internalization kinetics in three different cells types. In hippocampal neurons, p75 is internalized rapidly, whereas p75 internalization in PC12 cells is slower than in hippocampal neurons, but twice as fast as in sympathetic neurons (Bronfman et al. 2003, 2007). We have found that in cultures, these three different cell types exhibit substantial differences in the cholesterol content of their membranes, the greater the content of cholesterol in the cell, the slower the internalization of the receptor with sympathetic neurons presenting the highest levels of cholesterol and hippocampal neurons the lowest. Of note, although sympathetic neurons exhibit the greatest content of cholesterol in the plasma membrane the mobility of p75 in the plasma membrane seems to be similar than in PC12 cells (Fig. 5). These observations suggest that the content of cholesterol in the plasma membrane plays a role in the time of residence of the p75 receptor in the plasma membrane after ligand binding and thus regulates its internalization kinetics.

2.3 Neurotrophin Trafficking and Neurodegenerative Diseases

The role of the internalization and intracellular trafficking of neurotrophin receptors in signaling outcomes is emphasized by the fact that mutations in trafficking proteins cause neurodegeneration in humans and alteration of neurotrophin signaling. For example, missense mutants in the late endosomal Rab7 GTPase cause the autosomal dominant peripheral neuropathy Charcot–Marie–Tooth disease type 2B (CMT2B). Mutant Rab7 acts as a constitutively active GTPase, increasing the activity of Rab7 and downregulating NGF-induced differentiation through abnormal ERK1/2 signaling. Additionally, loss of function of alsin, an activator of the Rac1 and Rab5 small GTPases, causes ALS2, an autosomal recessive motor neuron disease with juvenile onset and slow progression. Als2(−/−) mice exhibit a marked diminution of Rab5-dependent endosome fusion activity, together with disturbances in the endosomal transport of the insulin-like growth factor 1 (IGF1) and BDNF receptors (BasuRay et al. 2010; Cogli et al. 2010; Devon et al. 2006). We have analyzed the consequences of loss of function of the Niemann–Pick type C 1 (NPC1) protein for neurotrophin signaling. NPC1 is a transmembrane protein that controls the efflux of cholesterol from endocytic pathways and causes abnormal endocytic function and neurodegeneration. Our analysis indicated that NPC1 loss of

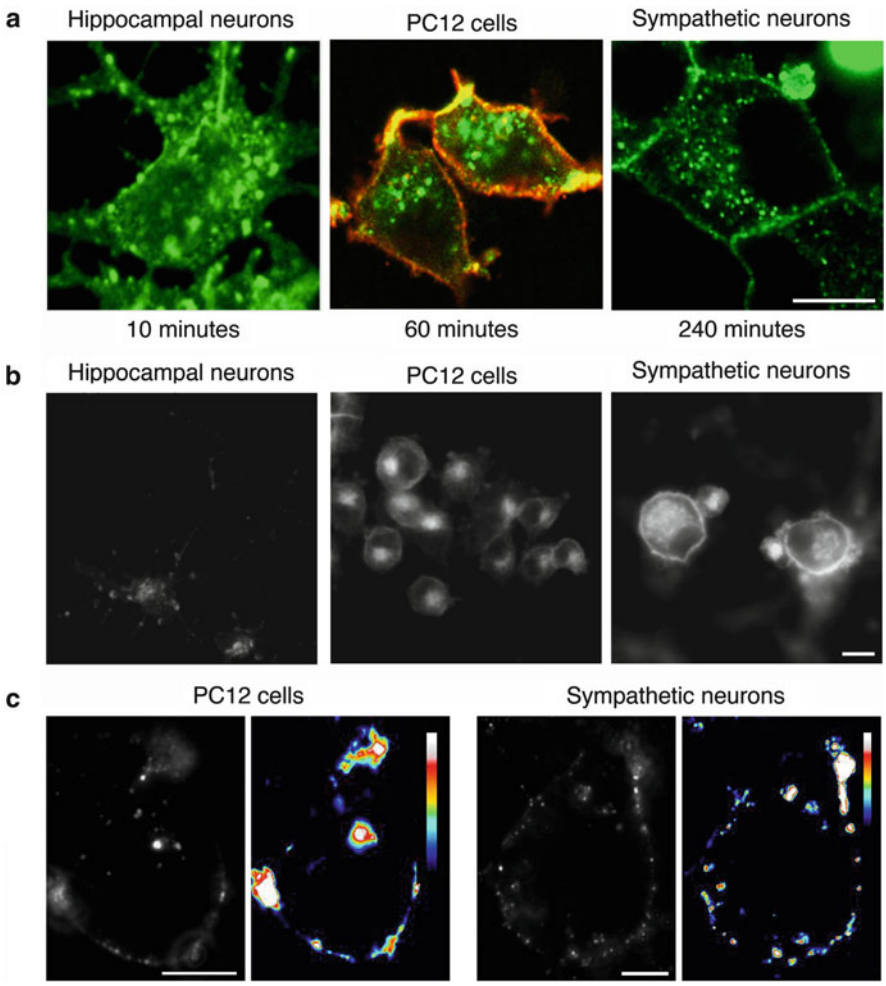

Fig. 5 Dynamics of p75 receptor in three different neuronal models. (**a**) p75 internalization in three different neuronal models. The peak of p75 internalization is observed at different time points in the three different models. p75 internalization was visualized as indicated in Bronfman et al. (2003, 2007). (**b**) Visualization of the levels of cellular cholesterol observed in three different neuronal models stained with filipin (a fluorescent drug that binds cholesterol). **a**, **b**, *scale bar*, 10 μm. (**c**) p75 localized in the plasma membrane of PC12 and sympathetic neurons was labeled with and antibody against the extracellular domain of p75 (MC192) labeled with Q-Dots. Movement of p75 in the plasma membrane was studied by real-time microscopy with a frequency of 1.5 frames/second (a total of 150 frames). The 150 frames were condensed to 1 and showed in a gray scale (*left panel*) or with segmentation of intensity ranges into a pseudo-colored scale (*right panel*). *Blue* indicates mobile p75. **c**, *scale bar*, 5 μm

function causes increased neurotrophin signaling and reduced recycling of TrkA in addition to increased pathological Tau phosphorylation (Cabeza et al. 2012). Other hereditary neurodegenerative diseases related to abnormal functioning of Rab

GTPases (including Rab5, Rab11) and alterations in BDNF and NGF transport are Huntington's disease, Alzheimer's disease, and Down syndrome (Gauthier et al. 2004; Ginsberg et al. 2010; Li et al. 2009; Pal et al. 2006; Salehi et al. 2006). A conclusion arising from these findings is that assembly of specific signaling complexes on specific endosomes provides a way to solve the problem of specificity in signal transduction; alteration of the trafficking properties of a neuron would alter this specificity, causing miss-regulation of signaling and contributing to diverse neurodegenerative diseases.

2.4 Mechanism of the Axonal Transport of Neurotrophin Signaling in Neurons

Neurotrophins were first discovered as target-derived factors essential for the survival and maturation of sensory neurons and sympathetic neurons of the peripheral nervous system (Glebova and Ginty 2005; Huang and Reichardt 2001; Korsching 1993; Levi-Montalcini 1966, 1987). The question then arose of how long-range projection neurons transmit the neurotrophic survival signal from the presynaptic terminal to the neuronal cell body to induce transcriptional changes? The first hint of an answer to this question came from the work of Hendry and colleagues, who showed that radiolabeled NGF was retrograde transported from adrenergic terminals in the mouse and rat iris to the cell body of sympathetic neurons in the superior cervical ganglia (SCGs). This transport was found to be sensitive to colchicine (a drug that destabilizes microtubules) and was inhibited by antibodies against NGF, indicating that the transport was specific and dependent on microtubules (Hendry et al. 1974a, b). Later, it was found that activated TrkA accumulated distally to a ligation site in the sciatic nerve, indicating that activated TrkA complexes are retrograde transported (Bhattacharyya et al. 1997; Ehlers et al. 1995). Additionally, Hendry and collaborators reported that the transport of radiolabeled NGF in sensory axons, mediated by the microtubule-associated molecular motor dynein, depends on signal transduction by different kinases, including TrkA and PI3-K (Reynolds et al. 1998). More details regarding the molecular mechanism of the transport of neurotrophin signaling came with the development of compartmentalized cultures of sensory and sympathetic neurons. In these cultures, neuronal cell bodies are located in a different compartment than axons. Thus, these cultures allow neurotrophin axonal stimulation without stimulation of the neuronal soma. Using them, Ginty, Segal, and collaborators reported that both the kinase activity and internalization of Trks are required for retrograde-transmitted nuclear responses, including the activation of transcription factors such as CREB and c-Fos. Furthermore, inhibition of dynein activity in the axons of compartmentalized sensory neuron cultures causes downregulation of the transport of activated TrkB, together with inhibition of the survival responses in the cell body. A biochemical explanation for these results was offered by detection of the direct interaction of Trks with the molecular motor dynein, as described by Chao

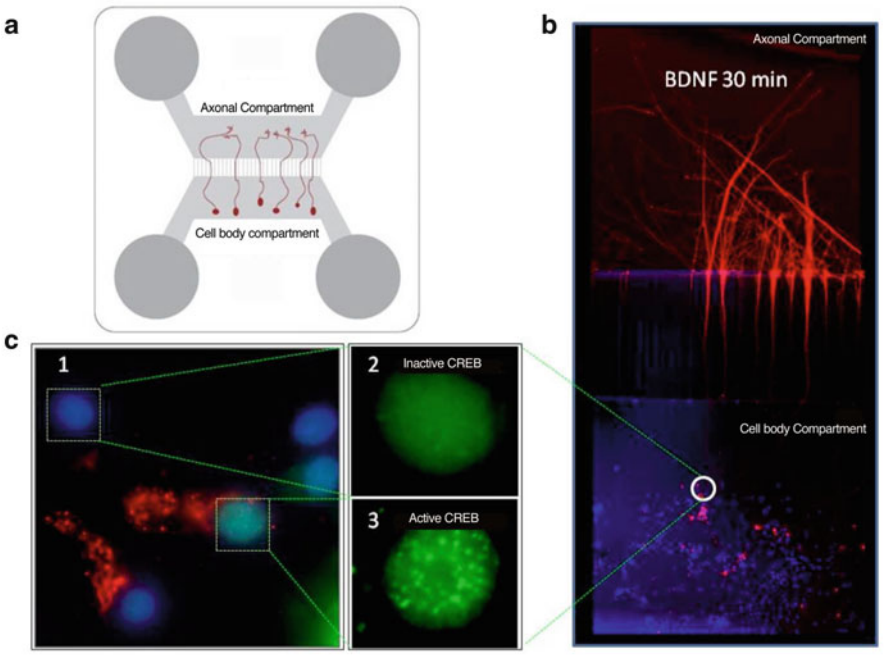

Fig. 6 Retrograde activation of CREB. (**a**) Scheme of a compartmentalized culture of cortical neurons. (**b**) Cortical neurons were cultured in microfluidic chambers and the axons were retrogradely labeled using a fluorescent (Alexa-555) subunit B of the cholera toxin (CTX). CTX was added only in the axonal compartment for 6 h, time that was enough to label only the somas of neurons that have crossed axons to the axonal compartment. Later, the axons were treated with BDNF for 30 min and the cultures were washed and fixed and the neuronal cell body compartment treated with an antibody to label pCREB. Neurons without axons in the axonal compartment are not labeled by CTX. Nucleus was labeled with Hoechst staining (shown in *blue*). (**c**) The image of neuronal cell bodies showed in **b** with a *circle* was magnified and the nuclear staining of pCREB is appreciated only in neurons retrogradely labeled with CTX

and coworkers (Heerssen et al. 2004; Riccio et al. 1997, 1999; Watson et al. 1999, 2001; Yano et al. 2001). These results, together with the isolation of endosomes derived from sciatic nerve axoplasm containing activated TrkA, p75, phospho-ERK1/2, PI3-K, phospho-p38, and Rap1 (Delcroix et al. 2003), led to the signaling endosome hypothesis, which postulated that after binding to neurotrophins in the synaptic terminal, activated Trks are internalized in endosomes that contain signaling molecules. These endosomes are retrograde transported back to the cell body in a dynein-dependent manner. Upon arrival to the cell body, signaling endosomes are expected to trigger nuclear responses (Heerssen and Segal 2002; Howe and Mobley 2004). Recent evidence has shown that, in central neurons, TrkB elicits a retrograde signaling that leads to CREB activation and an increase in dendritic ramification (Fig. 6) (Zhou et al. 2012). This retrograde response is mediated by the snapin

recruitment of dynein to TrkB signaling endosomes supporting the role of signaling endosomes in the retrograde signaling of peripheral and central neurons.

Other mechanisms have been postulated for the propagation of neurotrophin signaling along the axon, including the "wave propagation model" and the "retrograde effector model" (Bronfman and Kapon 2007; Howe and Mobley 2004). Additionally, Campenot's group has suggested that there might be signaling endosome-independent pathways of retrograde signaling because, using compartmentalized cultures of sympathetic neurons, they found that the addition of NGF to axonal terminals induces increased activation of TrkA in the cell body 1 min after NGF addition to axons and much earlier than the arrival of NGF-associated vesicles. However, these researchers have not yet provided a molecular mechanism for their findings, and a mechanism that has gained the most substantial support though experimental validation is the "signaling endosome model" (MacInnis and Campenot 2002; Senger and Campenot 1997; Ye et al. 2003). An interesting alternative to the signaling endosome hypothesis is that activated signaling complexes, such as ERKs, could undergo retrograde travel in an endosome-independent manner associated directly with dynein. Macromolecular complexes of ERK1/2 in association with locally synthesized importin, vimentin, and dynein have been described in sciatic nerves under injury conditions, supporting the existence of non-vesicular transport of activated signaling molecules (Hanz et al. 2003; Perlson et al. 2005).

Another controversial issue in this field is the nature of the transport organelle that carries retrograde neurotrophin signaling. Mobley and collaborators reported characterizing an early endosomal fraction (positive for Rab5 and EEA1) derived from sciatic nerve axoplasm where there are activated TrkA receptors and activated signaling molecules, such as Erk1/2, p38, and Akt. They have also provided evidence from electron microscopy and double immunostaining that the Rab5 GTPase co-localizes with activated TrkA and retrograde-transported NGF in axons. Another report also associates Rab5 with an axonal retrograde organelle formed by the Pincher chaperone. Intriguingly, the organelle is a multivesicular endosome/body (MVB) positive for Rab5 (Philippidou et al. 2011).

Multivesicular endosomes/bodies (MVBs) are organelles of the early-late endocytic pathway that sort endocytosed proteins to different destinations. Many lysosomally directed membrane proteins are sorted onto intraluminal vesicles, while recycling proteins remain on the perimeter membrane, from which they are removed via tubular extensions. Rab5–Rab7 conversion and the resulting change in the repertoire of Rab effector proteins on the endosome membrane mark the final progression of an MVB to a fusion-competent state, in which it can fuse with lysosomes. In the case of non-polarized cells in culture, MVBs are moved to the cell center during this maturation process by cytoplasmic dynein. An elegant study carried by Schiavo and collaborators provided evidence that the Rab5 GTPase is important for sorting to the retrograde transport pathway of vesicles. However, they found that it was the Rab7 GTPase, a classical marker of late endosomes, necessary for the retrograde transport of an organelle positive for p75 and TrkB in motor neurons. It is known that the Rab7 effector RIPL links Rab7-positive organelles to

dynein and to the subsequent movement of MBVs to the minus end of microtubules close to the perinuclear region of cells in culture. It is possible that Rab7 effectors are specifically distributed along the axon and that they play the role of linking transport carriers to dynein-mediated transport, rather than acting in late endosomes fusion. It has been described that there is a small proportion of NGF bound to axon terminals in primary cultures of sympathetic neurons that is actually retrograde transported and that a proportion of the NGF is recycled in the synaptic terminal (Deinhardt et al. 2006; Tsui-Pierchala and Ginty 1999; Ure and Campenot 1997; Weible et al. 2001). Thus, we can envision a model in which receptors are recycled in the synaptic terminal, and only a small proportion of the receptors are able to become active and recruit active Rab5. Rab5-tagged endosomes are sorted to retrograde pathways, and similar to the Rab5–Rab7 conversion that occurs in the cell body leading to maturation of late endosomes, there would be a conversion of Rab5 to Rab7 for retrograde transport. Therefore, it is possible that retrograde-transported vesicles are a heterogeneous group of vesicles with different degrees of Rab5/Rab7 loads that are not necessary multivesicular bodies since it has been described that MVBs are not frequently found in axons and that radiolabeled neurotrophins are often found with simple and small organelles more reminiscent than early endosomes (Fig. 7) (Altick et al. 2009).

It is of note that Rab5, Rab7, and Rab11 GTPases have all been functionally linked to dynein-mediated transport, and it is known that there are different dynein isoforms. Therefore, it is possible that each GTPase regulates the dynamics of different dynein isoforms. Additional research will be required to understand the heterogeneity of different retrograde-transported signaling organelles and the molecular machinery that generates them and regulates their transport (Horgan et al. 2010; Loubery et al. 2008; Satoh et al. 2008; Tan et al. 2011).

2.5 Retrograde Signaling and the Development of Proper Connectivity

Postganglionic sympathetic neurons have long served as a good model to study the molecular events underlying neuronal survival, axon growth, and the elaboration of dendrites in the PNS. These neurons express the TrkA and p75 receptors, and both NGF and NT3 are required for sympathetic nervous system development. It has been shown that during development of sympathetic axons, NT3 and NGF are required for proper axonal growth. While NT3 is required for the local growth of sympathetic axons through their local targets, such as the vasculature, NGF is required for final target innervation (for example, of the heart). This differential response is achieved by NT3 inducing a local response through the activation of TrkA in the growth cone, while NGF induces retrograde signaling that induces survival in the cell body, dependent on the activity of the CREB transcription factor. Different mechanisms have been developed by sympathetic neurons to increase their sensitivity to NGF once TrkA-NGF-mediated retrograde signaling has been initiated. NGF-TrkA retrograde signaling induces transcytosis of cell

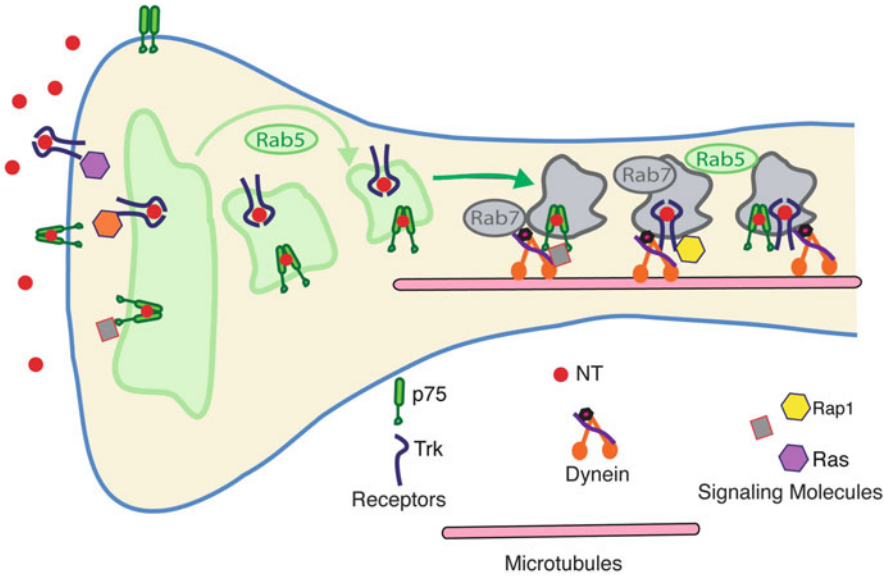

Fig. 7 Signaling endosomes in the axon. In response to neurotrophins (NTs) the neurotrophin receptors are internalized and a proportion of them are sorted to the dynein-dependent retrograde transport. Endosomes positive for p75, Trks only, or p75 and Trks are transported in the axon. The Rabs GTPases Rab5 and Rab7 are involved in the sorting and retrograde transport of potential signaling endosomes. Retrograde killing and survival signals are induced by p75 and Trks activation in distal axons, respectively. Several evidences indicate that these endosomes are associated with signaling molecules (see text for references)

body-associated TrkA receptors to the presynaptic terminal in a Rab11-dependent mechanism. The transcytosis of TrkA to axons results in increased sensitivity to NGF. Additionally, NGF-TrkA-mediated responses induce increased expression of TrkA and p75. p75 increases the specificity of TrkA binding to neurotrophins; thus, in the presence of p75, NT3 no longer binds TrkA. Another effect of NGF retrograde signaling is the increased expression of BDNF and NT4 that through the p75 receptor activate cell death signaling in neighboring neurons associated with low levels of TrkA-NGF signaling (Ascano et al. 2009; Deppmann et al. 2008; Kuruvilla et al. 2004). Therefore, there are at least three different positive feed-forward loops that increase sensitivity to NGF in sympathetic neurons and transform it into a "winner" in the competition for survival.

Contrary to what has been reported in PC12 cells regarding the p75 regulation of TrkA internalization (Geetha et al. 2005; Makkerh et al. 2005), p75 has no effect on the retrograde transport of TrkA signaling during neurotrophin-mediated target innervation of sympathetic neurons (Kuruvilla et al. 2004). However, it has been reported that p75 is internalized in the cell bodies and axons of sympathetic neurons through a mechanism that is partially dependent on clathrin coats and cholesterol. Additionally, internalized p75/BDNF vesicles appear more slowly than TrkA vesicles, suggesting (as observed in PC12 cells) that different populations of

vesicles are formed and transported in response to the binding of neurotrophins to p75 or TrkA (Bronfman et al. 2003; Hibbert et al. 2006). The significance of the retrograde transport of p75 for p75 signaling is largely unknown. It was recently reported that proNT3 is able to induce retrograde killing in sympathetic compartmentalized cultures when applied to axons (Yano et al. 2009), and we have found that BDNF is able to induce retrograde killing in compartmentalized cultures of sympathetic neurons in a dynein and c-Jun-amino-terminal kinase (JNK)-dependent manner (Escudero et al. unpoblished work). Therefore, it is possible that BDNF accumulates in DCVs in sympathetic synaptic terminals and it is secreted in an activity-dependent manner by the neurons that have established synaptic contacts and exhibit high levels of NGF-TrkA signaling. In the growth cone of neurons with low levels of TrkA-NGF signaling, BDNF through binding to p75 may induce retrograde killing of sympathetic neurons (Mok et al. 2009). A retrograde killing for neurotrophin withdrawal has also been recently described for sympathetic neurons. Determining whether this process is p75 dependent will require further research.

A standing question has been why the activation of TrkA by NT3 only induces local axonal growth while NGF induces the internalization of TrkA and retrograde signaling. Kuruvilla and Ginty and collaborators have recently provided the answer to this question proposing two different, but not mutually exclusive, explanations; NGF, but not NT3, promotes the endocytosis of TrkA through the calcineurin-mediated dephosphorylation of the endocytic GTPase dynamin1. NGF is able to induce specific dephosphorylation of dynamin1, increasing the internalization of TrkA and, thus, the retrograde transport associated with survival signaling, while the interaction of NT3 with TrkA does not have the same effect. Consistently, conditional deletion of *calcineurin* in sympathetic neurons disrupts NGF-dependent innervation of peripheral target tissues (Bodmer et al. 2011). On the other hand, NGF and not NT3 is able to activate, in endosomes, the actin regulators Rac1-GTP-cofilin, enabling the NGF/TrkA signaling endosomes to "escape" the actin network for retrograde transport (Harrington et al. 2011).

Another less explored mechanism for neurotrophin-induced axonal elongation is control of the local translation of axonal mRNAs. A recent study from Riccio and collaborators combined compartmentalized cultures of rat sympathetic neurons and sequential analysis of gene expression (SAGE) to analyze the mRNA content of sympathetic axons. Their screen yielded more than 11,000 tags in axons that matched known transcripts. Bioinformatics analysis revealed that many transcripts were highly enriched in axons compared to cell bodies, indicating that the accumulation of specific mRNAs in axons depends on active transport. The most abundant transcript in axons was myo-inositol monophosphatase-1 (Impa1), a key enzyme that regulates the inositol cycle and the main target of lithium in neurons. A novel localization element within the 3′ untranslated region of Impa1 mRNA was found to specifically target Impa1 transcripts to sympathetic neuron axons and regulate local Impa1 translation in response to axonally supplied NGF. Reduction of NGF-induced Impa1 synthesis in axons was observed to decrease nuclear CREB activation and induce axonal degeneration. Related findings have been reported by

the Twiss group in regenerating axons of adult sensory neurons grown in compartmentalized cultures. In their system, neurotrophins have been found to selectively increase the transport of specific RNAs to axons (Andreassi et al. 2010; Willis et al. 2007). Thus, target-derived NGF induces axonal elongation by cytoskeleton remodeling and axonal translation of proteins. Additionally, NGF-TrkA retrograde signaling in the cell body changes the repertoire of mRNAs that are anterograde transported to the synaptic terminal to increase target innervation and, thus, neuronal survival.

One interesting concept has emerged from the groups of Ginty and Segal and collaborators. In addition to regulating the number of neuronal cells that survive, long-distance neurotrophin signaling might regulate the degree of connectivity with preganglionic sympathetic neurons located in the central nervous system. In sympathetic neurons, NGF–TrkA signaling endosomes travel from distal axons to cell bodies and dendrites, where they promote postsynaptic density (PSD) formation. The presence of p75 restricts PSD formation, suggesting an important role for antagonistic NGF–TrkA and p75 signaling pathways during retrograde control of synapse establishment. A similar model has been suggested for sensory neurons, in which BDNF and NGF retrograde signaling induce the activation of ERK5 in the axon and cell body and the transcription factor MEF2D. MEF2-dependent transcriptional programs, in addition to inducing survival through the upregulation of the anti-apoptotic bcl-2 family member bcl-w, are also critical for establishing synaptic morphology and for regulating synapse numbers (Flavell et al. 2006; Pazyra-Murphy et al. 2009; Shalizi et al. 2006; Sharma et al. 2010).

In conclusion, proper connectivity of the nervous system is achieved by coordinating signal transduction with intracellular processes, such as secretion, endocytosis, and molecular motor transport, together with the local translation of localized mRNA in distal neuronal processes. To understand the molecular mechanisms that regulate these events through extracellular molecules (i.e., neurotrophins) will be useful to understand how the nervous system works and will provide better insight into how perturbation of intracellular trafficking may lead to neurodegenerative diseases.

Acknowledgments The authors gratefully acknowledge financial support from CARE (CONICYT PFB12/2007), FONDECYT 1085273 and 1120146 (FB) FONDECYT 3100076 (CF), and Proyecto Núcleo Milenio (MINREB) P07/011-F.

References

Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T (2007) Mouse and rat BDNF gene structure and expression revisited. J Neurosci Res 85:525–535

Altar CA, DiStefano PS (1998) Neurotrophin trafficking by anterograde transport. Trends Neurosci 21:433–437

Altick AL, Baryshnikova LM, Vu TQ, von Bartheld CS (2009) Quantitative analysis of multivesicular bodies (MVBs) in the hypoglossal nerve: evidence that neurotrophic factors do not use MVBs for retrograde axonal transport. J Comp Neurol 514:641–657

An JJ, Gharami K, Liao GY, Woo NH, Lau AG et al (2008) Distinct role of long 3′ UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell 134:175–187
Andreassi C, Zimmermann C, Mitter R, Fusco S, De Vita S et al (2010) An NGF-responsive element targets myo-inositol monophosphatase-1 mRNA to sympathetic neuron axons. Nat Neurosci 13:291–301
Arevalo JC, Yano H, Teng KK, Chao MV (2004) A unique pathway for sustained neurotrophin signaling through an ankyrin-rich membrane-spanning protein. EMBO J 23:2358–2368
Arevalo JC, Waite J, Rajagopal R, Beyna M, Chen ZY et al (2006) Cell survival through Trk neurotrophin receptors is differentially regulated by ubiquitination. Neuron 50:549–559
Arimura N, Kimura T, Nakamuta S, Taya S, Funahashi Y et al (2009) Anterograde transport of TrkB in axons is mediated by direct interaction with Slp1 and Rab27. Dev Cell 16:675–686
Ascano M, Richmond A, Borden P, Kuruvilla R (2009) Axonal targeting of Trk receptors via transcytosis regulates sensitivity to neurotrophin responses. J Neurosci 29:11674–11685
Assaife-Lopes N, Sousa VC, Pereira DB, Ribeiro JA, Chao MV, Sebastiao AM (2010) Activation of adenosine A2A receptors induces TrkB translocation and increases BDNF-mediated phospho-TrkB localization in lipid rafts: implications for neuromodulation. J Neurosci 30:8468–8480
Bagayogo IP, Dreyfus CF (2009) Regulated release of BDNF by cortical oligodendrocytes is mediated through metabotropic glutamate receptors and the PLC pathway. ASN Neuro 1(1): pii: e00001
Barker PA (2004) p75NTR is positively promiscuous: novel partners and new insights. Neuron 42:529–533
BasuRay S, Mukherjee S, Romero E, Wilson MC, Wandinger-Ness A (2010) Rab7 mutants associated with Charcot-Marie-Tooth disease exhibit enhanced NGF-stimulated signaling. PLoS One 5:e15351
Bath KG, Lee FS (2006) Variant BDNF (Val66Met) impact on brain structure and function. Cogn Affect Behav Neurosci 6:79–85
Beattie EC, Howe CL, Wilde A, Brodsky FM, Mobley WC (2000) NGF signals through TrkA to increase clathrin at the plasma membrane and enhance clathrin-mediated membrane trafficking. J Neurosci 20:7325–7333
Bessis A, Bechade C, Bernard D, Roumier A (2007) Microglial control of neuronal death and synaptic properties. Glia 55:233–238
Bhattacharyya A, Watson FL, Bradlee TA, Pomeroy SL, Stiles CD, Segal RA (1997) Trk receptors function as rapid retrograde signal carriers in the adult nervous system. J Neurosci 17:7007–7016
Bibel M, Barde YA (2000) Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev 14:2919–2937
Bilsland LG, Sahai E, Kelly G, Golding M, Greensmith L, Schiavo G (2010) Deficits in axonal transport precede ALS symptoms in vivo. Proc Natl Acad Sci U S A 107:20523–20528
Bodmer D, Ascano M, Kuruvilla R (2011) Isoform-specific dephosphorylation of dynamin1 by calcineurin couples neurotrophin receptor endocytosis to axonal growth. Neuron 70:1085–1099
Bonifacino JS, Traub LM (2003) Signals for sorting of transmembrane proteins to endosomes and lysosomes. Annu Rev Biochem 72:395–447
Brigadski T, Hartmann M, Lessmann V (2005) Differential vesicular targeting and time course of synaptic secretion of the mammalian neurotrophins. J Neurosci 25:7601–7614
Bronfman FC (2007) Metalloproteases and gamma-secretase: new membrane partners regulating p75 neurotrophin receptor signaling? J Neurochem 103(Suppl 1):91–100
Bronfman FC, Fainzilber M (2004) Multi-tasking by the p75 neurotrophin receptor: sortilin things out? EMBO Rep 5:867–871
Bronfman FC, Kapon R (2007) Commuting within the cell-mind the GAPs. Workshop on Systems Dynamics of Intracellular Communication: overcoming Distance in Signalling Networks. EMBO Rep 8:1011–1015

Bronfman FC, Tcherpakov M, Jovin TM, Fainzilber M (2003) Ligand-induced internalization of the p75 neurotrophin receptor: a slow route to the signaling endosome. J Neurosci 23:3209–3220

Bronfman FC, Escudero CA, Weis J, Kruttgen A (2007) Endosomal transport of neurotrophins: roles in signaling and neurodegenerative diseases. Dev Neurobiol 67:1183–1203

Bucci C, Parton RG, Mather IH, Stunnenberg H, Simons K et al (1992) The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. Cell 70:715–728

Bucci C, Thomsen P, Nicoziani P, McCarthy J, van Deurs B (2000) Rab7: a key to lysosome biogenesis. Mol Biol Cell 11:467–480

Butowt R, von Bartheld CS (2001) Sorting of internalized neurotrophins into an endocytic transcytosis pathway via the Golgi system: ultrastructural analysis in retinal ganglion cells. J Neurosci 21:8915–8930

Cabeza C, Figueroa A, Lazo OM, Galleguillos C, Pissani C et al (2012) Cholinergic abnormalities, endosomal alterations and up-regulation of nerve growth factor signaling in Niemann-Pick type C disease. Mol Neurodegener 7:11

Cao L, Zhu YL, Su Z, Lv B, Huang Z et al (2007) Olfactory ensheathing cells promote migration of Schwann cells by secreted nerve growth factor. Glia 55:897–904

Cavalli V, Corti M, Gruenberg J (2001) Endocytosis and signaling cascades: a close encounter. FEBS Lett 498:190–196

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK et al (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J Neurosci 24:4401–4411

Chen ZY, Ieraci A, Tanowitz M, Lee FS (2005a) A novel endocytic recycling signal distinguishes biological responses of Trk neurotrophin receptors. Mol Biol Cell 16:5761–5772

Chen ZY, Ieraci A, Teng H, Dall H, Meng CX et al (2005b) Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. J Neurosci 25:6156–6166

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T et al (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314:140–143

Chiaruttini C, Vicario A, Li Z, Baj G, Braiuca P et al (2009) Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. Proc Natl Acad Sci U S A 106:16481–16486

Cogli L, Progida C, Lecci R, Bramato R, Kruttgen A, Bucci C (2010) CMT2B-associated Rab7 mutants inhibit neurite outgrowth. Acta Neuropathol 120:491–501

Cohen-Cory S, Kidane AH, Shirkey NJ, Marshak S (2010) Brain-derived neurotrophic factor and the development of structural neuronal connectivity. Dev Neurobiol 70:271–288

Cuitino L, Matute R, Retamal C, Bu G, Inestrosa NC, Marzolo MP (2005) ApoER2 is endocytosed by a clathrin-mediated process involving the adaptor protein Dab2 independent of its Rafts' association. Traffic 6:820–838

Dai X, Qu P, Dreyfus CF (2001) Neuronal signals regulate neurotrophin expression in oligodendrocytes of the basal forebrain. Glia 34:234–239

de Melker AA, van der Horst G, Calafat J, Jansen H, Borst J (2001) c-Cbl ubiquitinates the EGF receptor at the plasma membrane and remains receptor associated throughout the endocytic route. J Cell Sci 114:2167–2178

Deinhardt K, Reversi A, Berninghausen O, Hopkins CR, Schiavo G (2007) Neurotrophins Redirect p75NTR from a clathrin-independent to a clathrin-dependent endocytic pathway coupled to axonal transport. Traffic 8:1736–1749

Deinhardt K, Salinas S, Verastegui C, Watson R, Worth D, Hanrahan S, Bucci C, Schiavo G. (2006) Rab5 and Rab7 control endocytic sorting along the retrograde transport pathway. Neuron 19;52(2):293–305

Delcroix JD, Valletta JS, Wu C, Hunt SJ, Kowal AS, Mobley WC (2003) NGF signaling in sensory neurons: evidence that early endosomes carry NGF retrograde signals. Neuron 39:69–84

Deppmann CD, Mihalas S, Sharma N, Lonze BE, Niebur E, Ginty DD (2008) A model for neuronal competition during development. Science 320:369–373

Devon RS, Orban PC, Gerrow K, Barbieri MA, Schwab C et al (2006) Als2-deficient mice exhibit disturbances in endosome trafficking associated with motor behavioral abnormalities. Proc Natl Acad Sci U S A 103:9595–9600

Di Fiore PP, De Camilli P (2001) Endocytosis and signaling. An inseparable partnership. Cell 106:1–4

Doherty GJ, McMahon HT (2009) Mechanisms of endocytosis. Annu Rev Biochem 78:857–902

Ehlers MD, Kaplan DR, Price DL, Koliatsos VE (1995) NGF-stimulated retrograde transport of trkA in the mammalian nervous system. J Cell Biol 130:149–156

Falley K, Schutt J, Iglauer P, Menke K, Maas C et al (2009) Shank1 mRNA: dendritic transport by kinesin and translational control by the 5′untranslated region. Traffic 10:844–857

Flavell SW, Cowan CW, Kim TK, Greer PL, Lin Y et al (2006) Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. Science 311:1008–1012

Fujitani M, Kawai H, Proia RL, Kashiwagi A, Yasuda H, Yamashita T (2005) Binding of soluble myelin-associated glycoprotein to specific gangliosides induces the association of p75NTR to lipid rafts and signal transduction. J Neurochem 94:15–21

Fukuda M (2008) Regulation of secretory vesicle traffic by Rab small GTPases. Cell Mol Life Sci 65:2801–2813

Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H et al (2004) Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 118:127–138

Geetha T, Jiang J, Wooten MW (2005) Lysine 63 polyubiquitination of the nerve growth factor receptor TrkA directs internalization and signaling. Mol Cell 20:301–312

Gentry JJ, Barker PA, Carter BD (2004) The p75 neurotrophin receptor: multiple interactors and numerous functions. Prog Brain Res 146:25–39

Georgieva MV, de Pablo Y, Sanchis D, Comella JX, Llovera M (2011) Ubiquitination of TrkA by Nedd4-2 regulates receptor lysosomal targeting and mediates receptor signaling. J Neurochem 117:479–493

Ginsberg SD, Alldred MJ, Counts SE, Cataldo AM, Neve RL et al (2010) Microarray analysis of hippocampal CA1 neurons implicates early endosomal dysfunction during Alzheimer's disease progression. Biol Psychiatry 68:885–893

Glebova NO, Ginty DD (2005) Growth and survival signals controlling sympathetic nervous system development. Annu Rev Neurosci 28:191–222

Gomes RA, Hampton C, El-Sabeawy F, Sabo SL, McAllister AK (2006) The dynamic distribution of TrkB receptors before, during, and after synapse formation between cortical neurons. J Neurosci 26:11487–11500

Greenberg ME, Xu B, Lu B, Hempstead BL (2009) New insights in the biology of BDNF synthesis and release: implications in CNS function. J Neurosci 29:12764–12767

Greene LA, Tischler AS (1976) Establishment of a noradrenergic clonal line of ral adrenal pheochromocytoma cells which respond to nerve growth factor. Proc Natl Acad Sci USA 73 (7):2424–2428

Grimes ML, Zhou J, Beattie EC, Yuen EC, Hall DE et al (1996) Endocytosis of activated TrkA: evidence that nerve growth factor induces formation of signaling endosomes. J Neurosci 16:7950–7964

Grimes ML, Beattie E, Mobley WC (1997) A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. Proc Natl Acad Sci U S A 94:9909–9914

Haglund K, Shimokawa N, Szymkiewicz I, Dikic I (2002) Cbl-directed monoubiquitination of CIN85 is involved in regulation of ligand-induced degradation of EGF receptors. Proc Natl Acad Sci U S A 99:12191–12196

Hanz S, Perlson E, Willis D, Zheng JQ, Massarwa R et al (2003) Axoplasmic importins enable retrograde injury signaling in lesioned nerve. Neuron 40:1095–1104

Harrington AW, St Hillaire C, Zweifel LS, Glebova NO, Philippidou P et al (2011) Recruitment of actin modifiers to TrkA endosomes governs retrograde NGF signaling and survival. Cell 146:421–434

Hawryluk MJ, Keyel PA, Mishra SK, Watkins SC, Heuser JE, Traub LM (2006) Epsin 1 is a polyubiquitin-selective clathrin-associated sorting protein. Traffic 7:262–281
Heerssen HM, Segal RA (2002) Location, location, location: a spatial view of neurotrophin signal transduction. Trends Neurosci 25:160–165
Heerssen HM, Pazyra MF, Segal RA (2004) Dynein motors transport activated Trks to promote survival of target-dependent neurons. Nat Neurosci 7:596–604
Hendry IA, Stach R, Herrup K (1974a) Characteristics of the retrograde axonal transport system for nerve growth factor in the sympathetic nervous system. Brain Res 82:117–128
Hendry IA, Stockel K, Thoenen H, Iversen LL (1974b) The retrograde axonal transport of nerve growth factor. Brain Res 68:103–121
Hibbert AP, Kramer BM, Miller FD, Kaplan DR (2006) The localization, trafficking and retrograde transport of BDNF bound to p75NTR in sympathetic neurons. Mol Cell Neurosci 32:387–402
Higuchi H, Yamashita T, Yoshikawa H, Tohyama M (2003) PKA phosphorylates the p75 receptor and regulates its localization to lipid rafts. EMBO J 22:1790–1800
Hirokawa N (2006) mRNA transport in dendrites: RNA granules, motors, and tracks. J Neurosci 26:7139–7142
Hirokawa N, Noda Y, Tanaka Y, Niwa S (2009) Kinesin superfamily motor proteins and intracellular transport. Nat Rev Mol Cell Biol 10:682–696
Holzbaur EL (2004) Motor neurons rely on motor proteins. Trends Cell Biol 14:233–240
Hong EJ, McCord AE, Greenberg ME (2008) A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. Neuron 60:610–624
Horgan CP, Hanscom SR, Jolly RS, Futter CE, McCaffrey MW (2010) Rab11-FIP3 links the Rab11 GTPase and cytoplasmic dynein to mediate transport to the endosomal-recycling compartment. J Cell Sci 123:181–191
Horton AC, Ehlers MD (2003a) Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. J Neurosci 23:6188–6199
Horton AC, Ehlers MD (2003b) Neuronal polarity and trafficking. Neuron 40:277–295
Howe CL, Mobley WC (2004) Signaling endosome hypothesis: a cellular mechanism for long distance communication. J Neurobiol 58:207–216
Howe CL, Valletta JS, Rusnak AS, Mobley WC (2001) NGF signaling from clathrin-coated vesicles: evidence that signaling endosomes serve as a platform for the Ras-MAPK pathway. Neuron 32:801–814
Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24:677–736
Huang EJ, Reichardt LF (2003) Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 72:609–642
Huang CS, Zhou J, Feng AK, Lynch CC, Klumperman J et al (1999) Nerve growth factor signaling in caveolae-like domains at the plasma membrane. J Biol Chem 274:36707–36714
Huang SH, Zhao L, Sun ZP, Li XZ, Geng Z et al (2009) Essential role of Hrs in endocytic recycling of full-length TrkB receptor but not its isoform TrkB.T1. J Biol Chem 284:15126–15136
Ibanez CF (2007) Message in a bottle: long-range retrograde signaling in the nervous system. Trends Cell Biol 17:519–528
Joset A, Dodd DA, Halegoua S, Schwab ME (2010) Pincher-generated Nogo-A endosomes mediate growth cone collapse and retrograde signaling. J Cell Biol 188:271–285
Jullien J, Guili V, Derrington EA, Darlix JL, Reichardt LF, Rudkin BB (2003) Trafficking of TrkA-green fluorescent protein chimerae during nerve growth factor-induced differentiation. J Biol Chem 278:8706–8716
Kanning KC, Hudson M, Amieux PS, Wiley JC, Bothwell M, Schecterson LC (2003) Proteolytic processing of the p75 neurotrophin receptor and two homologs generates C-terminal fragments with signaling capability. J Neurosci 23:5425–5436

Kao S, Jaiswal RK, Kolch W, Landreth GE (2001) Identification of the mechanisms regulating the differential activation of the mapk cascade by epidermal growth factor and nerve growth factor in PC12 cells. J Biol Chem 276:18169–18177

Kardon JR, Vale RD (2009) Regulators of the cytoplasmic dynein motor. Nat Rev Mol Cell Biol 10:854–865

Kenchappa RS, Zampieri N, Chao MV, Barker PA, Teng HK et al (2006) Ligand-dependent cleavage of the P75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. Neuron 50:219–232

Kirkham M, Parton RG (2005) Clathrin-independent endocytosis: new insights into caveolae and non-caveolar lipid raft carriers. Biochim Biophys Acta 1745:273–286

Korsching S (1993) The neurotrophic factor concept: a reexamination. J Neurosci 13:2739–2748

Kuruvilla R, Zweifel LS, Glebova NO, Lonze BE, Valdez G et al (2004) A neurotrophin signaling cascade coordinates sympathetic neuron development through differential control of TrkA trafficking and retrograde signaling. Cell 118:243–255

Lazo OM, Gonzalez A, Ascaño M, Kuruvilla R, Couve A, Bronfman FC (2013) BDNF regulates Rab11-mediated recycling endosome dynamics to induce dendritic branching. J Neurosci 33 (14):6112–6122

Lessmann V, Gottmann K, Malcangio M (2003) Neurotrophin secretion: current facts and future prospects. Prog Neurobiol 69:341–374

Levi-Montalcini R (1966) The nerve growth factor: its mode of action on sensory and sympathetic nerve cells. Harvey Lect 60:217–259

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

Li X, Standley C, Sapp E, Valencia A, Qin ZH et al (2009) Mutant huntingtin impairs vesicle formation from recycling endosomes by interfering with Rab11 activity. Mol Cell Biol 29:6106–6116

Limpert AS, Karlo JC, Landreth GE (2007) Nerve growth factor stimulates the concentration of TrkA within lipid rafts and extracellular signal-regulated kinase activation through c-Cbl-associated protein. Mol Cell Biol 27:5686–5698

Lin DC, Quevedo C, Brewer NE, Bell A, Testa JR et al (2006) APPL1 associates with TrkA and GIPC1 and is required for nerve growth factor-mediated signal transduction. Mol Cell Biol 26:8928–8941

Liu J, Lamb D, Chou MM, Liu YJ, Li G (2007) Nerve growth factor-mediated neurite outgrowth via regulation of Rab5. Mol Biol Cell 18:1375–1384

Lo KY, Kuzmin A, Unger SM, Petersen JD, Silverman MA (2011) KIF1A is the primary anterograde motor protein required for the axonal transport of dense-core vesicles in cultured hippocampal neurons. Neurosci Lett 491:168–173

Loubery S, Wilhelm C, Hurbain I, Neveu S, Louvard D, Coudrier E (2008) Different microtubule motors move early and late endocytic compartments. Traffic 9:492–509

Lu B, Pang PT, Woo NH (2005) The yin and yang of neurotrophin action. Nat Rev Neurosci 6:603–614

MacInnis BL, Campenot RB (2002) Retrograde support of neuronal survival without retrograde transport of nerve growth factor. Science 295:1536–1539

Makkerh JP, Ceni C, Auld DS, Vaillancourt F, Dorval G, Barker PA (2005) p75 neurotrophin receptor reduces ligand-induced Trk receptor ubiquitination and delays Trk receptor internalization and degradation. EMBO Rep 6:936–941

Marmor MD, Yarden Y (2004) Role of protein ubiquitylation in regulating endocytosis of receptor tyrosine kinases. Oncogene 23:2057–2070

Matsumoto T, Rauskolb S, Polack M, Klose J, Kolbeck R et al (2008) Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat Neurosci 11:131–133

Matsuoka I, Meyer M, Thoenen H (1991) Cell-type-specific regulation of nerve growth factor (NGF) synthesis in non-neuronal cells: comparison of Schwann cells with other cell types. J Neurosci 11:3165–3177

Mayor S, Pagano RE (2007) Pathways of clathrin-independent endocytosis. Nat Rev Mol Cell Biol 8:603–612

McCaffrey G, Welker J, Scott J, der Salm L, Grimes ML (2009) High-resolution fractionation of signaling endosomes containing different receptors. Traffic 10:938–950

Miaczynska M, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S et al (2004a) APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment. Cell 116:445–456

Miaczynska M, Pelkmans L, Zerial M (2004b) Not just a sink: endosomes in control of signal transduction. Curr Opin Cell Biol 16:400–406

Mochizuki N, Yamashita S, Kurokawa K, Ohba Y, Nagai T et al (2001) Spatio-temporal images of growth-factor-induced activation of Ras and Rap1. Nature 411:1065–1068

Mok SA, Lund K, Campenot RB (2009) A retrograde apoptotic signal originating in NGF-deprived distal axons of rat sympathetic neurons in compartmented cultures. Cell Res 19:546–560

Morris SM, Tallquist MD, Rock CO, Cooper JA (2002) Dual roles for the Dab2 adaptor protein in embryonic development and kidney transport. EMBO J 21:1555–1564

Mufson EJ, Kroin JS, Sendera TJ, Sobreviela T (1999) Distribution and retrograde transport of trophic factors in the central nervous system: functional implications for the treatment of neurodegenerative diseases. Prog Neurobiol 57:451–484

Nagappan G, Zaitsev E, Senatorov VV Jr, Yang J, Hempstead BL, Lu B (2009) Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. Proc Natl Acad Sci U S A 106:1267–1272

Ng BK, Chen L, Mandemakers W, Cosgaya JM, Chan JR (2007) Anterograde transport and secretion of brain-derived neurotrophic factor along sensory axons promote Schwann cell myelination. J Neurosci 27:7597–7603

Nishio M, Fukumoto S, Furukawa K, Ichimura A, Miyazaki H et al (2004) Overexpressed GM1 suppresses nerve growth factor (NGF) signals by modulating the intracellular localization of NGF receptors and membrane fluidity in PC12 cells. J Biol Chem 279:33368–33378

Nomura K, Kanemura H, Satoh T, Kataoka T (2004) Identification of a novel domain of Ras and Rap1 that directs their differential subcellular localizations. J Biol Chem 279:22664–22673

Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P et al (2004) Sortilin is essential for proNGF-induced neuronal cell death. Nature 427:843–848

Ohta K, Kuno S, Inoue S, Ikeda E, Fujinami A, Ohta M (2010) The effect of dopamine agonists: the expression of GDNF, NGF, and BDNF in cultured mouse astrocytes. J Neurol Sci 291:12–16

Pal A, Severin F, Lommer B, Shevchenko A, Zerial M (2006) Huntingtin-HAP40 complex is a novel Rab5 effector that regulates early endosome motility and is up-regulated in Huntington's disease. J Cell Biol 172:605–618

Pazyra-Murphy MF, Hans A, Courchesne SL, Karch C, Cosker KE et al (2009) A retrograde neuronal survival response: target-derived neurotrophins regulate MEF2D and bcl-w. J Neurosci 29:6700–6709

Pereira DB, Chao MV (2007) The tyrosine kinase Fyn determines the localization of TrkB receptors in lipid rafts. J Neurosci 27:4859–4869

Perlson E, Hanz S, Ben-Yaakov K, Segal-Ruder Y, Seger R, Fainzilber M (2005) Vimentin-dependent spatial translocation of an activated MAP kinase in injured nerve. Neuron 45:715–726

Perlson E, Maday S, Fu MM, Moughamian AJ, Holzbaur EL (2010) Retrograde axonal transport: pathways to cell death? Trends Neurosci 33:335–344

Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS et al (2004) The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci 24:10099–10102

Philippidou P, Valdez G, Akmentin W, Bowers WJ, Federoff HJ, Halegoua S (2011) Trk retrograde signaling requires persistent, Pincher-directed endosomes. Proc Natl Acad Sci U S A 108:852–857

Platta HW, Stenmark H (2011) Endocytosis and signaling. Curr Opin Cell Biol 23(4):393–403

Raju CS, Fukuda N, Lopez-Iglesias C, Goritz C, Visa N, Percipalle P (2011) In neurons, activity-dependent association of dendritically transported mRNA transcripts with the transacting factor CBF-A is mediated by A2RE/RTS elements. Mol Biol Cell 22(11):1864–1877

Reynolds AJ, Bartlett SE, Hendry IA (1998) Signalling events regulating the retrograde axonal transport of 125I-beta nerve growth factor in vivo. Brain Res 798:67–74

Riccio A, Pierchala BA, Ciarallo CL, Ginty DD (1997) An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons. Science 277:1097–1100

Riccio A, Ahn S, Davenport CM, Blendy JA, Ginty DD (1999) Mediation by a CREB family transcription factor of NGF-dependent survival of sympathetic neurons. Science 286:2358–2361

Rodriguez-Boulan E, Powell SK (1992) Polarity of epithelial and neuronal cells. Annu Rev Cell Biol 8:395–427

Salehi A, Delcroix JD, Belichenko PV, Zhan K, Wu C et al (2006) Increased App expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration. Neuron 51:29–42

Salinas S, Schiavo G, Kremer EJ (2010) A hitchhiker's guide to the nervous system: the complex journey of viruses and toxins. Nat Rev Microbiol 8:645–655

Satoh D, Sato D, Tsuyama T, Saito M, Ohkura H et al (2008) Spatial control of branching within dendritic arbors by dynein-dependent transport of Rab5-endosomes. Nat Cell Biol 10:1164–1171

Saxena S, Howe CL, Cosgaya JM, Hu M, Weis J, Kruttgen A (2004) Differences in the surface binding and endocytosis of neurotrophins by p75NTR. Mol Cell Neurosci 26:292–307

Saxena S, Bucci C, Weis J, Kruttgen A (2005a) The small GTPase Rab7 controls the endosomal trafficking and neuritogenic signaling of the nerve growth factor receptor TrkA. J Neurosci 25:10930–10940

Saxena S, Howe CL, Cosgaya JM, Steiner P, Hirling H et al (2005b) Differential endocytic sorting of p75NTR and TrkA in response to NGF: a role for late endosomes in TrkA trafficking. Mol Cell Neurosci 28:571–587

Schinder AF, Poo M (2000) The neurotrophin hypothesis for synaptic plasticity. Trends Neurosci 23:639–645

Schonteich E, Wilson GM, Burden J, Hopkins CR, Anderson K et al (2008) The Rip11/Rab11-FIP5 and kinesin II complex regulates endocytic protein recycling. J Cell Sci 121:3824–3833

Senger DL, Campenot RB (1997) Rapid retrograde tyrosine phosphorylation of trkA and other proteins in rat sympathetic neurons in compartmented cultures. J Cell Biol 138:411–421

Shalizi A, Gaudilliere B, Yuan Z, Stegmuller J, Shirogane T et al (2006) A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation. Science 311:1012–1017

Shao Y, Akmentin W, Toledo-Aral JJ, Rosenbaum J, Valdez G et al (2002) Pincher, a pinocytic chaperone for nerve growth factor/TrkA signaling endosomes. J Cell Biol 157:679–691

Sharma N, Deppmann CD, Harrington AW, St Hillaire C, Chen ZY et al (2010) Long-distance control of synapse assembly by target-derived NGF. Neuron 67:422–434

Shinoda Y, Sadakata T, Nakao K, Katoh-Semba R, Kinameri E et al (2011) Calcium-dependent activator protein for secretion 2 (CAPS2) promotes BDNF secretion and is critical for the development of GABAergic interneuron network. Proc Natl Acad Sci U S A 108:373–378

Slepnev VI, De Camilli P (2000) Accessory factors in clathrin-dependent synaptic vesicle endocytosis. Nat Rev Neurosci 1:161–172

Somsel Rodman J, Wandinger-Ness A (2000) Rab GTPases coordinate endocytosis. J Cell Sci 113 (Pt 2):183–192

Sonnichsen B, De Renzis S, Nielsen E, Rietdorf J, Zerial M (2000) Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of Rab4, Rab5, and Rab11. J Cell Biol 149:901–914

Sorkin A (2004) Cargo recognition during clathrin-mediated endocytosis: a team effort. Curr Opin Cell Biol 16:392–399

Sorkin A, Von Zastrow M (2002) Signal transduction and endocytosis: close encounters of many kinds. Nat Rev Mol Cell Biol 3:600–614

Stenmark H (2009) Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol 10:513–525

Tan SC, Scherer J, Vallee RB (2011) Recruitment of dynein to late endosomes and lysosomes through light intermediate chains. Mol Biol Cell 22:467–477

Tcherpakov M, Bronfman FC, Conticello SG, Vaskovsky A, Levy Z et al (2002) The p75 neurotrophin receptor interacts with multiple MAGE proteins. J Biol Chem 277:49101–49104

Tsui-Pierchala BA, Ginty DD (1999) Characterization of an NGF-P-TrkA retrograde-signaling complex and age-dependent regulation of TrkA phosphorylation in sympathetic neurons. J Neurosci 19:8207–8218

Ure DR, Campenot RB (1997) Retrograde transport and steady-state distribution of 125I-nerve growth factor in rat sympathetic neurons in compartmented cultures. J Neurosci 17:1282–1290

Urra S, Escudero CA, Ramos P, Lisbona F, Allende E et al (2007) TrkA receptor activation by nerve growth factor induces shedding of the p75 neurotrophin receptor followed by endosomal gamma-secretase-mediated release of the p75 intracellular domain. J Biol Chem 282:7606–7615

Vaegter CB, Jansen P, Fjorback AW, Glerup S, Skeldal S et al (2011) Sortilin associates with Trk receptors to enhance anterograde transport and neurotrophin signaling. Nat Neurosci 14:54–61

Valdez G, Akmentin W, Philippidou P, Kuruvilla R, Ginty DD, Halegoua S (2005) Pincher-mediated macroendocytosis underlies retrograde signaling by neurotrophin receptors. J Neurosci 25:5236–5247

Varsano T, Dong MQ, Niesman I, Gacula H, Lou X et al (2006) GIPC is recruited by APPL to peripheral TrkA endosomes and regulates TrkA trafficking and signaling. Mol Cell Biol 26:8942–8952

Verderio C, Bianco F, Blanchard MP, Bergami M, Canossa M et al (2006) Cross talk between vestibular neurons and Schwann cells mediates BDNF release and neuronal regeneration. Brain Cell Biol 35:187–201

Vilar M, Charalampopoulos I, Kenchappa RS, Simi A, Karaca E et al (2009) Activation of the p75 neurotrophin receptor through conformational rearrangement of disulphide-linked receptor dimers. Neuron 62:72–83

Watson FL, Heerssen HM, Moheban DB, Lin MZ, Sauvageot CM et al (1999) Rapid nuclear responses to target-derived neurotrophins require retrograde transport of ligand-receptor complex. J Neurosci 19:7889–7900

Watson FL, Heerssen HM, Bhattacharyya A, Klesse L, Lin MZ, Segal RA (2001) Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. Nat Neurosci 4:981–988

Weible MW 2nd, Bartlett SE, Reynolds AJ, Hendry IA (2001) Prolonged recycling of internalized neurotrophins in the nerve terminal. Cytometry 43:182–188

Willis DE, van Niekerk EA, Sasaki Y, Mesngon M, Merianda TT et al (2007) Extracellular stimuli specifically regulate localized levels of individual neuronal mRNAs. J Cell Biol 178:965–980

Willnow TE, Petersen CM, Nykjaer A (2008) VPS10P-domain receptors – regulators of neuronal viability and function. Nat Rev Neurosci 9:899–909

Wu C, Lai CF, Mobley WC (2001) Nerve growth factor activates persistent Rap1 signaling in endosomes. J Neurosci 21:5406–5416

Wu C, Ramirez A, Cui B, Ding J, Delcroix JD et al (2007) A functional dynein-microtubule network is required for NGF signaling through the Rap1/MAPK pathway. Traffic 8:1503–1520

Yang J, Siao CJ, Nagappan G, Marinic T, Jing D et al (2009) Neuronal release of proBDNF. Nat Neurosci 12:113–115

Yano H, Lee FS, Kong H, Chuang J, Arevalo J et al (2001) Association of Trk neurotrophin receptors with components of the cytoplasmic dynein motor. J Neurosci 21:RC125

Yano H, Torkin R, Martin LA, Chao MV, Teng KK (2009) Proneurotrophin-3 is a neuronal apoptotic ligand: evidence for retrograde-directed cell killing. J Neurosci 29:14790–14802

Ye H, Kuruvilla R, Zweifel LS, Ginty DD (2003) Evidence in support of signaling endosome-based retrograde survival of sympathetic neurons. Neuron 39:57–68

York RD, Molliver DC, Grewal SS, Stenberg PE, McCleskey EW, Stork PJ (2000) Role of phosphoinositide 3-kinase and endocytosis in nerve growth factor-induced extracellular signal-regulated kinase activation via Ras and Rap1. Mol Cell Biol 20:8069–8083

Yune TY, Lee JY, Jung GY, Kim SJ, Jiang MH et al (2007) Minocycline alleviates death of oligodendrocytes by inhibiting pro-nerve growth factor production in microglia after spinal cord injury. J Neurosci 27:7751–7761

Zhang Y, Moheban DB, Conway BR, Bhattacharyya A, Segal RA (2000) Cell surface Trk receptors mediate NGF-induced survival while internalized receptors regulate NGF-induced differentiation. J Neurosci 20:5671–5678

Zheng J, Shen WH, Lu TJ, Zhou Y, Chen Q et al (2008) Clathrin-dependent endocytosis is required for TrkB-dependent Akt-mediated neuronal protection and dendritic growth. J Biol Chem 283:13280–13288

Zhou B, Cai Q, Xie Y, Sheng ZH (2012) Snapin recruits dynein to BDNF-TrkB signaling endosomes for retrograde axonal transport and is essential for dendrite growth of cortical neurons. Cell Rep 2:42–51

Zwang Y, Yarden Y (2009) Systems biology of growth factor-induced receptor endocytosis. Traffic 10:349–363

Neurotrophins: Transcription and Translation

A.E. West, P. Pruunsild, and T. Timmusk

Abstract

Neurotrophins are powerful molecules. Small quantities of these secreted proteins exert robust effects on neuronal survival, synapse stabilization, and synaptic function. Key functions of the neurotrophins rely on these proteins being expressed at the right time and in the right place. This is especially true for BDNF, stimulus-inducible expression of which serves as an essential step in the transduction of a broad variety of extracellular stimuli into neuronal plasticity of physiologically relevant brain regions. Here we review the transcriptional and translational mechanisms that control neurotrophin expression with a particular focus on the activity-dependent regulation of BDNF.

Keywords

NGF • NT3 • NT4/5 • BDNF • Transcription • Translation • Activity-dependent • Plasticity

A.E. West (✉)
Department of Neurobiology, Duke University Medical Center, Durham, NC 27710, USA
e-mail: west@neuro.duke.edu

P. Pruunsild
Institute of Gene Technology, Tallinn University of Technology, 12618 Tallinn, Estonia

Department of Neurobiology, Interdisciplinary Center for Neurosciences, University of Heidelberg, 69120 Heidelberg, Germany

T. Timmusk
Institute of Gene Technology, Tallinn University of Technology, 12618 Tallinn, Estonia

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_4,

1 Introduction

Transcriptional regulation is mediated by the association of DNA binding proteins with gene regulatory elements, which confer developmental, cell-type-specific, and stimulus-dependent regulation on gene transcription. Protein–DNA interactions influence transcription by modulating the recruitment and/or activation of RNA polymerase II at nearby genes. Gene regulatory elements are defined by their function, and although many closely neighbor genes, regulatory elements can also act over long distances. Many regulatory elements are found in promoters, which are broadly defined as the region of genomic DNA immediately proximal to and up to about 2 kb upstream of the transcription start site (TSS) for a given gene. By contrast enhancer elements can be located at very great distances on either side of the TSS. Gene transcription can also be influenced by protein–DNA interactions at insulator and silencing elements, which impact transcription over large regions of the surrounding genome. All of these elements are subject to an additional level of regulation by the secondary and tertiary structure of chromatin, which can be modulated by modifications of both genomic DNA and its associated histone proteins. Once synthesized, mRNA is subject to several modes of posttranscriptional regulation that can impact levels of gene expression through regulation of RNA stability, transport, and translation. In addition to protein–RNA interactions, there is a growing awareness of the role of noncoding RNAs as mediators of these processes.

Here we describe the characterization of the transcriptional and translational processes that regulate expression of the neurotrophins. Though all the neurotrophins play important roles in neuronal physiology, commensurate with the importance of stimulus-dependent regulation of *Bdnf* mRNA expression for neural plasticity, the mechanisms underlying the dynamic regulation of this gene have received substantial attention and will be reviewed in the greatest detail.

2 Nerve Growth Factor

2.1 Expression Pattern and Regulation

Nerve growth factor (NGF) is expressed in both neuronal and non-neuronal cells of the peripheral and central nervous systems (Sofroniew et al. 2001). NGF is highly expressed in the target tissues of TrkA expressing neurons, which include dorsal root ganglia (DRG), cranial sensory neurons that mediate pain and temperature, sympathetic neurons, basal forebrain cholinergic neurons, striatal cholinergic neurons, and certain thalamic and brainstem neurons. Hippocampal and cortical neurons that are targets of cholinergic innervation express the highest levels of NGF mRNA in the brain (Lauterborn et al. 1993, 1995; Rocamora et al. 1996a), and interestingly, the majority of these NGF-positive neurons are GABAergic interneurons. In the striatum, NGF is also expressed by a population of small GABAergic interneurons (Bizon et al. 1999). In non-neuronal cells of the adult mouse, the highest levels of NGF mRNA are present in the salivary gland, vas

deferens, and heart. Expression of NGF mRNA in salivary gland is sex specific; in male animals the levels are much higher than in females (Sofroniew et al. 2001). Immature Schwann cells produce NGF during development, but in adults, NGF expression is undetectable in mature myelinating Schwann cells. However, after nerve injury the expression is induced in reactive and dedifferentiated Schwann cells (Heumann et al. 1987; Lindholm et al. 1987).

Expression of NGF is sensitive to regulation by both neuronal activity and stimuli related to inflammation. Limbic seizures induce *Ngf* expression by 1 h in the dentate gyrus, whereas expression appears in the neocortex and olfactory forebrain some hours later (Gall and Isackson 1989). Consistent with enhanced transcription as a mechanism underlying the activity-dependent increases in *Ngf* mRNA levels, membrane depolarization of cultured embryonic cortical neurons induces the association of RNA PolII with the *Ngf* gene promoter (Kim et al. 2010). CNS induction of *Ngf* is responsive to both glutamate (Zafra et al. 1990) and acetylcholine (da Penha Berzaghi et al. 1993). In addition, *Ngf* expression can be upregulated by glucocorticoids (Mocchetti et al. 1996; Barbany and Persson 1992) and activation of β2 adrenergic receptors (Colangelo et al. 1998). The interleukin IL-1 strongly induces expression of *Ngf* in non-neuronal cells of the peripheral nervous system after injury (Lindholm et al. 1987). Intraventricular injection of IL-1 also induces *Ngf* expression in the hippocampus, but it is not clear whether this induction is in neuronal or non-neuronal cells (Spranger et al. 1990).

2.2 Promoter Structure and Elements

The *Ngf* gene is found on chromosome 3qF2.2 in mouse, chromosome 2q34 in rat, and chromosome 1p13.2 in human. The mammalian *Ngf* gene contains several 5′ exons encoding the 5′ untranslated region (UTR) and one 3′ exon encoding the NGF protein (Metsis 2001) (Fig. 1). The structure of the mammalian *Ngf* gene and its transcripts has been studied most extensively in the mouse (Edwards et al. 1986; Selby et al. 1987). In mouse the *Ngf* gene comprises five exons, exons IA, IB, II, III, and IV covering about 50 kb. According to current knowledge, exons IA, IB, II, and III encode 5′ UTRs and exon IV the NGF pre-protein. Although exons IA and II both contain additional putative ATG codons, their usage for translation initiation of NGF protein has not been established. Four different splicing patterns have been described for the mouse *Ngf* gene leading to the following transcripts: transcripts containing exons IA, III, and IV, transcripts containing exons IB, III, and IV, transcripts containing exons IB, II, III, and IV, and transcripts containing 5′ extended exon III and exon IV. Exon IA-III-IV transcripts are the most abundant *Ngf* mRNAs in the submandibular gland comprising about 90 % of the pool of *Ngf* mRNAs. In other tissues, including heart, kidney, and brain, the most abundant transcript is exon IB-III-IV followed by exon IA-III-IV transcripts. The levels of exon IB-II-III-IV transcripts and exon III-IV transcripts are much lower. One major transcription initiation site has been determined both for exon IA and IB by primer extension and S1 nuclease protection assay showing that exons IA and IB, separated by only 142 bp, are linked to separate promoters. It has also been shown that the 5′

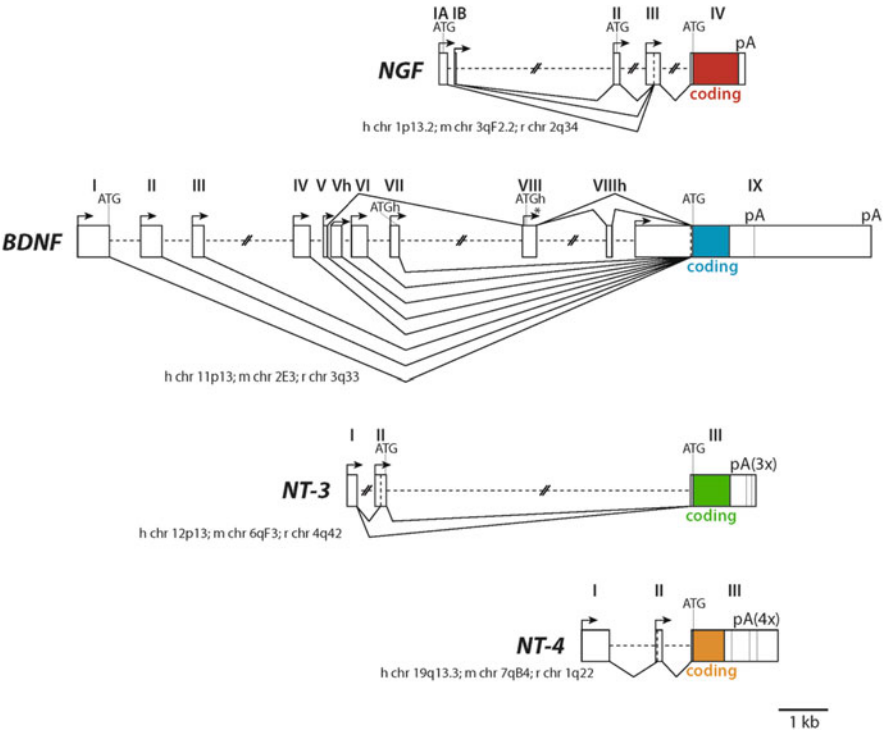

Fig. 1 Structures of mammalian neurotrophin genes. The structures of the genes include data published on the human, mouse, and rat neurotrophins. All neurotrophin genes consist of multiple 5′ exons linked to promoters that initiate transcription of distinct mRNAs. As a common feature, the 3′ exon that is included in all different transcripts of each neurotrophin comprises the open reading frame (ORF, *colored box*) encoding the respective prepro-neurotrophin. The beginning of the ORF is marked by the translation initiation codon ATG. There are variant upstream ATGs in all neurotrophin genes except *Ntf4*, but the usage of these translation initiation sites has not been verified. For all except the *Ngf* gene, usage of at least two alternative polyadenylation sites (pA, *thin vertical line*) has been detected. In the case of *Bdnf*, human-specific exons that are not present in rodent *Bdnf* are marked with the letter "h" following the Roman numeral representing the name of the exon brought above the box designating the exon. *Horizontal dashed lines* represent introns. *Vertical dashed lines* inside exons indicate alternative splicing acceptor sites used within that exon. Splicing patterns of neurotrophin mRNAs are shown by *lines* linking exons. The most upstream transcription start site (TSS) is indicated by an *arrow* for each exon. The *asterisk* marking the TSS of *BDNF* exon VIII stands for a rodent-specific transcription initiation site that has not been detected to be used in human. The genomic locations of human (h), mouse (m), and rat (r) genes are shown adjacent to each schematic. *Scale bar* is for exons and introns shown with uninterrupted *dashed lines*. Introns that are interrupted with *double slash* are longer and out of this scale

region of mouse exon IB is able to drive reporter gene expression when transiently expressed in cultured cells showing that this exon is linked to a functional promoter (Zheng and Heinrich 1988; D'Mello and Heinrich 1991). The putative promoter of mouse *Ngf* exon IA has not been studied.

The rat and human *NGF* genes have not been characterized in detail; however, bioinformatic analysis of GenBank suggests that, similar to mouse, exon IB-III-IV transcripts are the most abundant in several tissues, including brain. The transcription initiation site of exon IB has been determined for rat exon IB by S1 nuclease protection (Zheng and Heinrich 1988) and the 5′ region of both rat and human exon IB is able to direct reporter gene expression in various cultured cells using transient expression assays (Zheng and Heinrich 1988; Cartwright et al. 1992). In addition, transgenic mice expressing reporters under control of human and mouse *NGF* promoter regions have been characterized that partially recapitulate expression of the endogenous gene (Alexander et al. 1989; Kaisho et al. 1999; Kawaja et al. 2011).

The function of regulatory elements in the *Ngf* exon IB promoter has been studied in non-neuronal cells. Following cloning of the *Ngf* gene, attention focused on an AP-1 site found at +35 bp, mutation of which reduces activity of an *Ngf* promoter reporter plasmid in heterologous expression assays (D'Mello and Heinrich 1991). AP-1 elements are bound by members of the Fos/Jun family of transcription factors, and lesion of the sciatic nerve was known to induce both Fos protein and *Ngf* mRNA expression. Using a fibroblast line in which Fos could be inducibly overexpressed, it was shown that Fos increases *Ngf* mRNA expression through a mechanism that supports DNAse protection of the AP-1 containing fragment, suggesting that Fos binding to this AP-1 may contribute to lesion-induced increases in *Ngf* mRNA (Hengerer et al. 1990). By contrast elements 5′ to the TSS have been implicated in transcriptional regulation of *Ngf* in response to β2 adrenergic receptor activation. In C6-2B glioma cells, *Ngf* expression can be induced by addition of the β2 adrenergic receptor agonist clenbuterol. Activation requires an element mapped by DNAse footprinting and reporter transactivation to a region −90 to −70 bp relative to the TSS (Colangelo et al. 1998). Binding and reporter studies identified CCAAT/enhancer-binding protein δ (C/EBPδ) as a putative regulatory transcription factor for this site, and further studies showed that CREB binds to a CRE half-site at −65 bp. Importantly C/EBPδ knockout mice have significantly reduced β2 adrenergic receptor-induced NGF expression in the cortex, suggesting that similar transcriptional mechanisms may contribute to *Ngf* regulation in the brain (McCauslin et al. 2006).

2.3 Regulation of mRNA Stability

In addition to transcriptional regulation, *Ngf* mRNA is subject to stimulus-dependent changes in its stability. In cultured rat fibroblasts, in addition to a change in the transcriptional rate of *Ngf* synthesis as revealed by nuclear run-on, RNase protection assays demonstrate that IL-1 increases the half-life of *Ngf* mRNA (Lindholm et al. 1988). In smooth muscle cells, the secreted factors PDGF and TGFβ increase NGF secretion in the presence of the transcriptional inhibitor Actinomycin D and elevate the ratio of NGF protein to *Ngf* mRNA again suggesting an effect on RNA stability and/or processing (Sherer et al. 1998). AU-rich regions in the 3′UTR often serve as instability elements, and AU-rich regions of the *Ngf*

3′-UTR have been identified that appear to contribute to mRNA stability (Tang et al. 1997). However, the specific signaling mechanisms and proteins that regulate stability of *Ngf* mRNA under basal or stimulus-induced conditions remain unknown.

3 Neurotrophin-3

3.1 Expression Pattern and Regulation

Neurotrophin-3 (NT-3) is widely expressed in non-neuronal tissues during development and, in general, the levels are lower in the adult. In the adult rat the highest NT-3 protein levels have been detected in the pancreas and spleen (Katoh-Semba et al. 1996). In the nervous system, NT-3 is most highly expressed in the immature CNS when proliferation, migration, and differentiation of neuronal precursors are ongoing. NT-3 expression dramatically decreases with maturation of these regions (Maisonpierre et al. 1990b; Ernfors et al. 1992; Friedman et al. 1991b). The factors that regulate expression of NT-3 have been most highly studied in the developing cerebellum where expression of NT-3 is required for proper cerebellar development (Bates et al. 1999). Brain-derived neurotrophic factor (BDNF) can drive NT-3 expression in the cerebellum as can thyroid hormone T3 (Leingärtner et al. 1994). Strikingly, unlike BDNF and NGF, expression of NT-3 is not induced by traditional stimuli that increase neural activity in the CNS. For example, NT-3 shows no induction in the hippocampus following pilocarpine-induced seizures (da Penha Berzaghi et al. 1993) and reduced expression levels following kainate-induced seizure (Katoh-Semba et al. 1999).

3.2 Promoter Structure and Elements

NT-3 is encoded by the *Ntf3* gene on mouse chromosome 6qF3, rat chromosome 4q42, and human chromosome 12p13. In all mammals studied (mouse, rat, and human) the *Ntf3* gene comprises three exons giving rise to multiple *Ntf3* mRNA transcripts (Fig. 1). Several TSSs in both upstream exons and three different polyadenylation sites in exon III have been mapped by RNase protection assays and by RACE. Alternative promoter usage upstream of exons I and II leads to expression of transcripts that differ in the putative translation initiation ATGs (Leingärtner and Lindholm 1994; Kendall et al. 2000). Exon I-III transcripts contain an ATG in the beginning of exon III suggesting that it is used for initiation of protein translation. Exon I-II-III and exon II-III transcripts have two potential translation initiation codons; however, it has not been determined which of the ATGs is used for protein translation. Exon II-III transcripts appear to be the predominant transcripts in most tissues, including brain, and exon I-II-III transcripts have been demonstrated only in a few tissues in rat (Kendall et al. 2000). Both promoters are active when fused to reporter genes and transfected into cerebellar granule neurons (Leingärtner and Lindholm 1994). Transcripts

initiating from both promoters have been detected in cerebellar granule neurons; however, only promoter II is transcriptionally upregulated by tri-iodothyronine (T3).

In reporter assays, both promoters I and II of the *Ntf3* gene contain regions that function as enhancer and repressor elements (Leingärtner and Lindholm 1994; Katoh-Semba et al. 1996). One family of regulators that contributes to regulation of promoter II are the related zinc-finger transcription factors Sp4, Sp1, and Sp3. Sp4 and Sp1 bind directly to *Ntf3* promoter II in cerebellar granule neurons as shown by chromatin immunoprecipitation (Ramos et al. 2009). Knockdown of Sp4 expression leads to increased *Ntf3* expression in these cells suggesting that this interaction is required for *Ntf3* repression. However, the effects of Sp4 on NT-3 regulation may be context or cell-type dependent because mice with reduced Sp4 expression show reduced NT-3 in the hippocampus (Zhou et al. 2005). By contrast, BDNF-dependent activation of *Ntf3* promoter II in cerebellar granule cells is mediated by members of the MEF2 and CREB families of transcription factors (Shalizi et al. 2003). BDNF drives phosphorylation and activation of the MAP kinase family member Erk5, which then induces phosphorylation and activation of MEF2. BDNF-dependent induction of *Ntf3* requires a region −1087 to −838 bp relative to the TSS of exon II. Both MEF2 and CREB bind sequences within this region, and knockdown of MEF2 or overexpression of dominant-negative CREB inhibits BDNF-dependent induction of *Ntf3* suggesting that the two factors cooperate to mediate the regulation of this element (Shalizi et al. 2003). Finally the POU-domain transcription factor Brn-3c (POU4F3) has been implicated in *Ntf3* regulation in a cell line derived from organ of Corti (Clough et al. 2004). However, unlike the other factors, Brn-3c appears to be an activator of *Ntf3* promoter I.

4 Neurotrophin-4

4.1 Expression Pattern and Regulation

Although neurotrophin-4 (NT-4) (also called NT-4/5 or NT-5) binds and activates the TrkB receptor, regulation of NT-4 expression shares few similarities with the other TrkB ligand, BDNF. In the rat NT-4 is widely expressed in non-neuronal tissues both during embryonic and postnatal development and also in the adult. Highest NT-4 levels have been detected in early postnatal testis (Timmusk et al. 1993b). NT-4 is highly expressed in embryonic and adult skeletal muscle and it is strongly expressed by both neuronal and non-neuronal cells of the spinal cord (Ip et al. 1992; Scarisbrick et al. 1999). By contrast it is expressed at much lower levels in the CNS (Ip et al. 1992), both during development and in the adult animal (Timmusk et al. 1993b). Compared with *Bdnf* knockout mice, *Ntf4* null mice show minimal neurological phenotypes (Liu et al. 1995; Conover et al. 1995). NT-4 expression is induced in muscle by electrical stimulation (Funakoshi et al. 1995) and in spinal cord by systemic administration of the excitotoxic stimulus kainic acid (Scarisbrick et al. 1999). Analysis of *Ntf4* knockout mice has demonstrated that

muscle-derived NT-4 is required for maintenance of postsynaptic acetylcholinergic receptor clustering, normal muscular electrophysiological responses, and resistance to muscle fatigue. Thus, NT-4 is involved in activity-dependent feedback mechanisms involved in the maintenance of neuromuscular connections and muscular performance (Belluardo et al. 2001). Surprisingly, in the brain *Ntf4* is not activity regulated since there is no change in NT-4 expression in the hippocampus after pilocarpine-induced seizure (Mudo et al. 1996), a common method for inducing activity-regulated gene transcription.

4.2 Promoter Structure and Elements

NT-4 is encoded by the *Ntf5* gene on mouse chromosome 7qB4, the *Ntf4* gene on rat chromosome 1q22, and the *NTF4* gene on human chromosome 19q13.3. We refer here to the gene in all three species as "*Ntf4*". The *Ntf4* gene comprises three exons with two alternative promoters upstream of exons I and II (Fig. 1). The transcription initiation sites have been determined for rat *Ntf4* gene in newborn testis and adult skeletal muscle; however, there has been no comprehensive analysis of alternative promoter usage in other tissues and cell types in vivo. In cell lines promoter II confers significantly stronger transcriptional activity on a reporter plasmid than promoter I (Salin et al. 1997). Generation of transgenic mice that contain the full *Ntf4* gene plus 1.4 kb of additional upstream sequence show high levels of *Ntf4* expression in muscle and low but detectable expression in brain and thymus, indicating that this region is largely sufficient to confer proper expression of *Ntf4*. Importantly this transgene also recapitulates the activity-regulated expression of *Ntf4* in muscle (Funakoshi et al. 1995; Salin et al. 1997), suggesting that activity-responsive elements lie within this fragment. However, the position of these elements and their associated transcription factors has not yet been identified.

5 Brain-Derived Neurotrophic Factor

5.1 Expression Pattern and Regulation

Bdnf has a widespread expression pattern that is conserved among mammalian species (Maisonpierre et al. 1990a, b, 1991; Conner et al. 1997; Katoh-Semba et al. 1997). During development, *Bdnf* expression is more abundant in the nervous system compared with other tissues and its levels are dramatically increased in the brain postnatally (Kaisho et al. 1991; Katoh-Semba et al. 1997). In the adult nervous system, *Bdnf* displays a wide distribution pattern, with the highest levels of mRNA and protein in the hippocampus, amygdala, cerebral cortex, hypothalamus, and septum in the brain and in the dorsal root ganglia in the PNS. *Bdnf* mRNA expression is mostly confined to neurons and there are only a few brain areas where *Bdnf* transcripts are not detected (Ernfors et al. 1990; Hofer et al. 1990; Timmusk et al. 1994b; Conner et al. 1997; Katoh-Semba et al. 1997; Phillips et al. 1990;

Friedman et al. 1991a; Webster et al. 2006). *Bdnf* expression in adult tissues is also detectable outside of the nervous system. Similar *Bdnf* mRNA levels to those found in the brain have been detected in the heart and lung and lower levels in the thymus, liver, spleen, and muscle (Ernfors et al. 1990; Maisonpierre et al. 1990a; 1991; Katoh-Semba et al. 1997; Yamamoto et al. 1996).

Regulation of transcription is a major contributor to the pleiotropic functions of BDNF. Accordingly, *Bdnf* expression levels in neurons are regulated by many stimuli including ischemic and hypoglycemic insults (Lindvall et al. 1992), peripheral nerve axotomy (Michael et al. 1999), immobilization stress (Smith et al. 1995a, b), antidepressant treatment (Nibuya et al. 1995; Dias et al. 2003), drug craving after cocaine withdrawal (Grimm et al. 2003), and chronic social defeat stress (Tsankova et al. 2006). However, the best studied and probably the most potent *Bdnf* transcription-inducing stimulus is neuronal activity. Neuronal activity in the brain and *Bdnf* mRNA expression are both evoked by excitatory stimulus-evoked seizures by kainic acid treatment (Zafra et al. 1990; Ballarin et al. 1991; Metsis et al. 1993), electrical stimulation resulting in epileptogenesis (Ernfors et al. 1991), lesion-induced recurrent limbic seizures (Isackson et al. 1991), exposure to light as sensory input (Castren et al. 1992), electrical stimulation inducing LTP of synaptic transmission (Patterson et al. 1992; Castren et al. 1993), enriched environment (Falkenberg et al. 1992; Young et al. 1999), application of KCl to the cortical surface inducing spreading depression (Kokaia et al. 1993), mechanical stimulation of mystacial whiskers (Rocamora et al. 1996b; Nanda and Mack 2000), physical activity (Neeper et al. 1996; Russo-Neustadt et al. 2000), singing in birds (Li et al. 2000), hippocampus-dependent contextual learning (Hall et al. 2000), and amygdala-dependent learning (Rattiner et al. 2004). On the other hand, treatments or conditions that reduce neuronal activity, for example, inhibition of neuronal activity by gamma-aminobutyric acid (GABA) (Berninger et al. 1995) and monocular deprivation (Bozzi et al. 1995; Rossi et al. 1999), have been demonstrated to decrease *Bdnf* mRNA levels. Furthermore, expression of *Bdnf* undergoes circadian oscillation, mirroring variations in physiological activity (Bova et al. 1998; Berchtold et al. 1999). Thus, environmental stimuli that produce excitatory inputs onto neurons and increase their intracellular Ca^{2+} concentration, i.e., induce neuronal activity, have been found to be the key regulators of *Bdnf* transcription. The significance of this activity-regulated transcription of *Bdnf* is emphasized by the fact that BDNF is one of the major regulators of neuronal activity-dependent neurotransmission and plasticity in the brain (Schinder and Poo 2000; Poo 2001; Lu 2003; Bramham and Messaoudi 2005).

5.2 Promoter Structure

The *Bdnf* gene comprises nine exons that span 52.3 kb of chromosome 2qE3 in mouse, chromosome 3q33 in rat, and chromosome 11p14.1 in human. All three species appear to have at least eight homologous exons that contribute to alternate 5′ UTRs, each of which is linked to a separate promoter and can be spliced to form a

bipartite transcript and in some rare cases also a tripartite or a quadripartite transcript (V-VIII-VIIIh-IX, V-VIIIh-IX, and VI-IXb-IXd) with a common ninth exon that contains the coding sequence and 3′-UTR (Fig. 1) (Liu et al. 2005, 2006; Aid et al. 2007; Pruunsild et al. 2007). An ATG in exon I provides an alternative putative translation start site for exon I-IX variants (Timmusk et al. 1993a). Expression constructs encoding a human BDNF-GFP fusion protein containing both the exon I ATG and exon IX ATG are translated when transiently expressed in primary hippocampal neurons. However, it was not studied which of these two ATGs was used for translation initiation (Jiang et al. 2008). The pufferfish and zebrafish *Bdnf* genes preserve a similar multi-exon organization suggesting that this genomic structure may have a conserved function through evolution (Heinrich and Pagtakhan 2004). Rat and human BDNF genomic regions recapitulating tissue-specific, neuronal activity-, and axotomy-induced expression of rat *Bdnf* (Timmusk et al. 1995; Koppel et al. 2010) and human *BDNF* (Koppel et al. 2009) have been characterized in transgenic mice.

The functional importance of the multi-promoter organization of *Bdnf* is incompletely understood; however, it appears that the stimulus-selective activation of the distinct sets of transcription factors bound at each of these promoters serves to make BDNF expression responsive to a very diverse range of stimuli. Different 5′ *Bdnf* exons are induced by distinct kinds of stimuli (West 2008), consistent with the idea that transcription originating at each promoter may be differentially important for the myriad biological functions of BDNF. It should be noted that while the importance of *Bdnf* exon IV containing mRNA transcription in the development of GABAergic inhibition in the cortex has been studied relatively well in vivo using specific genetic manipulations that disrupt basal and activity-responsive *Bdnf* exon IV-derived production of BDNF protein (Hong et al. 2008; Sakata et al. 2009), the in vivo role of *Bdnf* exon I- and II-containing transcripts has not been addressed. In the light of the findings that exon I mRNAs of *Bdnf* are among the most strongly induced *Bdnf* transcripts upon neuronal activity (Metsis et al. 1993; Timmusk et al. 1993a) and that overexpression of *Bdnf* exon I, II, and III mRNAs without increasing other *Bdnf* transcripts is associated with enhanced LTP in mice (Barco et al. 2005), it would be especially interesting to elucidate the roles of all the multiple exons of *Bdnf*.

5.3 Promoter Regulation

5.3.1 Promoter I

The levels of *Bdnf* exon I increase markedly in the brain after kainic acid-induced seizures (Metsis et al. 1993; Timmusk et al. 1993a) and other experimental conditions that produce neuronal activity. The first transcription factors that were shown to contribute to this Ca^{2+}-mediated activation of *Bdnf* promoter I were the activating transcription factor (ATF)/cAMP/Ca^{2+}-response element binding protein (CREB) family basic leucine zipper protein CREB and the basic helix-loop-helix (bHLH) proteins upstream stimulatory factor (USF) 1 and USF2 (Fig. 2a) (Tabuchi

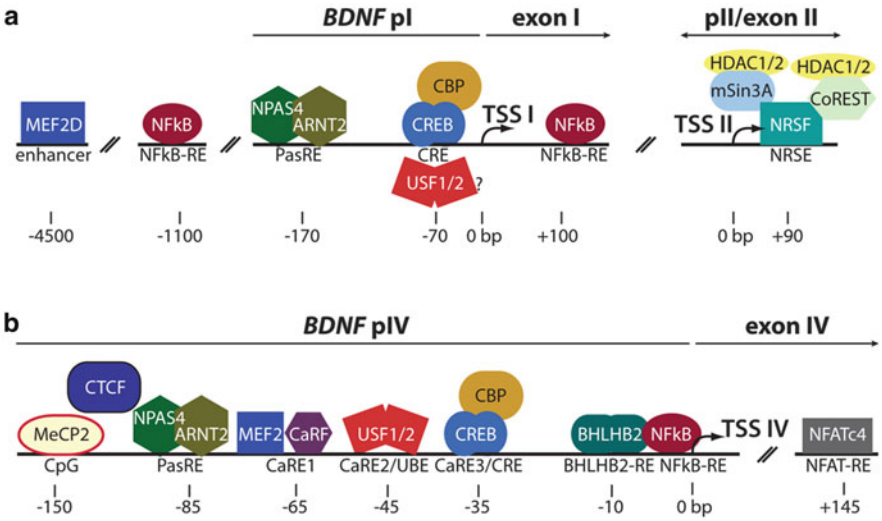

Fig. 2 Transcription factors and regulatory elements involved in the regulation of activity-dependent transcription from *BDNF* promoters I, II, and IV. *Bdnf* promoters (p) I, pII, and pIV are bound by multiple transcription factors (TFs) that regulate neuronal activity-dependent induction of *Bdnf* exon I, II, and IV mRNA transcription. (**a**) The TFs and *cis*-elements that have been shown to regulate activity-dependent *Bdnf* exon I or II transcription are myocyte-specific enhancer factor 2D (MEF2D); nuclear factor kappa B (NF-κB); neuronal PAS domain protein 4 (NPAS4); aryl hydrocarbon receptor nuclear translocator 2 (ARNT2); cAMP/Ca^{2+}-response element binding protein (CREB) bound by CREB binding protein (CBP); upstream stimulatory factors 1 and 2 (USF1/2); neuron-restrictive silencing factor (NRSF) bound by mSin3A (histone deacetylase complex subunit Sin3A), RE1-silencing transcription factor (REST) co-repressor 1 (CoREST), and histone deacetylase 1 and 2 (HDAC1/2); NF-κB response element (NF-κB-RE); bHLH-PAS transcription factor response element (PasRE); cAMP/Ca^{2+}-response element, in pI, a CRE-like element (CRE); and neuron-restrictive silencing element (NRSE). (**b**) The TFs and *cis*-elements that have been shown to regulate activity-dependent *Bdnf* exon IV transcription are methyl-CpG binding protein (MeCP2); CCCTC-binding factor (CTCF); NPAS4; ARNT2; MEF2; Ca^{2+}-response factor (CaRF); USF1 and USF2; CREB and CBP; basic helix-loop-helix domain containing, class B, 2 (BHLHB2); NF-κB; nuclear factor of activated T-cells cytoplasmic 4 (NFATc4); PasRE; Ca^{2+}-response element 1, 2 (UBE, USF-binding element), and 3 (CRE) (CaRE1, 2, and 3); and BHLHB2-RE, NF-κB, and response elements for the respective TFs (NFAT-RE). All factors that have been shown to bind specific *cis*-regulatory DNA elements in the promoters are depicted on the *line* representing DNA. The *cis*-elements are specified below the factors. The TFs that have been shown to contribute to regulation, but for which the binding site is not known, are depicted above the promoter. The *question mark* adjacent to the USF1/2 factors that are drawn below pI indicates that although USFs have been shown to regulate the rat promoter, regulation of human pI by the USF factors has not been confirmed and the regulatory element that has been found to bind USF in the rat promoter is not conserved in human. Transcription start sites (TSSs) are designated by *arrows*. Only the most upstream TSS for each promoter is shown. Distance in base pairs (bp) relative to the TSS is shown below the line representing DNA. This figure shows all the TFs and regulatory elements that have been shown by different groups, although data about some TFs are contradictory (for example, USF1/2 and CREB for pI, see text for details)

et al. 2002). Tabuchi et al. (2002) studied rat *Bdnf* promoter I regulation and found that Ca^{2+}-responsive DNA elements in *Bdnf* promoter I are located in two promoter regions: in a proximal and in a distal region that are located at approximately −70 to −100 bp and −180 to −280 bp, respectively, relative to the most 5′ transcription start site of rat exon I. In the proximal region, a cAMP/Ca^{2+}-response element (CRE)-like element overlapping with a USF-binding site was identified. These *cis*-elements were shown to be bound in vitro by CREB and USF1/USF2, correspondingly, and mutations in the CRE and USF-binding sites were shown to reduce rat *Bdnf* promoter I-dependent transcriptional activity in response to membrane depolarization of primary neurons. In addition, overexpression of dominant-negative forms of CREB and USF proteins in neurons was found to interfere with activity-dependent transcription from rat *Bdnf* promoter I (Tabuchi et al. 2002).

The transcription factors and *cis*-element that contributed to the Ca^{2+} responsiveness of the distal region of promoter I were identified when the regulation of the human *BDNF* promoter I in primary neurons was analyzed (Pruunsild et al. 2011). The deletion of these distal elements was even more potent in reducing the inducibility of *Bdnf* promoter I than deletions in the proximal region (Tabuchi et al. 2002), It was shown that the human as well as the rat *BDNF* promoter I is induced by neuronal activity by the bHLH-Per-Arnt-Sim (bHLH-PAS) transcription factors aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) and neuronal PAS domain protein 4 (NPAS4), which dimerize and bind to a Ca^{2+}-responsive element termed bHLH-PAS transcription factor response element (PasRE) located approximately −170 bp relative to the most 5′ transcription start site of human *BDNF* promoter I (Pruunsild et al. 2011). Pruunsild et al. demonstrated that mutating the PasRE drastically reduces neuronal activity-responsive induction of *BDNF* promoter I-dependent transcription. Also, it was shown that expression of dominant-negative ARNT2 and NPAS4 almost completely blocks and overexpression of ARNT2 and NPAS4 strongly enhances activity-responsive exon I transcription, respectively, in primary neurons. Moreover, ARNT2 binds *BDNF* promoter I in human brain in vivo (Pruunsild et al. 2011). In a separate study, NPAS4 has been detected to be bound on the mouse *Bdnf* promoter I region in mouse brain by chromatin immunoprecipitation (ChIP) (Lin et al. 2008), further strengthening involvement of the bHLH-PAS proteins in *BDNF* exon I regulation.

Some different results have been seen between the regulation of rodent and human *Bdnf* promoter I. Despite evidence for its use in rodents, the USF-binding element is not conserved in the human *BDNF* promoter I and the USF proteins have been found not to contribute to the neuronal activity-dependent regulation of the human promoter I (Pruunsild et al. 2011). Furthermore, the CRE-like element, although conserved, has been found in transient transfection assays of reporter constructs to be more important for basal transcription than for the activity-dependent induction of the human promoter I (Pruunsild et al. 2011). Nonetheless in vivo, a constitutively active form of CREB is able to enhance promoter I-dependent transcription suggesting the physiological relevance of this interaction (Barco et al. 2005). The potential importance of ARNT2 and NPAS4 factors is strongly supported by a study showing that neuronal activity-dependent

transcription of *BDNF* exon I transcripts is sensitive to protein synthesis inhibitors, indicating that immediate-early gene products are involved in activating promoter I (Lauterborn et al. 1996). As NPAS4 is one of the most strongly induced immediate-early genes by neuronal activity (Lin et al. 2008), it is conceivable that the ARNT2 and NPAS4 heterodimers, which would upregulate exon I transcription, form after the first wave of immediate-early genes have been transcribed and translated in response to the activating stimulus. This would also explain why the rise in the levels of *BDNF* exon I transcripts takes place with a delay compared to *BDNF* exon IV transcripts (Kokaia et al. 1994; Lauterborn et al. 1996; Pruunsild et al. 2011) that are predominantly under the control of the CREB/CRE system (Hong et al. 2008).

In addition to the factors described above, two other transcription factors have been implicated in mediating the neuronal activity-dependent induction of *Bdnf* exon I transcription: (1) nuclear factor kappa B (NF-κB), through binding two pairs of NF-κB response elements in proximity of *BDNF* promoter I (Lubin et al. 2007), and (2) myocyte-specific enhancer factor (MEF) 2D via binding a far upstream enhancer element (Flavell et al. 2008). Lubin et al. (2007) showed that pharmacological inhibition of the NF-κB pathway in rats decreased kainate-induced *Bdnf* exon I mRNA expression and that NF-κB was detectable on *Bdnf* promoter I with ChIP. MEF2D was shown to bind a far upstream *Bdnf* enhancer element with ChIP as well. Additionally, by mutation and deletion analyses of a *Bdnf* promoter I construct, the MEF2D binding site was demonstrated to significantly contribute to *Bdnf* promoter I neuronal activity-dependent induction in primary neurons, providing evidence that the *cis*-element whereby MEF2D augments *Bdnf* promoter I activity-responsive induction is the enhancer element approximately 4,500 bp upstream of *Bdnf* exon I (Flavell et al. 2008). The *cis*-elements for NF-κB in the activity-dependent regulation of *Bdnf* promoter I still need verification. Further insights into the role of NF-κB, as well as more understanding of the role of CREB and the possible rodent-specific function of the USFs in promoter I regulation, may reveal important new aspects of transcriptional control of *BDNF* expression.

5.3.2 Promoter II

Although to a lesser extent than *Bdnf* mRNAs containing exon I, BDNF exon II transcript levels also rise in response to neuronal activity in the brain (Metsis et al. 1993; Timmusk et al. 1993a). However no Ca^{2+}-responsive *cis*-elements or transcription factors have yet been described for *Bdnf* promoter II. Nonetheless promoter II-regulated transcripts have a unique role in the regulation of *Bdnf* expression since they are under the control of a neuron-restrictive silencer element (NRSE) (Palm et al. 1998; Timmusk et al. 1999). This element binds the zinc-finger protein neuron-restrictive silencer factor (NRSF) that recruits transcriptional co-repressors mSin3A and CoREST and in turn interacts with several other proteins, including HDACs, to regulate transcription (Fig. 2a) (Andres et al. 1999; Huang et al. 1999; Roopra et al. 2000). In transgenic mice with wild-type or mutated NRSE sequences, it has been shown that the *Bdnf* NRSE is involved in the repression of basal and kainic acid-induced transcription from *Bdnf* promoter II and, interestingly, also promoter I in neurons in vivo, indicating a role for this

element in modulating activity-dependent expression of *Bdnf* (Timmusk et al. 1999). One of the causes for Huntington's disease has been proposed to be mutant huntingtin-mediated NRSF-dependent decreases in *Bdnf* gene transcription, leading to reduced trophic support for striatal neurons (Zuccato et al. 2003). The remote effect of the NRSE in *Bdnf* promoter II on *Bdnf* exon I transcription suggests that *Bdnf* exons I and II, which are separated only by approximately 630 bp in the human genome, could be co-regulated as a single cluster. Although indications in this direction have been obtained by using reporter constructs encompassing the genomic region covering both promoters I and II of *Bdnf* (Timmusk et al. 1999; Hara et al. 2009), this hypothesis, and especially the role of Ca^{2+}-dependent *cis*-elements in front of *Bdnf* exon I in the activity-responsive induction of exon II, has yet to be proved by using additional control experiments where the expression of not only the reporter protein but also exon-specific mRNA is analyzed.

5.3.3 Promoter IV

Exon IV-containing *Bdnf* transcripts are broadly expressed and strongly stimulus responsive in the CNS. Exon IV-containing *Bdnf* transcripts are also found in some non-neuronal cells including those of the heart and lung (Timmusk et al. 1993a). Promoter IV is the most active of the inducible *Bdnf* promoters in the developing brain (Pattabiraman et al. 2005; Metsis et al. 1993) and its regulation has been strongly correlated with activity-regulated neuronal and synapse development (Hong et al. 2008; Sakata et al. 2009). RNAse protection and RACE assays have identified two major clusters of TSSs for promoter IV separated by about 80 bp. Both clusters are used for transcription initiation in all seven regions of the adult rat brain that have been analyzed (cerebral cortex, hippocampus, cerebellum, midbrain, thalamus, pons/medulla, and striatum). Also both TSSs are used under control conditions and 3 h after kainic acid treatment (Timmusk et al. 1993a, 1994a).

Sequences in the proximal region of *Bdnf* promoter IV (e.g., <250 bp upstream of the exon IV TSS) are sufficient to confer about a 5–6-fold induction on a luciferase reporter gene following KCl-induced membrane depolarization, suggesting that important calcium-response elements are found within region. Indeed promoter-luciferase reporter mutagenesis studies have led to the identification of several calcium-response elements (CaREs) within the proximal *Bdnf* promoter that are required for cooperative regulation of calcium-induced transcription of *Bdnf* exon IV (Chen et al. 2003b; Shieh et al. 1998; Tao et al. 1998; Pruunsild et al. 2011; Jiang et al. 2008). However, it is important to note that expression of endogenous exon IV-containing *Bdnf* transcripts shows over a 100-fold induction in response to the same stimulus (Tao et al. 2002). These data suggest that additional features of the endogenous *Bdnf* locus, such as epigenetic modifications of chromatin (Bird and Wolffe 1999) or the action of distant enhancers (Kim et al. 2010; Flavell et al. 2008), are likely to make a major contribution to activity-regulated *Bdnf* transcription.

A key insight from studies of *Bdnf* promoter IV is that the tight temporal, spatial, and stimulus-specific regulation of this single promoter is achieved by a complex

interplay between multiple activity-regulated transcriptional factors. At least eight different transcription factors have been shown to bind to CaREs in *Bdnf* promoter IV (Fig. 2b). Starting at the most upstream element, these factors include (1) the activity-inducible transcription factor NPAS4, heterodimerized with ARNT2, which has been shown to bind a PasRE in human *Bdnf* promoter IV (Pruunsild et al. 2011), (2) members of the myocyte enhancer factor 2 (MEF2) family of stimulus-regulated transcription factors, which bind to the upstream half of the element called CaRE1 (Hong et al. 2008; Lyons et al. 2012; Tao et al. 2002), (3) the unique transcription factor calcium-response factor (CaRF), which binds the downstream half of CaRE1 (Tao et al. 2002), (4) the upstream stimulatory factors USF1/2, which are basic helix-loop-helix family members that bind an E-box element referred to as CaRE2 (Chen et al. 2003b), (5) members of the CREB family, which bind a CRE half-site also called CaRE3 (Shieh et al. 1998; Tao et al. 1998), (6) the basic helix-loop-helix factor BHLHB2 which binds immediately upstream of the first TSS (Jiang et al. 2008), (7) the nuclear factor κB (NF-κB) which binds a site overlapping the first TSS (Lipsky et al. 2001), and (8) the nuclear factor of activated T cells (NFAT) which associates with an intragenic element +140 relative to the second TSS (Vashishta et al. 2009).

Distinct requirements for these transcription factors in the regulation of *Bdnf* promoter IV have been revealed through molecular genetic approaches that include RNA interference, the generation of transcription factor knockout mice, and the generation of transgenic mice that block the ability of specific factors to regulate *Bdnf*. For example, mice lacking *Bhlhb2* expression show enhanced hippocampal *Bdnf* exon IV expression under both basal and activity-induced conditions, implicating this protein as a repressor of *Bdnf* promoter IV (Jiang et al. 2008). Interestingly knockdown of *Npas4* or overexpression of dominant-negative forms of the PAS domain proteins ARNT2 and NPAS4 selectively impairs *Bdnf* exon IV expression at late time points after membrane depolarization (Lin et al. 2008; Pruunsild et al. 2011). *Npas4* is an immediate-early gene that shows very little expression prior to membrane depolarization, but very rapid and robust protein synthesis following stimuli that induce calcium influx into neurons (Lin et al. 2008). Recruitment of newly synthesized NPAS4 to *Bdnf* promoter IV appears to prolong the activation of transcription, allowing amplification of the initial transcription-inducing stimulus.

Despite the fact that CaRF binds the calcium-response element CaRE1 and is broadly expressed throughout the brain, studies in mice CaRF revealed that this factor appears to play a brain region-specific role in basal regulation of *Bdnf* transcription (McDowell et al. 2010). *Carf* knockout mice show reduced levels of *Bdnf* exon IV-containing mRNA transcripts and reduced BDNF protein in the frontal cortex compared with their wild-type littermates; however, *Bdnf* expression is unchanged in the hippocampus and striatum of the knockout mice (McDowell et al. 2010). Furthermore, although CaRE1 is required for activity-dependent transcription of *Bdnf* exon IV, CaRF is selectively required for the activity-*in*dependent regulation of *Bdnf* promoter IV activity (McDowell et al. 2010). By contrast, the MEF2 family transcription factor MEF2C appears to be selectively

required for the membrane depolarization-dependent activity of CaRE1 (Lyons et al. 2012). These data demonstrate that differential transcription factor binding to single gene regulatory elements can confer stimulus specificity upon the regulation of target genes.

By contrast with CaRF, the binding of CREB to CaRE3 is selectively required for the activity-dependent regulation of *Bdnf* exon IV transcription. The functional importance of this interaction was elegantly demonstrated by generation of a mouse strain bearing a mutation knocked into *Bdnf* promoter IV that selectively mutates the CRE/CaRE3 site (Hong et al. 2008). Neurons from CaRE3 mutant mice have normal basal levels of BDNF but lack activity-inducible transcription from promoter IV, validating the requirement for this CaRE in activity-dependent *Bdnf* gene regulation in vivo. Interestingly, disruption of CaRE3 is associated with impaired *Bdnf* promoter IV recruitment of other transcriptional regulators including MEF2, which binds to a DNA sequence distinct from CaRE3. These data provide experimental support for the role of a multifactor transcriptional complex at *Bdnf* promoter IV and suggest a function for CREB in nucleating the assembly of this complex.

5.4 Chromatin Regulation

In addition to the binding of sequence-specific transcription factors to gene regulatory elements, transcription is both gated and modulated by the secondary and tertiary structure of genomic DNA and its associated architectural proteins, which are collectively called chromatin. The core unit of chromatin is the nucleosome, which comprises ~146 bp of DNA wrapped around an octamer of histone proteins with two copies each of histone H2A, H2B, H3, and H4. The positioning and stability of nucleosomes impact transcription by modulating the accessibility of gene regulatory elements for transcription factor binding. Chromatin structure is sensitive to modifications of both genomic DNA and histone proteins. Differences in chromatin structure are a major determinant of cell-type-specific programs of gene transcription, and as we will discuss below, stimulus-dependent changes in chromatin regulation are emerging as an important mechanism that contributes to the plasticity of *Bdnf* transcription.

5.4.1 Posttranslational Histone Modifications

Dynamic acetylation of specific lysine (K) residues on the N-terminal tails of histones H3 (at K9 and K14) and H4 (at K5, K8, K12, and K16) bound to gene promoters is highly associated with transcriptional activation (Roh et al. 2004). A wide variety of environmental stimuli that induce *Bdnf* transcription have been demonstrated to drive increased acetylation of histones selectively at induced *Bdnf* promoters in physiologically relevant brain regions. Stimuli that have been shown to induce histone acetylation in conjunction with *Bdnf* transcription include seizure (Tsankova et al. 2004; Huang et al. 2002), membrane depolarization (Chen et al. 2003a; Martinowich et al. 2003), antidepressant treatment (Tsankova

et al. 2006), cocaine administration (Kumar et al. 2005), forced cocaine abstinence (Sadri-Vakili et al. 2010), dopamine D1 receptor agonist administration (Schroeder et al. 2008), and extinction of conditioned fear (Bredy et al. 2007). Among the molecular mechanisms that mediate steady-state changes in histone acetylation at *Bdnf*, the histone acetyltransferase CBP has been shown to be recruited to the CREB binding site of *Bdnf* promoter IV in an activity-dependent manner (Hong et al. 2008), and the histone deacetylase HDAC2 has been found to be preferentially associated compared to HDAC1 with *Bdnf* promoters I and II in vivo (Guan et al. 2009).

Activity-dependent regulation of histone methylation has also been observed on *Bdnf* promoters, implicating an additional set of regulatory enzymes in transcriptional control. Histone methylation has been associated with both transcriptional activation and repression depending on the particular lysine that is methylated, with H3K4 and H3K36 correlating with transcriptionally active genes, whereas H3K9, H3K27, and H4K20 correlate with transcriptionally repressed genes (Barski et al. 2007; Lachner and Jenuwein 2002). Furthermore, the mono-, di-, or tri-methylation (me1, me2, or me3) of lysines can mediate differential recruitment of methyl-sensitive binding partners to histones (Shi et al. 2006). On *Bdnf* promoter IV, chronic membrane depolarization of cultured cortical neurons drives increased H3K4me2, a modification associated with transcriptional activation (Martinowich et al. 2003), while on the same promoter, repressive methylation events including H3K9me2, H3K9me3, and H3K27me3 are reduced by acute membrane depolarization (Chen et al. 2003a) or exposure to an enriched environment (Kuzumaki et al. 2011). Large families of enzymes mediate the site-specific methylation and demethylation of histones suggesting a potential source of specificity for the regulation of histone methylation (Shi 2007). However, which specific enzymes act at *Bdnf* promoters and how their function and/or recruitment is coupled to neuronal activity remain largely unknown.

The observation that histone modifications are subject to stimulus-dependent plasticity at *Bdnf* promoters is intriguing because the persistent nature of many chromatin structural changes suggests that these changes could provide a mechanism of molecular memory. To address this possibility, a growing number of studies are examining correlations between histone modifications and *Bdnf* gene expression in chronic stimulation paradigms. For example, in vivo, downregulation of *Bdnf* exons III and IV is seen in hippocampus in a paradigm of chronic social defeat stress in mice (Tsankova et al. 2006). This decrease in *Bdnf* transcription is correlated with an increase in repressive histone H3K27me2 on both promoters III and IV. Interestingly, acute treatment of defeated mice with the antidepressant imipramine restores *Bdnf* expression and induces the activating mark H3K4me2 *without* diminishing the "repressive" H3K27me2 mark. A similar dissociation between H3K27 methylation and *Bdnf* gene expression has also been observed following light deprivation in mice. One week of light deprivation leads to reduced expression of multiple *Bdnf* isoforms in the visual cortex while *Bdnf* expression in the hippocampus remains unchanged (Karpova et al. 2010). However, H3K27me3 levels rise on *Bdnf* promoter IV in both brain regions and are elevated at all active

Bdnf promoters in the hippocampus. One hypothesis suggested by these data is that persistent histone modifications may chronically alter the transcriptional state of *Bdnf* in subtle ways that modulate but do not eliminate stimulus-dependent promoter regulation. Future studies that address more subtle aspects of transcriptional regulation such as the kinetics of gene activation or the cell-type specificity of induction may yield more insight into the functional relevance of these long-lasting changes in histone modifications.

5.4.2 DNA Methylation

In mammalian cells, genomic DNA is extensively modified by the addition of methyl-groups, predominantly at cytosine residues in CpG dinucleotides (Lister et al. 2009). DNA methylation of gene promoters has traditionally been associated with the persistent transcriptional repression that characterizes X-chromosome inactivation, gene imprinting, and long-term silencing of retrotransposons (Bird 2002). More recently, genome-wide studies have shown that substantial DNA methylation is also found over active gene bodies and in intergenic regions (Hellman and Chess 2007; Meissner et al. 2008), where it is thought to modulate gene expression by influencing diverse processes that include maintenance of active chromatin states, alternative promoter choice, and RNA splicing (Luco et al. 2011; Maunakea et al. 2010; Wu et al. 2010).

DNA methylation can be very persistent. For example, the differential methylation of imprinting regions can impact selective parent-of-origin gene expression for the life of a cell (Reik 2007). However a growing body of data indicates that DNA methylation is also subject to neuronal activity-regulated changes suggesting that modulation of DNA methylation may impact the transcriptional regulation of plasticity genes. Consistent with this possibility, stimulus-dependent changes in DNA methylation at *Bdnf* promoters have been correlated with regulation of *Bdnf* mRNA expression. Martinowich et al. (Martinowich et al. 2003) were the first to suggest that chronic membrane depolarization of cortical neurons in culture could lead to activity-regulated loss of methylation in *Bdnf* promoter IV. Subsequent studies have shown changes in the level of DNA methylation that are negatively correlated with *Bdnf* mRNA expression following contextual fear conditioning (Lubin et al. 2008), exercise (Gomez-Pinilla et al. 2011), and early life adversity (Roth et al. 2009).

Intriguing data suggest that the stimulus-regulated demethylation of DNA in the CNS is mediated by activation of DNA repair mechanisms. In the hippocampus, seizure drives rapid, transient demethylation of a highly methylated region of the *Bdnf* gene that is found just upstream of and overlapping the coding exon, exon IX (Ma et al. 2009). Loss of DNA methylation is maximal 4 h following seizure initiation but returns to baseline by 24 h. Seizure-induced DNA demethylation of *Bdnf* requires the enzyme Tet1, and demethylation fails to occur when *Tet1* expression is knocked down (Guo et al. 2011). In the absence of Tet1, seizure also fails to induce *Bdnf* expression, suggesting the causal importance of this demethylation reaction for *Bdnf* gene expression. Tet1 is part of a family of enzymes that mediate the conversion of 5-methyl-cytosine (5mC) to the

intermediate 5-hydroxymethyl-cytosine (5hmC) (Ito et al. 2010). Once induced by Tet1, 5hmC is a substrate for demethylation by the *Aid*/*Apobec* family of Zn^{2+}-dependent cytidine deaminases. Overexpression of *Aid* in the dentate gyrus demethylates *Bdnf* exon IX and induces *Bdnf* mRNA expression, whereas knockdown of *Apobec* reduces seizure-induced DNA demethylation at *Bdnf* and impairs stimulus-dependent *Bdnf* induction (Guo et al. 2011). It will be of great interest in the future to understand how neural activity modulates the activity of this demethylation pathway.

DNA methylation impacts transcription by inhibiting or recruiting the association of DNA binding proteins with methylated regions of the genome (Klose and Bird 2006). Two methyl-DNA-sensitive proteins implicated as effectors of DNA methylation for the regulation of *Bdnf* are the methyl-CpG binding protein 2 (MeCP2) (Chen et al. 2003a; Martinowich et al. 2003) and the insulator protein CTCF (Chang et al. 2010). MeCP2 is of particular interest in the CNS because loss-of-function mutations in human *MECP2* cause the neurodevelopmental disorder Rett syndrome (RTT) (Chahrour and Zoghbi 2007; Amir et al. 1999). Several lines of evidence suggest that MeCP2 modulates both synapse development and function (Deng et al. 2010; Medrihan et al. 2008; Nelson et al. 2006; Dani et al. 2005; Chao et al. 2007; Tropea et al. 2009; Armstrong 2005), and loss of MeCP2-dependent regulation of *Bdnf* expression has been suggested to make a major contribution to these defects. Although MeCP2 has been shown to associate with both a histone deacetylase and a histone H3-K9 methyltransferase (Fuks et al. 2003; Nan et al. 1998), and traditionally has been studied for its role in transcriptional repression, adult *Mecp2* null mice show impaired expression of *Bdnf* suggesting a more complex role for MeCP2 in regulation of this and likely other genes. Unlike classic transcriptional regulators, which bind discrete gene regulatory elements, MeCP2 is bound widely across the genome in a pattern that closely tracks the distribution of DNA methylation. This binding pattern suggests that MeCP2 is a global regulator of chromatin, perhaps via effects of chromosome architecture or long-distance genomic interactions. How global chromatin regulation of this kind would affect *Bdnf* expression in particular and activity-regulated gene transcription in general is an exciting question that remains to be understood.

5.5 Translational Regulation

Although transcriptional regulation is thought to make the major contribution to determining the expression levels of BDNF, several lines of evidence suggest that once synthesized, *Bdnf* mRNA is subject to additional modes of regulation that refine the spatial and temporal synthesis of BDNF protein. Neuronal activity may also play a role in sculpting translational regulation. The *Bdnf* 3′-UTR has been shown to confer activity-regulated stability on a luciferase reporter gene, and elements mediating this effect have been mapped in the 3′-UTR though the regulatory mechanisms remain to be determined (Fukuchi and Tsuda 2010). Another way that neuronal activity may influence the translation of *Bdnf* has been shown for

Exon VI-containing *Bdnf* transcripts, for which RNAse protection analyses have revealed that in response to membrane depolarization of neurons, a different TSS is activated that is well downstream of the primary TSS (Timmusk et al. 1994a). The shorter transcript generated from this new TSS lacks a GC-rich region near the 5′ end of Exon VI and is predicted to be more easily translated, potentially enhancing the activity-dependent expression of BDNF protein. Here we review described mechanisms that may modulate the stability and/or translation of *Bdnf* mRNA as well as regulatory pathways that direct *Bdnf* mRNA trafficking in the cell.

5.5.1 MicroRNAs Targeting the *Bdnf* 3′-UTR

MicroRNAs (miRNAs) are short noncoding RNA molecules encoded within conserved regions of the genome. These regulatory RNAs bind to complementary sequences that are usually located in the 3′ untranslated region (UTR) of their target messenger RNAs. Although miRNA binding can regulate protein expression by repressing translation, miRNA–mRNA pairs most often lead to degradation of the target messenger RNA (Guo et al. 2010).

Bioinformatics-based in silico analyses of putative miRNA binding sites have suggested that multiple miRNAs may be capable of binding the *Bdnf* 3′ UTR (Konopka et al. 2010; Lewis et al. 2003). For example, one panel of prediction algorithms identified potential binding sites for 26 different miRNAs in the 3′ UTR of human *BDNF* (Mellios et al. 2008). Five of these miRNA families were shown to be highly expressed in the prefrontal cortex, a brain region where control of *BDNF* levels is important for cognitive function. The authors of this study demonstrated that overexpression of either of two of these miRNAs, miR-30a-5p and miR-195, was sufficient to reduce the expression of luciferase when transfected into heterologous cells along with a luciferase construct fused to the *BDNF* 3′-UTR (Mellios et al. 2008). Interestingly, overexpression of the miR-30a-5p precursor in cultured rat forebrain neurons was shown to reduce BDNF protein levels without changing levels of *Bdnf* mRNA (Mellios et al. 2008). These data raise the possibility that miR-30a-5p may modulate *Bdnf* translation rather than inducing degradation of *Bdnf* mRNA; however, the mechanisms of this effect remain to be determined. Other studies have started with screens for miRNAs of relevance to a biological phenomenon and then addressed *Bdnf* as a potential target gene. For example, the miR-22 gene contains a single-nucleotide polymorphism that is linked to panic disorder (Muiños-Gimeno et al. 2011). *Bdnf* was identified bioinformatically as a potential target of miR22 and overexpression studies in heterologous cells were used to demonstrate that miR-22 can degrade a luciferase report fused to the *BDNF* 3′-UTR. In another study miR-15-a was identified as a miRNA genetically required for inner ear development, which is a process that is highly sensitive to BDNF levels (Ernfors et al. 1995). *Bdnf* was again identified and tested as a candidate target of regulation using a combination of in silico analysis and heterologous expression assays (Friedman et al. 2009).

As is apparent from these examples, the challenge that remains for miRNA studies is to demonstrate the physiological relevance of endogenous miRNA–target gene interactions for the modulation of gene expression levels in vivo. In support of

a role for endogenous miRNAs in the regulation of BDNF, expression levels of BDNF have been shown to be elevated in the hippocampus of *Camk2a*-Cre conditional *Dicer* knockout mice (Konopka et al. 2010). However this observation does not demonstrate that the effect on BDNF protein is the result of a direct interaction between miRNAs and the *Bdnf* 3′-UTR. Future studies of miRNA knockout strains and/or targeted knockin mutations of miRNA binding sites in the 3′-UTRs of *Bdnf* will enhance our understanding of the functional relevance of this regulatory mechanism for BDNF expression during neuronal development and plasticity.

5.5.2 Natural Antisense *BDNF* Transcripts

In humans, the opposite strand of the *BDNF* gene encodes a variably spliced, apparently noncoding transcript spanning 11 exons transcribed in reverse orientation to *BDNF* (Pruunsild et al. 2007; Liu et al. 2005). This *antiBDNF* gene spans ~191 kb and consists of ten exons with no evidence of open reading frames. *5′* RACE indicates that there is a single promoter upstream of exon I (Pruunsild et al. 2007). Exons I–IV of *antiBDNF* are located 3′ to the *BDNF* gene, and exons VII–X overlap *BDNF* introns. However, exons V and VI of the *antiBDNF* transcript overlap the coding exon of *BDNF*. *AntiBDNF* mRNA is expressed in many tissues where *BDNF* is also expressed, raising the possibility that these two RNAs could form complementary double-stranded RNA species. Consistent with this model, RNAseA/T1 treatment of RNA harvested from human cerebellum supports recovery of double-strand RNA templates of the *BDNF* coding exon for cDNA synthesis (Pruunsild et al. 2007). Natural antisense transcripts are a heterogeneous class of regulatory RNAs that can form sense–antisense RNA duplexes to lead to RNA degradation or translational repression (Faghihi and Wahlestedt 2009). Although *AntiBDNF* was first reported to be expressed only in humans by two groups (Liu et al. 2006; Aid et al. 2007), there has been a recent identification of an antisense *Bdnf* transcript in mice (Modarresi et al. 2012). Inhibition of this *Bdnf* antisense transcript leads to increased expression of BDNF protein; however, this appears to be through a mechanism that is independent of changes in *Bdnf* transcript stability (Modarresi et al. 2012). Although both mouse and human *Bdnf* antisense transcripts overlap the coding region of the *Bdnf* gene, the transcription start sites and exon organization of these transcripts are otherwise entirely different. One hypothesis of the origin of species-specific antisense transcripts is that insertion of the long-terminal repeats of human-specific endogenous retroviruses may create new promoters that drive the formation of these antisense transcripts (Gogvadze et al. 2009). Regardless, this evidence for a species-specific mechanism that may modulate expression of BDNF adds a new and interesting dimension to the intricate complexity of this highly regulated gene.

5.5.3 Dendritic Trafficking of *Bdnf* mRNA

At synapses, BDNF is hypothesized to activate local signaling cascades that modulate synaptic strength and structure (Poo 2001). Though not as robustly targeted to dendrites as the classic dendritic RNAs (*Camk2a*, *Mtap2*, and *Arc*)

(Schuman 1999) the evidence that *Bdnf* mRNA can be even weakly detected in dendrites (Tongiorgi et al. 1997, 2004) raised intense interest in the possibility that regulated trafficking and localized synthesis of *Bdnf* might impact the specificity of neuronal plasticity.

Expression analyses suggest that multiple regions of the *Bdnf* mRNA contribute to its dendritic localization. Most commonly, RNA targeting determinants have been mapped to 3′-UTRs. Through the use of two different alternative polyadenylation sites, *Bdnf* transcripts fall into two categories with either a short or long 3′-UTR (Hofer et al. 1990; Timmusk et al. 1993a). RNAs containing the long UTR are preferentially localized to dendrites and genetic truncation of the long 3′-UTR of *Bdnf* leads to impaired dendritic *Bdnf* mRNA localization, consistent with a localization of a positive dendritic target sequence to this region (An et al. 2008). However the coding sequence and 5′-UTRs of *Bdnf* appear to contribute to cellular mRNA localization as well. In situ analyses show that *Bdnf* mRNAs with different 5′-UTRs are differentially localized in the cell. For example, exon VI-containing forms of *Bdnf* are targeted to dendrites after stimulation of visual cortical neurons, whereas exon IV-containing forms of *Bdnf* are localized only to the somata of the same cell (Pattabiraman et al. 2005). Furthermore, in the hippocampus, exon II and exon VI probes detect *Bdnf* mRNA in apical dendrites after kainate-induced seizure, whereas exon I- and IV-containing transcripts remain restricted to the somata despite being strongly induced in levels by the stimulus (Chiaruttini et al. 2008). Overexpression analyses in hippocampus neurons show that when fused to GFP alone, the coding sequence of *Bdnf* is trafficked to the dendrites whereas addition of exon I or exon IV sequences to the 5′-UTR of the reporter construct leads to retention of *Bdnf* in the somata. These data raise the possibility that competing dendritic targeting and somatic retention signals may be found in the coding sequence and 5′-UTRs of the *Bdnf* mRNA, respectively (Chiaruttini et al. 2008).

The identification of *Bdnf* mRNA binding proteins is just beginning to yield insights into the regulation of its trafficking. Using bioinformatics, Chiaruttini et al. (2008) identified a putative binding site for the RNA binding/trafficking protein Translin (Li et al. 2008) in the coding sequence of *Bdnf*. Intriguingly, this binding site overlaps the sequence encoding the common nonsynonymous Val66Met SNP in BDNF, which has been shown to impact BDNF synthesis and secretion (Egan et al. 2003; Chen et al. 2004).There is reduced dendritic targeting of *Bdnf* mRNA in the apical dendrites of the hippocampus following pilocarpine seizure in *Bdnf* Met/met mice compared with Val/Val (Chiaruttini et al. 2008). Translin and its associated protein Trax are in dendrites, and Translin knockouts do show moderately reduced levels of dendritic *Bdnf* mRNA under baseline conditions. However these mice show robust dendritic trafficking of *Bdnf* mRNA following pilocarpine seizure demonstrating that Translin expression is not required for trafficking under these conditions (Wu et al. 2011). Another RNA binding protein that may influence *Bdnf* mRNA trafficking and/or translation is the heterogeneous nuclear ribonucleoprotein CArG box binding factor A (CBF-A) (Raju et al. 2011). CBF-A is found in dendrites and synaptosomes as well as somata

and nuclei, suggesting that it could have functions in regulation of dendritic mRNAs. CBF-A coimmunopreciptiates with *Bdnf*, *Arc*, and *Camk2a* RNA from synaptosomes, and electrophoretic mobility shift assays demonstrate that CBF-A can form a direct interaction with hnRNP A2 response elements (RTS) located in the 3′ untranslated regions of all three mRNAs. However, rather than selectively inhibiting dendritic localization of these mRNAs, knockdown of CBF-A reduces overall NMDA-R-dependent induction of *Bdnf*, *Arc*, and *Camk2a* mRNAs, suggesting a more general role for CBF-A in stability and/or processing of mRNAs including *Bdnf* (Raju et al. 2011).

Despite the presence of *Bdnf* mRNA in dendrites, it remains to be determined whether *Bdnf* is actually locally translated in dendrites or at synapses. Nonetheless, several lines of evidence suggest the importance of translational regulation of BDNF expression for its functions at synapses. For example, truncation of the long 3′-UTR of *Bdnf* not only reduces dendritic *Bdnf* levels but also causes defects in pruning of dendritic spines and a selective impairment of long-term potentiation at synapses onto the dendrites of hippocampal neurons (An et al. 2008). *Bdnf* transcripts with the long 3′-UTR are more likely to be recovered in the polysome fraction from cells, suggesting that they are more readily translated (Lau et al. 2010; Timmusk et al. 1994a). Under basal conditions, addition of the long 3′-UTR of *Bdnf* to a reporter suppresses translation, whereas following neuronal activity the long 3′-UTR enhances reporter translation, raising the possibility that stimulus-sensitive translational regulatory elements lie within this domain (Lau et al. 2010). One signaling pathway that has been shown to modulate neuronal BDNF translation in a stimulus-regulated fashion is the eukaryotic elongation factor 2 kinase (eEF2K, also known as CaMKIII). eEF2 is a critical component of the translational machinery that promotes ribosomal translocation during protein synthesis. Under resting conditions in neurons, basal activity of NMDA receptors promotes phosphorylation of eEF2 by eEF2K, which inhibits general translation (Sutton et al. 2007). However, upon NMDA receptor blockade, reduced activity of eEF2K permits dephosphorylation of eEF2 that promotes translation of target mRNAs including *Bdnf* (Autry et al. 2011). Intriguingly, translation induction of BDNF by the NMDA-receptor antagonist ketamine is positively correlated with the antidepressant actions of this drug (Autry et al. 2011). Thus these data raise the possibility that translational regulation of BDNF could contribute to the modulation of complex cognitive and emotional behaviors.

Conclusions

Two decades of research into the transcriptional and translational mechanisms that control expression of the neurotrophins have yielded a wealth of molecular information about fundamental regulatory pathways that contribute to neuronal development and plasticity. These regulatory pathways offer promising targets for the development of therapeutics that could be used to extrinsically regulate neurotrophin levels for the correction of neurological disorders. The challenge for the future is to understand how these pathways are integrated in vivo to sculpt

subtle aspects of the gene expression program that underlies the complexity of the mammalian brain.

References

Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T (2007) Mouse and rat BDNF gene structure and expression revisited. J Neurosci Res 85:525–535

Alexander JM, Hsu D, Penchuk L, Heinrich G (1989) Cell-specific and developmental regulation of a nerve growth factor-human growth hormone fusion gene in transgenic mice. Neuron 3:133–139

Amir R, Van den Veyver I, Wan M, Tran C, Francke U, Zoghbi H (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 23:185–188

An JJ, Gharami K, Liao G-Y, Woo NH, Lau AG, Vanevski F, Torre ER, Jones KR, Feng Y, Lu B et al (2008) Distinct role of long 3′ UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell 134:175–187

Andres M, Burger C, Peral-Rubio M, Battagliolo E, Anderson M, Grimes J, Dallman J, Ballas N, Mandel G (1999) CoREST: a functional corepressor required for regulation of neural-specific gene expression. Proc Natl Acad Sci U S A 96:9873–9878

Armstrong DD (2005) Neuropathology of Rett syndrome. J Child Neurol 20:747–753

Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng P-F, Kavalali ET, Monteggia LM (2011) NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature 475:91–95

Ballarin M, Ernfors P, Lindefors N, Persson H (1991) Hippocampal damage and kainic acid injection induce a rapid increase in mRNA for BDNF and NGF in the rat brain. Exp Neurol 114:35–43

Barbany G, Persson H (1992) Regulation of neurotrophin mRNA expression in the rat brain by glucocorticoids. Eur J Neurosci 4:396–403

Barco A, Patterson SL, Patterson S, Alarcón JM, Gromova P, Mata-Roig M, Morozov A, Kandel ER (2005) Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. Neuron 48:123–137

Barski A, Cuddapah S, Cui K, Roh T, Schones D, Wang Z, Wei G, Chepelev I, Zhao K (2007) High-resolution profiling of histone methylations in the human genome. Cell 129:823–837

Bates B, Rios M, Trumpp A, Chen C, Fan G, Bishop JM, Jaenisch R (1999) Neurotrophin-3 is required for proper cerebellar development. Nat Neurosci 2:115–117

Belluardo N, Westerblad H, Mudo G, Casabona A, Bruton J, Caniglia G, Pastoris O, Grassi F, Ibáñez CF (2001) Neuromuscular junction disassembly and muscle fatigue in mice lacking neurotrophin-4. Mol Cell Neurosci 18:56–67

Berchtold NC, Oliff HS, Isackson P, Cotman CW (1999) Hippocampal BDNF mRNA shows a diurnal regulation, primarily in the exon III transcript. Brain Res Mol Brain Res 71:11–22

Berninger B, Marty S, Zafra F, da Penha Berzaghi M, Thoenen H, Lindholm D (1995) GABAergic stimulation switches from enhancing to repressing BDNF expression in rat hippocampal neurons during maturation in vitro. Development 121:2327–2335

Bird A (2002) DNA methylation patterns and epigenetic memory. Genes Dev 16:6–21

Bird A, Wolffe A (1999) Methylation-induced repression – belts, braces, and chromatin. Cell 99:451–454

Bizon JL, Lauterborn JC, Gall CM (1999) Subpopulations of striatal interneurons can be distinguished on the basis of neurotrophic factor expression. J Comp Neurol 408:283–298

Bova R, Micheli MR, Qualadrucci P, Zucconi GG (1998) BDNF and trkB mRNAs oscillate in rat brain during the light-dark cycle. Brain Res Mol Brain Res 57:321–324

Bozzi Y, Pizzorusso T, Cremisi F, Rossi F, Barsacchi G, Maffei L (1995) Monocular deprivation decreases the expression of messenger RNA for brain-derived neurotrophic factor in the rat visual cortex. Neuroscience 69:1133–1144

Bramham CR, Messaoudi E (2005) BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. Prog Neurobiol 76:99–125

Bredy TW, Wu H, Crego C, Zellhoefer J, Sun YE, Barad M (2007) Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. Learning Mem 14:268–276

Cartwright M, Martin S, D'Mello S, Heinrich G (1992) The human nerve growth factor gene: structure of the promoter region and expression in L929 fibroblasts. Brain Res Mol Brain Res 15:67–75

Castren E, Zafra F, Thoenen H, Lindholm D (1992) Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. Proc Natl Acad Sci U S A 89:9444–9448

Castren E, Pitkänen M, Sirviö J, Parsadanian A, Lindholm D, Thoenen H, Riekkinen PJ (1993) The induction of LTP increases BDNF and NGF mRNA but decreases NT-3 mRNA in the dentate gyrus. Neuroreport 4:895–898

Chahrour M, Zoghbi H (2007) The story of Rett syndrome: from clinic to neurobiology. Neuron 56:422–437

Chang J, Zhang B, Heath H, Galjart N, Wang X, Milbrandt J (2010) Nicotinamide adenine dinucleotide (NAD)-regulated DNA methylation alters CCCTC-binding factor (CTCF)/cohesin binding and transcription at the BDNF locus. Proc Natl Acad Sci U S A 107:21836–21841

Chao H, Zoghbi H, Rosenmund C (2007) MeCP2 controls excitatory synaptic strength by regulating glutamatergic synapse number. Neuron 56:58–65

Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, Jaenisch R, Greenberg ME (2003a) Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science 302:885–889

Chen WG, West AE, Tao X, Corfas G, Szentirmay MN, Sawadogo M, Vinson C, Greenberg ME (2003b) Upstream stimulatory factors are mediators of Ca2+-responsive transcription in neurons. J Neurosci 23:2572–2581

Chen Z, Patel P, Sant G, Meng C, Teng K, Hempstead B, Lee F (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J Neurosci 24:4401–4411

Chiaruttini C, Sonego M, Baj G, Simonato M, Tongiorgi E (2008) BDNF mRNA splice variants display activity-dependent targeting to distinct hippocampal laminae. Mol Cell Neurosci 37:11–19

Clough RL, Sud R, Davis-Silberman N, Hertzano R, Avraham KB, Holley M, Dawson SJ (2004) Brn-3c (POU4F3) regulates BDNF and NT-3 promoter activity. Biochem Biophys Res Commun 324:372–381

Colangelo AM, Johnson PF, Mocchetti I (1998) beta-adrenergic receptor-induced activation of nerve growth factor gene transcription in rat cerebral cortex involves CCAAT/enhancer-binding protein delta. Proc Natl Acad Sci U S A 95:10920–10925

Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S (1997) Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. J Neurosci 17:2295–2313

Conover JC, Erickson JT, Katz DM, Bianchi LM, Poueymirou WT, McClain J, Pan L, Helgren M, Ip NY, Boland P (1995) Neuronal deficits, not involving motor neurons, in mice lacking BDNF and/or NT4. Nature 375:235–238

D'Mello SR, Heinrich G (1991) Structural and functional identification of regulatory regions and cis elements surrounding the nerve growth factor gene promoter. Brain Res Mol Brain Res 11:255–264

da Penha Berzaghi M, Cooper J, Castren E, Zafra F, Sofroniew M, Thoenen H, Lindholm D (1993) Cholinergic regulation of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) but not neurotrophin-3 (NT-3) mRNA levels in the developing rat hippocampus. J Neurosci 13:3818–3826

Dani V, Chang Q, Maffei A, Turrigiano G, Jaenisch R, Nelson S (2005) Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. Proc Natl Acad Sci U S A 102:12560–12565

Deng JV, Rodriguiz RM, Hutchinson AN, Kim I-H, Wetsel WC, West AE (2010) MeCP2 in the nucleus accumbens contributes to neural and behavioral responses to psychostimulants. Nat Neurosci 13:1128–1136

Dias B, Banerjee S, Duman R, Vaidya V (2003) Differential regulation of brain derived neurotrophic factor transcripts by antidepressant treatments in the adult rat brain. Neuropharmacology 45:553–563

Edwards RH, Selby MJ, Rutter WJ (1986) Differential RNA splicing predicts two distinct nerve growth factor precursors. Nature 319:784–787

Egan M, Kojima M, Callicott J, Goldberg T, Kolachana B, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M et al (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112:257–269

Ernfors P, Wetmore C, Olson L, Persson H (1990) Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. Neuron 5:511–526

Ernfors P, Bengzon J, Kokaia Z, Persson H, Lindvall O (1991) Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. Neuron 7:165–176

Ernfors P, Merlio J-P, Persson H (1992) Cells expressing mRNA for neurotrophins and their receptors during embryonic rat development. Eur J Neurosci 4:1140–1158

Ernfors P, Van De Water T, Jaenisch R (1995) Complementary roles of BDNF and NT-3 in vestibular and auditory development. Neuron 14:1153–1164

Faghihi MA, Wahlestedt C (2009) Regulatory roles of natural antisense transcripts. Nat Rev Mol Cell Biol 10:637–643

Falkenberg T, Mohammed AK, Henriksson B, Persson H, Winblad B, Lindefors N (1992) Increased expression of brain-derived neurotrophic factor mRNA in rat hippocampus is associated with improved spatial memory and enriched environment. Neurosci Lett 138:153–156

Flavell S, Kim T, Gray J, Harmin D, Hong E, Markenscoff-Papadimitriou E, Bear D, Greenberg ME (2008) Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. Neuron 60:1022–1038

Friedman WJ, Olson L, Persson H (1991a) Cells that express brain-derived neurotrophic factor mRNA in the developing postnatal rat brain. Eur J Neurosci 3:688–697

Friedman WJ, Ernfors P, Persson H (1991b) Transient and persistent expression of NT-3/HDNF mRNA in the rat brain during postnatal development. J Neurosci 11:1577–1584

Friedman LM, Dror AA, Mor E, Tenne T, Toren G, Satoh T, Biesemeier DJ, Shomron N, Fekete DM, Hornstein E et al (2009) MicroRNAs are essential for development and function of inner ear hair cells in vertebrates. Proc Natl Acad Sci U S A 106:7915–7920

Fuks F, Hurd PJ, Wolf D, Nan X, Bird AP, Kouzarides T (2003) The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. J Biol Chem 278:4035–4040

Fukuchi M, Tsuda M (2010) Involvement of the 3′-untranslated region of the brain-derived neurotrophic factor gene in activity-dependent mRNA stabilization. J Neurochem 115:1222–1233

Funakoshi H, Belluardo N, Arenas E, Yamamoto Y, Casabona A, Persson H, Ibáñez CF (1995) Muscle-derived neurotrophin-4 as an activity-dependent trophic signal for adult motor neurons. Science 268:1495–1499

Gall CM, Isackson PJ (1989) Limbic seizures increase neuronal production of messenger RNA for nerve growth factor. Science 245:758–761

Gogvadze E, Stukacheva E, Buzdin A, Sverdlov E (2009) Human-specific modulation of transcriptional activity provided by endogenous retroviral insertions. J Virol 83:6098–6105
Gomez-Pinilla F, Zhuang Y, Feng J, Ying Z, Fan G (2011) Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. Eur J Neurosci 33:383–390
Grimm J, Lu L, Hayashi T, Hope B, Su T, Shaham Y (2003) Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. J Neurosci 23:742–747
Guan J-S, Haggarty SJ, Giacometti E, Dannenberg J-H, Joseph N, Gao J, Nieland TJF, Zhou Y, Wang X, Mazitschek R et al (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459:55–60
Guo H, Ingolia NT, Weissman JS, Bartel DP (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466:835–840
Guo JU, Su Y, Zhong C, Ming G-L, Song H (2011) Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. Cell 145:423–434
Hall J, Thomas K, Everitt B (2000) Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. Nat Neurosci 3:533–535
Hara D, Fukuchi M, Miyashita T, Tabuchi A, Takasaki I, Naruse Y, Mori N, Kondo T, Tsuda M (2009) Remote control of activity-dependent BDNF gene promoter-I transcription mediated by REST/NRSF. Biochem Biophys Res Commun 384:506–511
Heinrich G, Pagtakhan C (2004) Both 5″ and 3″ flanks regulate Zebrafish brain-derived neurotrophic factor gene expression. BMC Neurosci 5:19
Hellman A, Chess A (2007) Gene body-specific methylation on the active X chromosome. Science 315:1141–1143
Hengerer B, Lindholm D, Heumann R, Rüther U, Wagner EF, Thoenen H (1990) Lesion-induced increase in nerve growth factor mRNA is mediated by c-fos. Proc Natl Acad Sci U S A 87:3899–3903
Heumann R, Lindholm D, Bandtlow C, Meyer M, Radeke MJ, Misko TP, Shooter E, Thoenen H (1987) Differential regulation of mRNA encoding nerve growth factor and its receptor in rat sciatic nerve during development, degeneration, and regeneration: role of macrophages. Proc Natl Acad Sci U S A 84:8735–8739
Hofer M, Pagliusi S, Hahn A, Leibrock J, Barde Y-A (1990) Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J 9:2459–2464
Hong EJ, McCord AE, Greenberg ME (2008) A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. Neuron 60:610–624
Huang Y, Myers SJ, Dingledine R (1999) Transcriptional repression by REST: recruitment of Sin3A and histone deacetylase to neuronal genes. Nat Neurosci 2:867–872
Huang Y, Doherty J, Dingledine R (2002) Altered histone acetylation at glutamate receptor 2 and brain-derived neurotrophic factor genes is an early event triggered by status epilepticus. J Neurosci 22:8422–8428
Ip NY, Ibáñez CF, Nye SH, McClain J, Jones PF, Gies DR, Belluscio L, Le Beau MM, Espinosa R, Squinto SP (1992) Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity. Proc Natl Acad Sci U S A 89:3060–3064
Isackson P, Huntsman M, Murray K, Gall C (1991) BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: temporal patterns of induction distinct from NGF. Neuron 6:937–948
Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y (2010) Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 466:1129–1133

Jiang X, Tian F, Du Y, Copeland N, Jenkins N, Tessarollo L, Wu X, Pan H, Hu X, Xu K et al (2008) BHLHB2 controls Bdnf promoter 4 activity and neuronal excitability. J Neurosci 28:1118–1130

Kaisho Y, Shintani A, Ono Y, Kato K, Igarashi K (1991) Regional expression of the nerve growth factor gene family in rat brain during development. Biochem Biophys Res Commun 174:379–385

Kaisho Y, Ohta H, Miyamoto M, Igarashi K (1999) Nerve growth factor promoter driven neurotrophin-3 overexpression in the mouse and the protective effect of transgene on age-related behavioral deficits. Neurosci Lett 277:181–184

Karpova NN, Rantamäki T, Di Lieto A, Lindemann L, Hoener MC, Castrén E (2010) Darkness reduces BDNF expression in the visual cortex and induces repressive chromatin remodeling at the BDNF gene in both hippocampus and visual cortex. Cell Mol Neurobiol 30:1117–1123

Katoh-Semba R, Kaisho Y, Shintani A, Nagahama M, Kato K (1996) Tissue distribution and immunocytochemical localization of neurotrophin-3 in the brain and peripheral tissues of rats. J Neurochem 66:330–337

Katoh-Semba R, Takeuchi IK, Semba R, Kato K (1997) Distribution of brain-derived neurotrophic factor in rats and its changes with development in the brain. J Neurochem 69:34–42

Katoh-Semba R, Takeuchi IK, Inaguma Y, Ito H, Kato K (1999) Brain-derived neurotrophic factor, nerve growth and neurotrophin-3 selected regions of the rat brain following kainic acid-induced seizure activity. Neurosci Res 35:19–29

Kawaja MD, Smithson LJ, Elliott J, Trinh G, Crotty A-M, Michalski B, Fahnestock M (2011) Nerve growth factor promoter activity revealed in mice expressing enhanced green fluorescent protein. J Comp Neurol 519:2522–2545

Kendall S, Yeo M, Henttu P, Tomlinson DR (2000) Alternative splicing of the neurotrophin-3 gene gives rise to different transcripts in a number of human and rat tissues. J Neurochem 75:41–47

Kim T-K, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbara-Haley K, Kuersten S et al (2010) Widespread transcription at neuronal activity-regulated enhancers. Nature 465(7295):182–187

Klose R, Bird A (2006) Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci 31:89–97

Kokaia Z, Gidö G, Ringstedt T, Bengzon J, Kokaia M, Siesjö BK, Persson H, Lindvall O (1993) Rapid increase of BDNF mRNA levels in cortical neurons following spreading depression: regulation by glutamatergic mechanisms independent of seizure activity. Brain Res Mol Brain Res 19:277–286

Kokaia Z, Metsis M, Kokaia M, Bengzon J, Elmer E, Smith M, Timmusk T, Siesjo B, Persson H, Lindvall O (1994) Brain insults in rats induce increased expression of the BDNF gene through differential use of multiple promoters. Eur J Neurosci 6:587–596

Konopka W, Kiryk A, Novak M, Herwerth M, Parkitna JR, Wawrzyniak M, Kowarsch A, Michaluk P, Dzwonek J, Arnsperger T et al (2010) MicroRNA loss enhances learning and memory in mice. J Neurosci 30:14835–14842

Koppel I, Aid-Pavlidis T, Jaanson K, Sepp M, Pruunsild P, Palm K, Timmusk T (2009) Tissue-specific and neural activity-regulated expression of human BDNF gene in BAC transgenic mice. BMC Neurosci 10:68

Koppel I, Aid-Pavlidis T, Jaanson K, Sepp M, Palm K, Timmusk T (2010) BAC transgenic mice reveal distal cis-regulatory elements governing BDNF gene expression. Genesis 48:214–219

Kumar A, Choi K, Renthal W, Tsankova N, Theobald D, Truong H, Russo S, LaPlant Q, Sasaki T, Whistler K et al (2005) Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 48:303–314

Kuzumaki N, Ikegami D, Tamura R, Hareyama N, Imai S, Narita M, Torigoe K, Niikura K, Takeshima H, Ando T et al (2011) Hippocampal epigenetic modification at the brain-derived neurotrophic factor gene induced by an enriched environment. Hippocampus 21:127–132

Lachner M, Jenuwein T (2002) The many faces of histone lysine methylation. Curr Opin Cell Biol 14:286–298

Lau AG, Irier HA, Gu J, Tian D, Ku L, Liu G, Xia M, Fritsch B, Zheng JQ, Dingledine R et al (2010) Distinct 3′UTRs differentially regulate activity-dependent translation of brain-derived neurotrophic factor (BDNF). Proc Natl Acad Sci U S A 107:15945–15950

Lauterborn JC, Tran TM, Isackson PJ, Gall CM (1993) Nerve growth factor mRNA is expressed by GABAergic neurons in rat hippocampus. Neuroreport 5:273–276

Lauterborn JC, Bizon JL, Tran TM, Gall CM (1995) NGF mRNA is expressed by GABAergic but not cholinergic neurons in rat basal forebrain. J Comp Neurol 360:454–462

Lauterborn J, Rivera S, Stinis C, Haynes V, Isackson P, Gall C (1996) Differential effects of protein synthesis inhibition on the activity-dependent expression of BDNF transcripts: evidence for immediate-early gene responses from specific promoters. J Neurosci 16:7428–7436

Leingärtner A, Lindholm D (1994) Two promoters direct transcription of the mouse NT-3 gene. Eur J Neurosci 6:1149–1159

Leingärtner A, Heisenberg CP, Kolbeck R, Thoenen H, Lindholm D (1994) Brain-derived neurotrophic factor increases neurotrophin-3 expression in cerebellar granule neurons. J Biol Chem 269:828–830

Lewis B, Shih I, Jones-Rhoades M, Bartel D, Burge C (2003) Prediction of mammalian microRNA targets. Cell 115:787–798

Li X, Jarvis E, Alvarez-Borda B, Lim D, Nottebohm F (2000) A relationship between behavior, neurotrophin expression, and new neuron survival. Proc Natl Acad Sci U S A 97:8584–8589

Li Z, Wu Y, Baraban JM (2008) The Translin/Trax RNA binding complex: clues to function in the nervous system. Biochim Biophys Acta 1779:479–485

Lin Y, Bloodgood B, Hauser J, Lapan A, Koon A, Kim T, Hu L, Malik A, Greenberg M (2008) Activity-dependent regulation of inhibitory synapse development by Npas4. Nature 455:1198–1204

Lindholm D, Heumann R, Meyer M, Thoenen H (1987) Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. Nature 330:658–659

Lindholm D, Heumann R, Hengerer B, Thoenen H (1988) Interleukin 1 increases stability and transcription of mRNA encoding nerve growth factor in cultured rat fibroblasts. J Biol Chem 263:16348–16351

Lindvall O, Ernfors P, Bengzon J, Kokaia Z, Smith M, Siesjo B, Persson H (1992) Differential regulation of mRNAs for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3 in the adult rat brain following cerebral ischemia and hypoglycemic coma. Proc Natl Acad Sci U S A 89:648–652

Lipsky R, Xu K, Zhu D, Kelly C, Terhakopian A, Novelli A, Marini A (2001) Nuclear factor kappaB is a critical determinant in N-methyl-D-aspartate receptor-mediated neuroprotection. J Neurochem 78:254–264

Lister R, Pelizzola M, Dowen R, Hawkins R, Hon G, Tonti-Filippini J, Nery J, Lee L, Ye Z, Ngo Q et al (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 462:315–322

Liu X, Ernfors P, Wu H, Jaenisch R (1995) Sensory but not motor neuron deficits in mice lacking NT4 and BDNF. Nature 375:238–241

Liu Q, Walther D, Drgon T, Polesskaya O, Lesnick T, Strain K, de Andrade M, Bower J, Maraganore D, Uhl G (2005) Human brain derived neurotrophic factor (BDNF) genes, splicing patterns, and assessments of associations with substance abuse and Parkinson's Disease. Am J Med Genet B Neuropsychiatr Genet 134:93–103

Liu Q, Lu L, Gong J, Shaham Y, Uhl G (2006) Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. Brain Res 1067:1–12

Lu B (2003) BDNF and activity-dependent synaptic modulation. Learn Mem 10:86–98

Lubin FD, Ren Y, Xu X, Anderson AE (2007) Nuclear factor-kappa B regulates seizure threshold and gene transcription following convulsant stimulation. J Neurochem 103:1381–1395

Lubin F, Roth T, Sweatt J (2008) Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. J Neurosci 28:10576–10586

Luco RF, Allo M, Schor IE, Kornblihtt AR, Misteli T (2011) Epigenetics in alternative pre-mRNA splicing. Cell 144:16–26

Lyons MR, Schwarz CM, West AE (2012) Members of the myocyte enhancer factor 2 transcription factor family differentially regulate Bdnf transcription in response to neuronal depolarization. J Neurosci 32:12780–12785

Ma D, Jang M, Guo J, Kitabatake Y, Chang M, Pow-Anpongkul N, Flavell R, Lu B, Ming G, Song H (2009) Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. Science 323:1074–1077

Maisonpierre PC, Belluscio L, Squinto S, Ip NY, Furth ME, Lindsay RM, Yancopoulos GD (1990a) Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. Science 247:1446–1451

Maisonpierre PC, Belluscio L, Friedman B, Alderson RF, Wiegand SJ, Furth ME, Lindsay RM, Yancopoulos GD (1990b) NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. Neuron 5:501–509

Maisonpierre P, Le Beau M, Espinosa R, Ip N, Belluscio L, Monte L, De S, Squinto S, Furth M, Yancopoulos G (1991) Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions and chromosomal localizations. Genomics 10:558–568

Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, Fan G, Sun Y (2003) DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science 302:890–893

Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, Johnson BE, Hong C, Nielsen C, Zhao Y et al (2010) Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature 466:253–257

McCauslin CS, Heath V, Colangelo AM, Malik R, Lee S, Mallei A, Mocchetti I, Johnson PF (2006) CAAT/enhancer-binding protein delta and cAMP-response element-binding protein mediate inducible expression of the nerve growth factor gene in the central nervous system. J Biol Chem 281:17681–17688

McDowell KA, Hutchinson AN, Wong-Goodrich SJE, Presby MM, Su D, Rodriguiz RM, Law KC, Wiliams CL, Wetsel WC, West AE (2010) Reduced cortical BDNF expression and aberrant memory in *Carf* knockout mice. J Neurosci 30:7453–7465

Medrihan L, Tantalaki E, Aramuni G, Sargsyan V, Dudanova I, Missler M, Zhang W (2008) Early defects of GABAergic synapses in the brain stem of a MeCP2 mouse model of Rett syndrome. J Neurophysiol 99:112–121

Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, Zhang X, Bernstein BE, Nusbaum C, Jaffe DB et al (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature 454:766–770

Mellios N, Huang H-S, Grigorenko A, Rogaev E, Akbarian S (2008) A set of differentially expressed miRNAs, including miR-30a-5p, act as post-transcriptional inhibitors of BDNF in prefrontal cortex. Hum Mol Genet 17:3030–3042

Metsis M (2001) Genes for neurotrophic factors and their receptors: structure and regulation. Cell Mol Life Sci 58:1014–1020

Metsis M, Timmusk T, Arenas E, Persson H (1993) Differential usage of multiple brain-derived neurotrophic factor promoters in the rat brain following neuronal activation. Proc Natl Acad Sci U S A 90:8802–8806

Michael GJ, Averill S, Shortland PJ, Yan Q, Priestley JV (1999) Axotomy results in major changes in BDNF expression by dorsal root ganglion cells: BDNF expression in large trkB and trkC cells, in pericellular baskets, and in projections to deep dorsal horn and dorsal column nuclei. Eur J Neurosci 11:3539–3551

Mocchetti I, Spiga G, Hayes VY, Isackson PJ, Colangelo A (1996) Glucocorticoids differentially increase nerve growth factor and basic fibroblast growth factor expression in the rat brain. J Neurosci 16:2141–2148

Modarresi F, Faghihi MA, Lopez-Toledano MA, Fatemi RP, Magistri M, Brothers SP, van der Brug MP, Wahlestedt C (2012) Inhibition of natural antisense transcripts in vivo results in gene-specific transcriptional upregulation. Nat Biotechnol 30:453–459

Mudo G, Jiang XH, Timmusk T, Bindoni M, Belluardo N (1996) Change in neurotrophins and their receptor mRNAs in the rat forebrain after status epilepticus induced by pilocarpine. Epilepsia 37:198–207

Muiños-Gimeno M, Espinosa-Parrilla Y, Guidi M, Kagerbauer B, Sipilä T, Maron E, Pettai K, Kananen L, Navinés R, Martín-Santos R et al (2011) Human microRNAs miR-22, miR-138-2, miR-148a, and miR-488 are associated with panic disorder and regulate several anxiety candidate genes and related pathways. Biol Psychiatry 69:526–533

Nan X, Ng H, Johnson C, Laherty C, Turner B, Eisenman R, Bird A (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393:386–389

Nanda S, Mack K (2000) Seizures and sensory stimulation result in different patterns of brain derived neurotrophic factor protein expression in the barrel cortex and hippocampus. Brain Res Mol Brain Res 78:1–14

Neeper S, Gomez-Pinilla F, Choi J, Cotman C (1996) Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. Brain Res 726:49–56

Nelson E, Kavalali E, Monteggia L (2006) MeCP2-dependent transcriptional repression regulates excitatory neurotransmission. Curr Biol 16:710–716

Nibuya M, Morinobu S, Duman R (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 15:7539–7547

Palm K, Belluardo N, Metsis M, Timmusk T (1998) Neuronal expression of zinc finger transcription factor REST/NRSF/XBR gene. J Neurosci 18:1280–1296

Pattabiraman P, Tropea D, Chiaruttini C, Tongiorgi E, Cattaneo A, Domenici L (2005) Neuronal activity regulates the developmental expression and subcellular localization of cortical BDNF mRNA isoforms in vivo. Mol Cell Neurosci 28:556–570

Patterson S, Grover L, Schwartzkroin P, Bothwell M (1992) Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. Neuron 9:1081–1088

Phillips HS, Hains JM, Laramee GR, Rosenthal A, Winslow JW (1990) Widespread expression of BDNF but not NT3 by target areas of basal forebrain cholinergic neurons. Science 250:290–294

Poo M-M (2001) Neurotrophins as synaptic modulators. Nat Rev Neurosci 2:24–32

Pruunsild P, Kazantseva A, Aid T, Palm K, Timmusk T (2007) Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters. Genomics 90:397–406

Pruunsild P, Sepp M, Orav E, Koppel I, Timmusk T (2011) Identification of cis-elements and transcription factors regulating neuronal activity-dependent transcription of human BDNF gene. J Neurosci 31:3295–3308

Raju CS, Fukuda N, López-Iglesias C, Göritz C, Visa N, Percipalle P (2011) In neurons, activity-dependent association of dendritically transported mRNA transcripts with the transacting factor CBF-A is mediated by A2RE/RTS elements. Mol Biol Cell 22:1864–1877

Ramos B, Valín A, Sun X, Gill G (2009) Sp4-dependent repression of neurotrophin-3 limits dendritic branching. Mol Cell Neurosci 42:152–159

Rattiner L, Davis M, French C, Ressler K (2004) Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. J Neurosci 24:4796–4806

Reik W (2007) Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 447:425–432

Rocamora N, Pascual M, Acsàdy L, de Lecea L, Freund TF, Soriano E (1996a) Expression of NGF and NT3 mRNAs in hippocampal interneurons innervated by the GABAergic septohippocampal pathway. J Neurosci 16:3991–4004

Rocamora N, Welker E, Pascual M, Soriano E (1996b) Upregulation of BDNF mRNA expression in the barrel cortex of adult mice after sensory stimulation. J Neurosci 16:4411–4419
Roh T-Y, Ngau WC, Cui K, Landsman D, Zhao K (2004) High-resolution genome-wide mapping of histone modifications. Nat Biotechnol 22:1013–1016
Roopra A, Sharling L, Wood IC, Briggs T, Bachfischer U, Paquette AJ, Buckley NJ (2000) Transcriptional repression by neuron-restrictive silencer factor is mediated via the Sin3-histone deacetylase complex. Mol Cell Biol 20:2147–2157
Rossi FM, Bozzi Y, Pizzorusso T, Maffei L (1999) Monocular deprivation decreases brain-derived neurotrophic factor immunoreactivity in the rat visual cortex. Neuroscience 90:363–368
Roth TL, Lubin FD, Funk AJ, Sweatt JD (2009) Lasting epigenetic influence of early-life adversity on the BDNF gene. Biol Psychiatry 65:760–769
Russo-Neustadt A, Beard R, Huang Y, Cotman C (2000) Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. Neuroscience 101:305–312
Sadri-Vakili G, Kumaresan V, Schmidt HD, Famous KR, Chawla P, Vassoler FM, Overland RP, Xia E, Bass CE, Terwilliger EF et al (2010) Cocaine-induced chromatin remodeling increases brain-derived neurotrophic factor transcription in the rat medial prefrontal cortex, which alters the reinforcing efficacy of cocaine. J Neurosci 30:11735–11744
Sakata K, Woo N, Martinowich K, Greene J, Schloesser R, Shen L, Lu B (2009) Critical role of promoter IV-driven BDNF transcription in GABAergic transmission and synaptic plasticity in the prefrontal cortex. Proc Natl Acad Sci U S A 106:5942–5947
Salin T, Timmusk T, Lendahl U, Metsis M (1997) Structural and functional characterization of the rat neurotrophin-4 gene. Mol Cell Neurosci 9:264–275
Scarisbrick IA, Isackson PJ, Windebank AJ (1999) Differential expression of brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 in the adult rat spinal cord: regulation by the glutamate receptor agonist kainic acid. J Neurosci 19:7757–7769
Schinder AF, Poo M (2000) The neurotrophin hypothesis for synaptic plasticity. Trends Neurosci 23:639–645
Schroeder FA, Penta KL, Matevossian A, Jones SR, Konradi C, Tapper AR, Akbarian S (2008) Drug-induced activation of dopamine D1 receptor signaling and inhibition of class I/II histone deacetylase induce chromatin remodeling in reward circuitry and modulate cocaine-related behaviors. Neuropsychopharmacology 33:2981–2992
Schuman EM (1999) mRNA trafficking and local protein synthesis at the synapse. Neuron 23:645–648
Selby MJ, Edwards R, Sharp F, Rutter WJ (1987) Mouse nerve growth factor gene: structure and expression. Mol Cell Biol 7:3057–3064
Shalizi A, Lehtinen M, Gaudillière B, Donovan N, Han J, Konishi Y, Bonni A (2003) Characterization of a neurotrophin signaling mechanism that mediates neuron survival in a temporally specific pattern. J Neurosci 23:7326–7336
Sherer TB, Neff PS, Tuttle JB (1998) Increased nerve growth factor mRNA stability may underlie elevated nerve growth factor secretion from hypertensive vascular smooth muscle cells. Brain Res Mol Brain Res 62:167–174
Shi Y (2007) Histone lysine demethylases: emerging roles in development, physiology and disease. Nat Rev Genet 8:829–833
Shi X, Hong T, Walter K, Ewalt M, Michishita E, Hung T, Carney D, Pena P, Lan F, Kaadige M et al (2006) ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. Nature 442:96–99
Shieh P, Hu S-C, Bobb K, Timmusk T, Ghosh A (1998) Identification of a signaling pathway involved in calcium regulation of BDNF expression. Neuron 20:727–740
Smith MA, Makino S, Kvetnansky R, Post RM (1995a) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 15:1768–1777

Smith MA, Makino S, Kim SY, Kvetnansky R (1995b) Stress increases brain-derived neurotropic factor messenger ribonucleic acid in the hypothalamus and pituitary. Endocrinology 136:3743–3750

Sofroniew MV, Howe CL, Mobley WC (2001) Nerve growth factor signaling, neuroprotection, and neural repair. Annu Rev Neurosci 24:1217–1281

Spranger M, Lindholm D, Bandtlow C, Heumann R, Gnahn H, Näher-Noé M, Thoenen H (1990) Regulation of nerve growth factor (NGF) synthesis in the rat central nervous system: comparison between the effects of interleukin-1 and various growth factors in astrocyte cultures and in vivo. Eur J Neurosci 2:69–76

Sutton MA, Taylor AM, Ito HT, Pham A, Schuman EM (2007) Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. Neuron 55:648–661

Tabuchi A, Sakaya H, Kisukeda T, Fushiki H, Tsuda M (2002) Involvement of an upstream stimulatory factor as well as cAMP-responsive element-binding protein in the activation of brain-derived neurotrophic factor gene promoter I. J Biol Chem 277:35920–35931

Tang B, Wang M, Wise BC (1997) Nerve growth factor mRNA stability is controlled by a cis-acting instability determinant in the 3′-untranslated region. Brain Res Mol Brain Res 46:118–126

Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME (1998) Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron 20:709–726

Tao X, West AE, Chen WG, Corfas G, Greenberg ME (2002) A calcium-responsive transcription factor, CaRF, that regulates neuronal activity-dependent expression of BDNF. Neuron 33:383–395

Timmusk T, Palm K, Metsis M, Reintam T, Paalme V, Saarma M, Persson H (1993a) Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron 10:475–489

Timmusk T, Belluardo N, Metsis M, Persson H (1993b) Widespread and developmentally regulated expression of neurotrophin-4 mRNA in rat brain and peripheral tissues. Eur J Neurosci 5:605–613

Timmusk T, Persson H, Metsis M (1994a) Analysis of transcriptional initiation and translatability of brain-derived neurotrophic factor mRNAs in the rat brain. Neurosci Lett 177:27–31

Timmusk T, Belluardo N, Persson H, Metsis M (1994b) Developmental regulation of brain-derived neurotrophic factor messenger RNAs transcribed from different promoters in the rat brain. Neuroscience 60:287–291

Timmusk T, Lendahl U, Funakoshi H, Arenas E, Persson H, Metsis M (1995) Identification of brain-derived neurotrophic factor promoter regions mediating tissue-specific, axotomy-, and neuronal activity-induced expression in transgenic mice. J Cell Biol 128:185–199

Timmusk T, Palm K, Lendahl U, Metsis M (1999) Brain-derived neurotrophic factor expression in vivo is under the control of neuron-restrictive silencer element. J Biol Chem 274:1078–1084

Tongiorgi E, Righi M, Cattaneo A (1997) Activity-dependent dendritic targeting of BDNF and TrkB mRNAs in hippocampal neurons. J Neurosci 17:9492–9505

Tongiorgi E, Armellin M, Giulianini PG, Bregola G, Zucchini S, Paradiso B, Steward O, Cattaneo A, Simonato M (2004) Brain-derived neurotrophic factor mRNA and protein are targeted to discrete dendritic laminas by events that trigger epileptogenesis. J Neurosci 24:6842–6852

Tropea D, Giacometti E, Wilson N, Beard C, McCurry C, Fu D, Flannery R, Jaenisch R, Sur M (2009) Partial reversal of Rett syndrome-like symptoms in MeCP2 mutant mice. Proc Natl Acad Sci U S A 106:2029–2034

Tsankova N, Kumar A, Nestler E (2004) Histone modifications at gene promoter regions in rat hippocampus after acute and chronic electroconvulsive seizures. J Neurosci 24:5603–5610

Tsankova N, Berton O, Renthal W, Kumar A, Neve R, Nestler E (2006) Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 9:519–525

Vashishta A, Habas A, Pruunsild P, Zheng J-J, Timmusk T, Hetman M (2009) Nuclear factor of activated T-cells isoform c4 (NFATc4/NFAT3) as a mediator of antiapoptotic transcription in NMDA receptor-stimulated cortical neurons. J Neurosci 29:15331–15340

Webster MJ, Herman MM, Kleinman JE, Shannon Weickert C (2006) BDNF and trkB mRNA expression in the hippocampus and temporal cortex during the human lifespan. Gene Expr Patterns 6:941–951

West AE (2008) Activity-dependent regulation of brain-derived neurotrophic factor transcription. In: Dukek S (ed) Transcriptional regulation by neuronal activity. Springer, New York, pp 155–174

Wu H, Coskun V, Tao J, Xie W, Ge W, Yoshikawa K, Li E, Zhang Y, Sun YE (2010) Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. Science 329:444–448

Wu Y-C, Williamson R, Li Z, Vicario A, Xu J, Kasai M, Chern Y, Tongiorgi E, Baraban JM (2011) Dendritic trafficking of brain-derived neurotrophic factor mRNA: regulation by translin-dependent and -independent mechanisms. J Neurochem 116:1112–1121

Yamamoto M, Sobue G, Yamamoto K, Terao S, Mitsuma T (1996) Expression of mRNAs for neurotrophic factors (NGF, BDNF, NT-3, and GDNF) and their receptors (p75NGFR, trkA, trkB, and trkC) in the adult human peripheral nervous system and nonneural tissues. Neurochem Res 21:929–938

Young D, Lawlor PA, Leone P, Dragunow M, During MJ (1999) Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. Nat Med 5:448–453

Zafra F, Hengerer B, Leibrock J, Thoenen H, Lindholm D (1990) Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. EMBO J 9:3545–3550

Zheng M, Heinrich G (1988) Structural and functional analysis of the promoter region of the nerve growth factor gene. Brain Res 427:133–140

Zhou X, Long JM, Geyer MA, Masliah E, Kelsoe JR, Wynshaw-Boris A, Chien KR (2005) Reduced expression of the Sp4 gene in mice causes deficits in sensorimotor gating and memory associated with hippocampal vacuolization. Mol Psychiatry 10:393–406

Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, Cataudella T, Leavitt B, Hayden M, Timmusk T et al (2003) Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nat Genet 35:76–83

Part II

Neurotrophin Receptors

Trk Receptors

Katrin Deinhardt and Moses V. Chao

Abstract

The tropomyosin-related tyrosine kinase (Trk) receptors were initially described as a family of growth factor receptors required for neuronal survival. They have since been shown to influence many aspects of neuronal development and function, including differentiation, outgrowth, and synaptic plasticity. This chapter will give an overview on the biology of Trk receptors within the nervous system. The structure and downstream signaling pathways of the full-length receptors will be described, as well as the biological functions of their truncated isoforms. Finally, the role of Trk receptors in the nervous system in health and disease will be discussed.

Keywords

Trk receptors • Neurotrophins • Survival • Neurite outgrowth • Apoptosis • Signaling endosome

1 Introduction

Widespread programmed cell death occurs during the formation of the vertebrate nervous system. In this way, correct neuronal numbers and appropriate target innervation are ensured during nervous system development. The neurotrophic hypothesis proposes that during development, neurons approaching the same final target compete for limited amounts of target-derived trophic factors, which in turn accounts for selective cell survival (Levi-Montalcini 1987b). In this way, the

K. Deinhardt
Centre for Biological Sciences, University of Southampton, Southampton 5017 1BJ, UK

M.V. Chao (✉)
Molecular Neurobiology Program, Skirball Institute of Biomolecular Medicine, New York University School of Medicine, 540 First Avenue, New York, NY 10016, USA
e-mail: moses.chao@nyumc.org

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_5,

nervous system shapes itself to maintain only the appropriate connections. This hypothesis implies that (1) the efficacy of neuronal survival depends upon the amounts of trophic factors produced and (2) specific receptor expression in distinct cell populations confers neuronal responsiveness.

Within the peripheral nervous system, neurotrophins and their cognate Trk receptors fit well with the neurotrophic hypothesis, as many peripheral neuronal subpopulations exhibit a predominant dependence on a specific neurotrophin during the period of naturally occurring cell death. However, in the central nervous system, the overlapping expression of multiple neurotrophin receptors and their cognate ligands allows for the creation of diverse connectivity, which extends well into adulthood. Moreover, the activities of neurotrophin-Trk signaling extend well beyond neuronal survival and include molecular mechanisms underlying neuronal growth and arborization, as well as the strengthening of synaptic transmission. Importantly, Trk signaling is not confined to the nervous system, but is instead increasingly recognized in non-neuronal tissues such as the vasculature (Kermani and Hempstead 2007).

This chapter will focus upon several aspects of Trk receptor biology within the nervous system. We will discuss the receptor structure, signal transduction, and retrograde transport and discuss the impact of Trk signaling on the nervous system in health and disease.

2 Structure

The Trk family of tyrosine kinase receptors comprises three single-pass type I transmembrane proteins. Their extracellular domains are heavily glycosylated and each contains three leucine-rich repeats flanked by two cysteine repeats and immunoglobulin-C2 (Ig) domains proximal to the transmembrane region. Intracellularly, Trk receptors possess a tyrosine kinase domain.

Trks interact with their ligands using the second of their Ig domains (Ultsch et al. 1999; Wiesmann et al. 1999), and the receptor expression profile confers ligand responsiveness to a cell. In addition, differential splicing of Trk mRNA results in the generation of Trk isoforms with different extracellular domains, affecting ligand binding. For example, a short insert in the juxtamembrane region of TrkA increases affinity for NT-3 without affecting nerve growth factor (NGF) binding (Clary and Reichardt 1994), and in TrkB, the presence of a similar insert allows for activation through NT-3 and NT-4, while lack of this insert confers specificity for brain-derived neurotrophic factor (BDNF) (Boeshore et al. 1999; Strohmaier et al. 1996). Other Trk isoforms lacking large parts of the intracellular domain including the tyrosine kinase domain ("truncated Trks") are discussed in more detail below (see also Sect. 8).

Ligand binding to full-length Trk receptors results in receptor dimerization and subsequent activation in trans of the receptor. Three tyrosine residues are found within the autoregulatory loop of the Trk kinase domain, and phosphorylation of these sites further activates the kinase, thereby initiating downstream signaling pathways.

3 Ligands

The ligands for the Trk receptors are a family of basic growth factors called neurotrophins. They comprise NGF, BDNF, and neurotrophins (NT) 3 and 4, which bind selectively and with affinities of 10^{-9}–10^{-10} M to their respective Trk receptors, TrkA, TrkB, and TrkC. NGF binds most specifically to TrkA, BDNF and NT-4 to TrkB, and NT-3 to TrkC. The p75 can bind to all four neurotrophins and additionally regulate the affinity of Trk receptors for individual neurotrophins (Bothwell 1995; Chao and Hempstead 1995). Although Trk and p75 receptors do not appear to bind directly to each other, there is evidence that complexes form between these receptors. As a result, p75 can increase ligand affinity and selectivity for Trk receptors. For example, an excess of p75 over TrkA increases TrkA's affinity for NGF to 10^{-11} M. Moreover, the presence of p75 restricts TrkA signaling to NGF, not NT-3 (Benedetti et al. 1993), and increases TrkB specificity for BDNF over NT-3 and NT-4 (Bibel et al. 1999).

All neurotrophins are initially synthesized as proneurotrophins, which are then cleaved by proteases either intra- or extracellularly to generate the mature ligand. The prodomain is essential for the correct folding of the mature ligand as well as for its targeting to the secretory pathway. In addition, proneurotrophins themselves are active signaling molecules, which have opposing effects compared to their mature counterparts (Lu et al. 2005) and bind with high affinity to the p75 receptor. The roles of p75 and proneurotrophins are discussed in more detail in chapter "Deciphering Proneurotrophin Actions".

4 Signaling

The signaling pathways activated by Trk receptors impact on many diverse neuronal functions, including cell survival and differentiation, axonal and dendritic growth and arborization, synapse formation, and synaptic plasticity. Much of the groundwork describing Trk signaling pathways was initially performed in rat adrenal pheochromocytoma (PC12) cells, which express TrkA and p75 and are used as a model cell line for sympathetic neurons. However, these cells differ from primary neurons in many aspects, such as lack of axonal and dendritic specification and continuing cell cycling. Therefore, many studies investigating neurotrophin signaling focused on NGF and TrkA. These results have been extrapolated to TrkB and TrkC signaling. However, more recently Trk signaling has been studied in a large variety of primary neurons as well as outside the nervous system, and it has become clear that while many pathways are indeed shared between the individual Trk receptors, others are activated in a Trk- or cell type-specific manner.

The mechanism of Trk signaling involves phosphorylation of specific tyrosine residues upon neurotrophin binding. These phosphorylated tyrosine sites mediate signaling by creating docking sites for effector proteins that initiate the activation of intracellular signaling pathways. The Y490 and Y785 tyrosine residues in human TrkA receptor, and their corresponding residues in TrkB and TrkC, serve as the

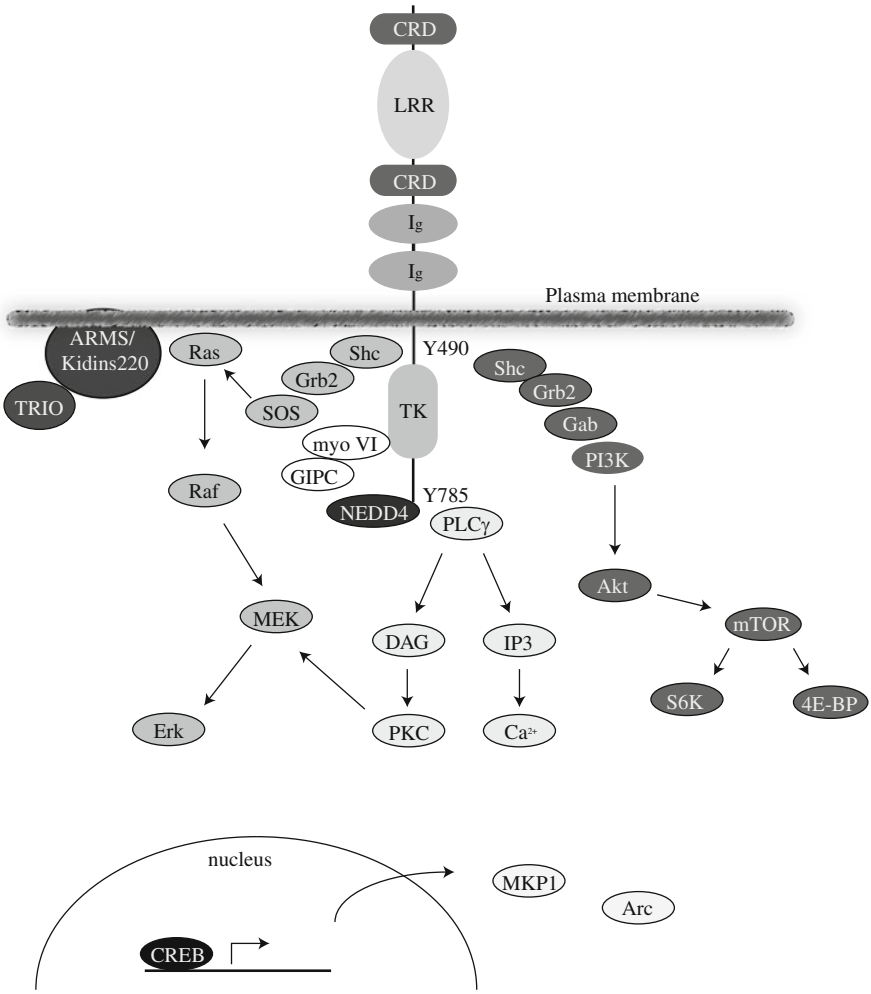

Fig. 1 Schematic of signaling pathways downstream of Trk tyrosine kinase receptors. Each Trk receptor undergoes ligand-dependent dimerization that results in the recruitment of multiple cytoplasmic proteins, which in turn increase the activities of PLCγ, PI3K, and Erk

main docking sites to initiate downstream signaling pathways such as the Shc-extracellular signal-regulated kinase (ERK) or phospholipase C-γ (PLC-γ) pathways, respectively. The Y670, Y674, and Y675 residues located within the tyrosine kinase domain can also recruit adaptor proteins after phosphorylation, including the Grb2 and SH2B adaptor proteins. Canonical Trk receptor signaling has been reviewed extensively [e.g., see Arevalo and Wu (2006), Huang and Reichardt (2003), Reichardt (2006)]. Below, we describe in more detail the three most studied pathways downstream of Trk receptor activation (see also Fig. 1).

4.1 PLC-γ

Phosphorylation of Trk at the most C-terminal tyrosine, Y785, leads to recruitment and activation of PLC-γ, which hydrolyzes phosphatidylinositol(4, 5)bisphosphate ($PI(4,5)P_2$) into diacylglycerol (DAG) and inositol tris-phosphate (IP_3). IP_3 leads to release of intracellular Ca^{2+}, which in turn activates Ca^{2+}-dependent enzymes such as Ca^{2+}-calmodulin-regulated protein kinases (CaM kinases) and the phosphatase calcineurin. Additionally, the release of Ca^{2+} and the production of DAG activate protein kinase C (PKC), which subsequently stimulates Erk signaling via Raf, as well as the capsaicin VR1 channel (Chuang et al. 2001) and the transient receptor potential channel (TRPC), which contributes to the BDNF-induced rise of Ca^{2+} at growth cones and synapses. Other activities that are affected include the formation of TrkB-postsynaptic density 95 (PSD95) complexes at synapses and cAMP response element binding protein (CREB)-dependent transcription. PLC-γ signaling in response to both NGF and BDNF has been implicated in chemoattraction of axonal growth cones, and prolonged activation in response to an NGF pulse induces the transcription of a sodium channel. Furthermore, mice harboring a targeted mutation at the TrkB PLC-γ docking site, Y816 (Minichiello et al. 1999), have impaired hippocampal long-term potentiation (LTP), along with impaired induction of CREB and CaM kinase signaling. However, there are potential contributions of other proteins, such as ARMS/Kidins220 scaffold protein, which is phosphorylated by Trk and influences LTP (Wu et al. 2010).

4.2 PI3K-Akt

Phosphorylation of Trk at the tyrosine residue closest to the transmembrane domain, Y490 in TrkA or Y515 in TrkB, creates a Shc binding site. This in turn leads to activation of phosphatidylinositol 3-kinase (PI3K) via Grb2 and Gab1 and to phosphorylation of inositol phospholipids at the 3′ position resulting in a change of the local membrane composition. As a consequence, Akt translocates to the plasma membrane and becomes activated. Akt activity results in increased protein translation via the mammalian target of rapamycin (mTOR)-p70S6 kinase and 4E-BP1 pathways and in enhancing axonal growth through phosphorylation and inactivation of GSK-3β. Additionally, activated Akt promotes neuronal survival by inhibiting a forkhead transcription factor, FKHRL1, which regulates expression of pro-apoptotic genes, by phosphorylating and therefore inhibiting the pro-apoptotic protein Bad, and by phosphorylating the inhibitor of the NFκB pro-survival pathway, IκB, and thus promoting its degradation.

4.3 Erk

In addition to PI3K-Akt signaling, the creation of a Shc binding site at Y490 initiates downstream Ras-extracellular signal-regulated kinase (Erk) signaling.

Recruitment of a complex of growth factor receptor bound protein 2 (Grb2) and the Ras activator son of sevenless (SOS) stimulates activation of Ras and downstream, transient activation of the c-Raf/MEK/Erk cascade. Prolonged Erk activation is also initiated at the Y490 site, but requires the ARMS/Kidins220 protein, which recruits Crk, another adaptor protein. Binding to Crk activates the exchange factor C3G and thus initiates Rap1/Raf-dependent MEK/Erk signaling (Arevalo et al. 2004). Ultimately, Erk signaling may lead to local axonal growth as well as to the initiation of CREB-mediated transcriptional events.

5 Alternatives to Ligand Binding: Transactivation of Trk Receptors

In addition to direct activation through neurotrophins, Trk receptors are, similar to EGF receptors, also transactivated intracellularly using alternative, neurotrophin-independent pathways. The ability to transactivate Trks was first demonstrated for adenosine and pituitary adenylate cyclase-activating polypeptide (PACAP) signaling through G-protein-coupled receptors (GPCRs), a mechanism requiring Src family kinases (SFK) and intracellular calcium (Lee and Chao 2001; Lee et al. 2002). In contrast to neurotrophin-mediated direct activation, transactivation is a relatively slow process that happens within hours, not minutes, and is thought to occur on intracellular membranes, primarily at the Golgi (Rajagopal et al. 2004). Ligand binding to the low-density lipoprotein receptor LRP1 also transactivates Trks in an SFK-dependent manner and is required for LRP-dependent neurite outgrowth in PC12 cells (Shi et al. 2009).

Transactivation of Trk receptors is not restricted to in vitro paradigms, as recent studies demonstrated that transactivation of Trk occurs in embryonic cortical neurons by EGF (Puehringer et al. 2013). Also, both glucocorticoids (Jeanneteau et al. 2008) and zinc (Huang et al. 2008) are able to transactivate Trk receptors in vivo. Indeed, zinc-mediated transactivation of TrkB affects synaptic transmission by modulating in mossy fiber LTP.

6 Membrane Trafficking

Activation of Trk receptors by neurotrophins leads to the endocytosis of the receptor–ligand complexes via clathrin-mediated endocytosis (Grimes et al. 1996; Zheng et al. 2008). Internalized receptors continue to signal either locally or are transported over long distances to relay a signal from or to distant parts of the cell. Eventually, the endocytosed receptors will be either degraded or recycled back to the plasma membrane.

6.1 Receptor Recycling and Degradation

The decision between receptor recycling or degradation following stimulation and internalization determines the responsiveness of the cell to ligand. Additionally, post-endocytic sorting of Trks controls the strength and duration of intracellular receptor signaling. In general, Trk receptors can either undergo recycling or degradation or enter the retrograde axonal transport pathway to carry trophic signals over long distances (see Sect. 6.2). However, there are differences between the individual Trk receptors. A detailed study in PC12 cells revealed that a juxtamembrane motif in TrkA biases this receptor for rapid recycling and delays degradation. In contrast, TrkB receptors, which lack this sequence, are primarily sorted into the degradative route (Chen et al. 2005; Sommerfeld et al. 2000). Accordingly, the biological response is different, with NGF–TrkA signaling leading to prolonged downstream signaling compared to BDNF–TrkB.

6.2 Retrograde Axonal Transport

In the peripheral nervous system, neurotrophins are released by the target tissue and bind to their cognate Trk receptors at the nerve terminals. Here, they are internalized into membranous vesicles, which are then transported retrogradely along the axon to the cell body to convey the survival signal. These Trk-containing vesicles have been termed "signaling endosomes" (Grimes et al. 1996). Experiments using compartmented in vitro systems, where the distal axon is isolated from the somatodendritic compartment and can therefore be selectively exposed to ligands, have demonstrated that distal stimulation of Trk receptors with neurotrophins can indeed lead to a nuclear response, and this process requires the internalization of the ligand–receptor complex. For example, the NGF–TrkA complex can signal to CREB this way to mediate neuronal survival (Riccio et al. 1997, 1999).

Measurements of ^{125}I-NGF transport from distal axons to the cell body in mice indicated a rate from 3 to 10 mm/h in vivo (Stockel et al. 1975). Later, in vitro assays directly visualizing retrograde axonal transport of fluorescently labeled NGF, BDNF, or TrkB in cultured dorsal root ganglia and motor neurons confirmed these transport rates and furthermore identified proteins that participate in this process, such as the molecular motor dynein (Heerssen et al. 2004). In addition, purification of signaling endosomes from sciatic nerve and in vitro neuronal cultures identified the endocytic Rab GTPases Rab 5 and 7 (Deinhardt et al. 2006; Delcroix et al. 2003) as well as the actin modulators Rac and cofilin (Harrington et al. 2011) as essential components for the trafficking of Trk-containing signaling endosomes along the retrograde transport route. These experiments also provided insights into the nature of signals that are relayed from the terminal to the soma (Perlson et al. 2009). While these studies have begun to shed light on the complex process of axonal retrograde Trk trafficking and signaling in peripheral neurons, much less is known about axonal transport of Trk receptors in

the brain, where neurons do not necessarily depend on target-derived neurotrophins for their survival.

7 Effects of Trk Signaling on the Nervous System

Individual Trk receptors are expressed in separate subsets of neurons. For example, TrkB is predominantly expressed within the central nervous system, while both TrkA and TrkC are largely found on peripheral neuronal populations. Accordingly, the analysis of Trk receptor knockout mice revealed limited overlap between the phenotypes of different Trk-deficient mice (Snider 1994). For example, TrkA null animals have normal motor function, but display severe sensory and sympathetic neuropathies and die within 1 month of birth. This is accompanied by a profound loss in superior cervical, dorsal root, and trigeminal ganglion neurons, but TrkA null mice show very limited defects within the central nervous system (Smeyne et al. 1994).

TrkC null animals appear largely normal at birth, but display subsequent growth defects, and mostly die by 3 weeks of age. During postnatal development, these animals develop abnormal postures and movements but can sense pain, suggesting that proprioception is specifically affected. In these mice, motor neuron afferents are decreased and a population of dorsal root ganglia neurons is lost, while the central nervous system appears grossly normal (Klein et al. 1994).

Animals deficient for TrkB in contrast fail to feed and die within hours after birth. These animals lack populations of motor neurons as well as dorsal root and trigeminal ganglia neurons. Interestingly, despite the broad expression of TrkB throughout the central nervous system there is no profound neuronal loss within the brain of TrkB null animals (Klein et al. 1993) or even within the brains of TrkB; TrkC double knockout mice (Silos-Santiago et al. 1997). These findings highlight the profound difference between central and peripheral neurons with regard to their dependence on trophic factors for survival.

Expression of Trk receptors is not just restricted to the nervous system, and accordingly, Trk null animals have additional severe defects, for example, in the cardio vasculature (Kermani and Hempstead 2007). TrkB is expressed in cardiac endothelial cells, and TrkB null animals show increased apoptosis of these cells and a decrease in intramyocardial blood vessels (Wagner et al. 2005). TrkC expression was found in developing cardiomyocytes, and consequently TrkC null animals have severe cardiac deficiencies, such as atrial and ventricular septal defects and valvular defects (Tessarollo et al. 1997). The potential role of TrkA in cardiac and vascular development is less well studied. However, restoring TrkA expression specifically in the nervous system of TrkA null animals showed that the resulting animals are viable and grossly normal, with minor immune defects (Coppola et al. 2004). This argues against a prominent role of TrkA signaling in the formation and maintenance of a healthy vasculature.

7.1 Neuronal Survival

As described above, individual Trk receptors mediate the survival of specific subpopulations of peripheral neurons, as their absence results in loss of defined subsets of cells. An underlying molecular mechanism has been described for NGF–TrkA signaling in superior cervical ganglion neurons. Here, Trk receptors are activated by target tissue-derived neurotrophins and thus relay a retrograde signal along the axon to the neuronal soma, which triggers the PI3 kinase–Akt pathway and CREB activation, thereby resulting cell survival (Riccio et al. 1999). Interestingly, within the same system NT-3, which also activates TrkA, cannot substitute for NGF in supporting survival from the distal axon (Kuruvilla et al. 2004), suggesting that not all ways of Trk receptor activation are equal.

Withdrawal of growth factors in compartmented in vitro cultures of superior cervical ganglion neurons showed that in addition to the absence of a survival signal, a negative signal is generated and transmitted along the axon to the cell body, contributing to the cell death response (Mok et al. 2009). Along these lines, a recent study suggested that TrkA and TrkC but not TrkB act as so-called dependence receptors in vitro and in vivo, which not only require ligand-dependent activation to promote neuronal survival, but can also actively induce neuronal death in absence of sufficient ligand (Nikoletopoulou et al. 2010). These observations may explain in part why neurons of the central nervous system, which mainly express TrkB, are less sensitive to apoptosis following lack of neurotrophic support.

7.2 Morphological Effects

In addition to promoting neuronal survival, neurotrophin-Trk signaling enhances neuronal outgrowth and arborization in a wide range of different neuronal subtypes. Indeed, the TrkA ligand NGF was initially described as a soluble factor promoting the axon outgrowth from chick sensory ganglia explants (Levi-Montalcini 1987a).

Within the central nervous system, where neurons are not dependent on neurotrophin-Trk signaling for their survival, enhancing neuronal growth and synaptic strengthening (see Sect. 7.3) appear to be the primary functions of Trk signaling. Accordingly, interfering with BDNF–TrkB signaling leads to axonal and dendritic outgrowth and arborization defects in multiple animal models ranging from frog to mouse [e.g., see Chen et al. (2006), Hu et al. (2005)]. Molecular mechanisms that lead to Trk-dependent neurite growth and branching include signaling via the scaffolding protein ARMS/Kidins220 (Wu et al. 2009), ubiquitination and downregulation of a RhoA activator (Lin et al. 2011), and induction of a MAP kinase phosphatase to regulate microtubule dynamics (Jeanneteau et al. 2010). In addition to axonal and dendritic growth and arborization, BDNF–TrkB signaling also has a well-established role in dendritic spine formation and therefore synapse development (see Sect. 7.3).

During the development of sympathetic neurons, TrkA mediates both the initial axon extension toward the target tissue and then terminal branching and synaptic innervation once the target is reached. The switch between extension to branching and innervation is marked by a change in ligands from NT-3 to NGF-dependent TrkA signaling (Kuruvilla et al. 2004), thereby demonstrating Trk receptors can respond differentially, depending on the stimulus provided.

7.3 Synaptic Plasticity

The role of neurotrophins in modulating synaptic transmission is best described for BDNF–TrkB signaling. BDNF–TrkB not only have a well-established role in promoting dendritic spine formation, and therefore providing a structural basis for synapse formation, but also enhance synaptic transmission in paradigms of LTP (Cohen-Cory et al. 2010; Minichiello 2009). Indeed, blocking BDNF-dependent TrkB activation leads to a decrease in hippocampal LTP, in both the early and late response. Several of the signaling cascades triggered by BDNF–TrkB signaling are essential in LTP maintenance, such as the Erk and Ca^{2+}/ calmodulin pathways, therefore providing a molecular mechanism of how BDNF–TrkB signaling may influence synaptic strength. In addition, activation of TrkB enhances glutamate release and synaptic transmission in a myosin6/ GIPC-dependent manner (Yano et al. 2006). Conversely, defects in postsynaptic events during synaptic transmission and plasticity are observed in mouse models with reduced BDNF secretion (Ninan et al. 2010; Pattwell et al. 2012). Hence, BDNF is capable of regulating both presynaptic and postsynaptic events in central neurons (Manabe 2002). These effects are not without behavioral consequences, as these mice display increased anxiety and decreased responsiveness to antidepressant treatment (Bath et al. 2012; Chen et al. 2006; Yu et al. 2012).

8 Truncated Trk Receptors

In addition to the full-length receptor tyrosine kinases, truncated isoforms of Trk receptors exist, which lack the intracellular kinase domain. These shorter isoforms are expressed at high levels throughout the mature nervous system. However, compared to their full-length counterparts, relatively little is known about the biological function of these truncated isoforms. Initially it was thought that the truncated Trk receptors act as dominant negatives, which sequester free neurotrophin ligands away from the active full-length receptors. However, more recently it has become clear that these truncated versions are not just neurotrophin sinks, but are also actively signaling molecules (Fenner 2012) (see also Fig. 2). For example, binding of NT-3 to truncated TrkC leads to recruitment of the scaffolding protein tamalin, which results in activation of Arf6 and the small Rho family GTPase Rac1 and ultimately membrane ruffling (Esteban et al. 2006). Truncated TrkB on the other hand interacts with a Rho GDP dissociation inhibitor (GDI), and

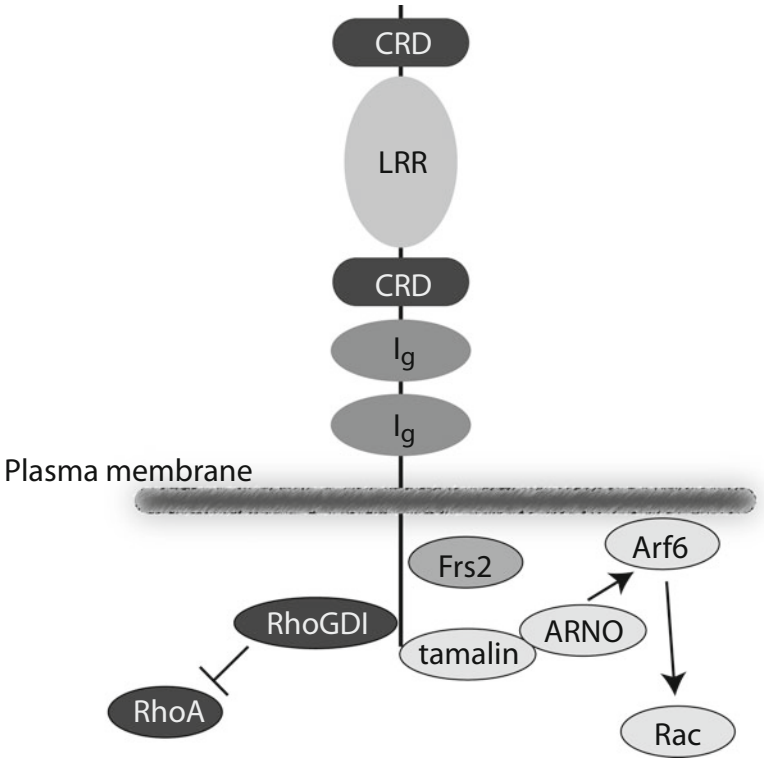

Fig. 2 Active signaling downstream of truncated Trk receptors. Truncated Trk receptors lack the tyrosine kinase domain of their full-length counterparts, but are nevertheless competent to recruit cytoplasmic proteins and initiate signaling cascades following ligand-dependent dimerization

binding of BDNF to truncated TrkB leads to the release of the Rho-GDI and thus to the inhibition of Rho (Ohira et al. 2005). Analysis of mice lacking selectively the truncated form of TrkB revealed a decrease in dendritic complexity specifically within the amygdala, while the dentate gyrus area of the hippocampus was not affected. This defect in neuronal morphology was associated with an increase in anxiety (Carim-Todd et al. 2009). In addition, mice lacking truncated TrkB display increased neuromuscular function (Dorsey et al. 2012).

9 Trk Signaling in Disease

Neurotrophic factors have been proposed as a treatment for Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, and peripheral neuropathy. Considerable evidence in rodents and primates has shown the efficacy of neurotrophins, such as NGF and BDNF, to prevent death of neurons; improve cell signaling; restore learning and memory, and prevent age-related

cognitive decline. Neurotrophins carry out their trophic functions through Trk receptor signaling (see Sect. 4). However, many clinical trials with NGF and BDNF have met with disappointing results, in part due to difficulties of delivery and uncertain pharmacokinetics (Thoenen and Sendtner 2002). Neurotrophic factors are large, sticky proteins that do not diffuse well into tissues and do not readily cross the blood–brain barrier. The problems in managing the dose and pharmacokinetics of these proteins have hindered the application of neurotrophic factors as a therapeutic intervention for many aging and neurodegenerative diseases.

An extensive amount of preclinical research in the past 25 years indicates that neurotrophic factor-based therapies can reverse deficits in learning and memory in disorders such as Alzheimer's disease (Nagahara et al. 2009; Tuszynski 2007) and promote neuronal regeneration (Lu and Tuszynski 2008). Indeed, activation of Trk receptors results in neuroprotective effects upon cortical, hippocampal, striatal, basal forebrain cholinergic and motor neurons after nerve injury (Lee and Chao 2001; Rajagopal et al. 2004). These observations are significant since cholinergic neurons in the basal forebrain degenerate in Alzheimer's disease; motor neurons undergo cell death in ALS; and striatal neurons in Parkinson's and Huntington's diseases. Activation of TrkB receptors prevents motor neurons from cell death after injury. In addition, there is evidence to support a causal role of BDNF in Huntington's disease (Zuccato and Cattaneo 2009).

Activation of Trk receptors results in increases in Akt, CREB, and ERK activities, as well as phosphoinositide lipid phosphorylation and activation of GTPases, such as Ras and Rap1, to promote neuronal cell survival and differentiation (Chao 2003) (also see Sect. 4). A unique substrate of Trk receptors is the ARMS/Kidins protein, which is rapidly phosphorylated by neurotrophin treatment. Indeed, a deficit in the levels of ARMS protein results in age-dependent neurodegeneration in the entorhinal cortex accompanied by impairments of spatial memory that mimic Alzheimer's disease (Duffy et al. 2011).

As described above, low-molecular-weight compounds are capable of activating Trk receptors, through receptor transactivation (Domeniconi and Chao 2010; Jeanneteau et al. 2008; Lee and Chao 2001) (also see Sect. 5). Administration of adenosine agonists rescues lesioned motor neurons (Wiese et al. 2007) and ameliorates motor deficits in a Huntington's disease mouse model (Chou et al. 2005). Therefore, small molecules that transactivate the TrkB receptor could be used for the treatment of many neurodegenerative diseases, in lieu of using BDNF. Because the identified compounds (adenosine, steroids) have many systemic effects, additional small molecules of high specificity and potency capable of targeting and activating Trk receptors in the brain are needed. Transactivation of Trk receptors is not only a potential mechanism to prevent age-related degeneration, but can also be applied to mood disorders (anxiety, depression). Mood and anxiety disorders are among the most prevalent of all medical disorders. BDNF has been implicated in both depression and anxiety (Martinowich et al. 2007). Decreases in BDNF levels occur with stress-induced depressive behaviors, hippocampal atrophy, and increased anxiety-related behaviors. Identification of specific

Trk agonists will be relevant not only to the treatment of aging diseases affecting the nervous system, but also conditions of pain, anxiety, and depression.

Conclusion

Within this chapter, we have attempted to cover major aspects of Trk receptor biology in the nervous system. We have discussed the molecular structure of Trk receptors, their cognate ligands, and the molecular basis of ligand-dependent receptor activation, as well as the possibility for ligand-independent receptor transactivation at intracellular membranes. We further described intracellular mechanisms of signal transduction downstream of Trk receptor activation, using the most studied pathways as examples, and depicted how distinct routes of Trk receptor membrane trafficking can influence both the duration and location of Trk receptor signaling. Finally, we have portrayed multiple effects of Trk receptor signaling on neuronal physiology, such as mediating the survival of neuronal populations during development, and higher order functions in adulthood, such as supporting learning and memory by increasing synaptic transmission. Together, this emphasizes not only the essential role of Trk receptors within a healthy nervous system, but also highlights the potential of Trk receptors as a therapeutic target in neurodegenerative diseases, as discussed in the final part of this chapter.

References

Arevalo JC, Yano H, Teng KK, Chao MV (2004) A unique pathway for sustained neurotrophin signaling through an ankyrin-rich membrane-spanning protein. EMBO J 23:2358–2368

Arevalo JC, Wu SH (2006) Neurotrophin signaling: many exciting surprises! Cell Mol Life Sci 63:1523–1537

Bath KG, Jing DQ, Dincheva I, Neeb CC, Pattwell SS, Chao MV, Lee FS, Ninan I (2012) BDNF Val66Met impairs fluoxetine-induced enhancement of adult hippocampus plasticity. Neuropsychopharmacology 37:1297–1304

Benedetti M, Levi A, Chao MV (1993) Differential expression of nerve growth factor receptors leads to altered binding affinity and neurotrophin responsiveness. Proc Natl Acad Sci USA 90:7859–7863

Bibel M, Hoppe E, Barde YA (1999) Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR. EMBO J 18:616–622

Boeshore KL, Luckey CN, Zigmond RE, Large TH (1999) TrkB isoforms with distinct neurotrophin specificities are expressed in predominantly nonoverlapping populations of avian dorsal root ganglion neurons. J Neurosci 19:4739–4747

Bothwell M (1995) Functional interactions of neurotrophins and neurotrophin receptors. Annu Rev Neurosci 18:223–253

Carim-Todd L, Bath KG, Fulgenzi G, Yanpallewar S, Jing D, Barrick CA, Becker J, Buckley H, Dorsey SG, Lee FS et al (2009) Endogenous truncated TrkB.T1 receptor regulates neuronal complexity and TrkB kinase receptor function in vivo. J Neurosci 29:678–685

Chao MV (2003) Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci 4:299–309

Chao MV, Hempstead BL (1995) p75 and Trk: a two-receptor system. Trends Neurosci 18:321–326

Chen ZY, Ieraci A, Tanowitz M, Lee FS (2005) A novel endocytic recycling signal distinguishes biological responses of Trk neurotrophin receptors. Mol Biol Cell 16:5761–5772

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS et al (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314:140–143

Chou SY, Lee YC, Chen HM, Chiang MC, Lai HL, Chang HH, Wu YC, Sun CN, Chien CL, Lin YS et al (2005) CGS21680 attenuates symptoms of Huntington's disease in a transgenic mouse model. J Neurochem 93:310–320

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 411:957–962

Clary DO, Reichardt LF (1994) An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. Proc Natl Acad Sci USA 91:11133–11137

Cohen-Cory S, Kidane AH, Shirkey NJ, Marshak S (2010) Brain-derived neurotrophic factor and the development of structural neuronal connectivity. Dev Neurobiol 70:271–288

Coppola V, Barrick CA, Southon EA, Celeste A, Wang K, Chen B, el Haddad B, Yin J, Nussenzweig A, Subramaniam A et al (2004) Ablation of TrkA function in the immune system causes B cell abnormalities. Development 131:5185–5195

Deinhardt K, Salinas S, Verastegui C, Watson R, Worth D, Hanrahan S, Bucci C, Schiavo G (2006) Rab5 and Rab7 control endocytic sorting along the axonal retrograde transport pathway. Neuron 52:293–305

Delcroix JD, Valletta JS, Wu C, Hunt SJ, Kowal AS, Mobley WC (2003) NGF signaling in sensory neurons: evidence that early endosomes carry NGF retrograde signals. Neuron 39:69–84

Domeniconi M, Chao MV (2010) Transactivation of Trk receptors in spinal motor neurons. Histol Histopathol 25:1207–1213

Dorsey SG, Lovering RM, Renn CL, Leitch CC, Liu X, Tallon LJ, Sadzewicz LD, Pratap A, Ott S, Sengamalay N et al (2012) Genetic deletion of trkB.T1 increases neuromuscular function. Am J Physiol Cell Physiol 302:C141–C153

Duffy AM, Schaner MJ, Wu SH, Staniszewski A, Kumar A, Arevalo JC, Arancio O, Chao MV, Scharfman HE (2011) A selective role for ARMS/Kidins220 scaffold protein in spatial memory and trophic support of entorhinal and frontal cortical neurons. Exp Neurol 229:409–420

Esteban PF, Yoon HY, Becker J, Dorsey SG, Caprari P, Palko ME, Coppola V, Saragovi HU, Randazzo PA, Tessarollo L (2006) A kinase-deficient TrkC receptor isoform activates Arf6-Rac1 signaling through the scaffold protein tamalin. J Cell Biol 173:291–299

Fenner BM (2012) Truncated TrkB: beyond a dominant negative receptor. Cytokine Growth Factor Rev 23:15–24

Grimes ML, Zhou J, Beattie EC, Yuen EC, Hall DE, Valletta JS, Topp KS, LaVail JH, Bunnett NW, Mobley WC (1996) Endocytosis of activated TrkA: evidence that nerve growth factor induces formation of signaling endosomes. J Neurosci 16:7950–7964

Harrington AW, St Hillaire C, Zweifel LS, Glebova NO, Philippidou P, Halegoua S, Ginty DD (2011) Recruitment of actin modifiers to TrkA endosomes governs retrograde NGF signaling and survival. Cell 146:421–434

Heerssen HM, Pazyra MF, Segal RA (2004) Dynein motors transport activated Trks to promote survival of target-dependent neurons. Nat Neurosci 7:596–604

Hu B, Nikolakopoulou AM, Cohen-Cory S (2005) BDNF stabilizes synapses and maintains the structural complexity of optic axons in vivo. Development 132:4285–4298

Huang EJ, Reichardt LF (2003) Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 72:609–642

Huang YZ, Pan E, Xiong ZQ, McNamara JO (2008) Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. Neuron 57:546–558

Jeanneteau F, Garabedian MJ, Chao MV (2008) Activation of Trk neurotrophin receptors by glucocorticoids provides a neuroprotective effect. Proc Natl Acad Sci USA 105:4862–4867
Jeanneteau F, Deinhardt K, Miyoshi G, Bennett AM, Chao MV (2010) The MAP kinase phosphatase MKP-1 regulates BDNF-induced axon branching. Nat Neurosci 13:1373–1379
Kermani P, Hempstead B (2007) Brain-derived neurotrophic factor: a newly described mediator of angiogenesis. Trends Cardiovasc Med 17:140–143
Klein R, Smeyne RJ, Wurst W, Long LK, Auerbach BA, Joyner AL, Barbacid M (1993) Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. Cell 75:113–122
Klein R, Silos-Santiago I, Smeyne RJ, Lira SA, Brambilla R, Bryant S, Zhang L, Snider WD, Barbacid M (1994) Disruption of the neurotrophin-3 receptor gene trkC eliminates la muscle afferents and results in abnormal movements. Nature 368:249–251
Kuruvilla R, Zweifel LS, Glebova NO, Lonze BE, Valdez G, Ye H, Ginty DD (2004) A neurotrophin signaling cascade coordinates sympathetic neuron development through differential control of TrkA trafficking and retrograde signaling. Cell 118:243–255
Lee FS, Chao MV (2001) Activation of Trk neurotrophin receptors in the absence of neurotrophins. Proc Natl Acad Sci USA 98:3555–3560
Lee FS, Rajagopal R, Kim AH, Chang PC, Chao MV (2002) Activation of Trk neurotrophin receptor signaling by pituitary adenylate cyclase-activating polypeptides. J Biol Chem 277:9096–9102
Levi-Montalcini R (1987a) The nerve growth factor 35 years later. Science 237:1154–1162
Levi-Montalcini R (1987b) The nerve growth factor: thirty-five years later. Biosci Rep 7:681–699
Lin MY, Lin YM, Kao TC, Chuang HH, Chen RH (2011) PDZ-RhoGEF ubiquitination by Cullin3-KLHL20 controls neurotrophin-induced neurite outgrowth. J Cell Biol 193:985–994
Lu P, Tuszynski MH (2008) Growth factors and combinatorial therapies for CNS regeneration. Exp Neurol 209:313–320
Lu B, Pang PT, Woo NH (2005) The yin and yang of neurotrophin action. Nat Rev Neurosci 6:603–614
Manabe T (2002) Does BDNF have pre- or postsynaptic targets? Science 295:1651–1653
Martinowich K, Manji H, Lu B (2007) New insights into BDNF function in depression and anxiety. Nat Neurosci 10:1089–1093
Minichiello L (2009) TrkB signalling pathways in LTP and learning. Nat Rev Neurosci 10:850–860
Minichiello L, Korte M, Wolfer D, Kuhn R, Unsicker K, Cestari V, Rossi-Arnaud C, Lipp HP, Bonhoeffer T, Klein R (1999) Essential role for TrkB receptors in hippocampus-mediated learning. Neuron 24:401–414
Mok SA, Lund K, Campenot RB (2009) A retrograde apoptotic signal originating in NGF-deprived distal axons of rat sympathetic neurons in compartmented cultures. Cell Res 19:546–560
Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, Wang L, Blesch A, Kim A, Conner JM et al (2009) Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. Nat Med 15:331–337
Nikoletopoulou V, Lickert H, Frade JM, Rencurel C, Giallonardo P, Zhang L, Bibel M, Barde YA (2010) Neurotrophin receptors TrkA and TrkC cause neuronal death whereas TrkB does not. Nature 467:59–63
Ninan I, Bath KG, Dagar K, Perez-Castro R, Plummer MR, Lee FS, Chao MV (2010) The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the hippocampus. J Neurosci 30:8866–8870
Ohira K, Kumanogoh H, Sahara Y, Homma KJ, Hirai H, Nakamura S, Hayashi M (2005) A truncated tropomyosin-related kinase B receptor, T1, regulates glial cell morphology via Rho GDP dissociation inhibitor 1. J Neurosci 25:1343–1353

Pattwell SS, Bath KG, Perez-Castro R, Lee FS, Chao MV, Ninan I (2012) The BDNF Val66Met polymorphism impairs synaptic transmission and plasticity in the infralimbic medial prefrontal cortex. J Neurosci 32:2410–2421

Perlson E, Jeong GB, Ross JL, Dixit R, Wallace KE, Kalb RG, Holzbaur EL (2009) A switch in retrograde signaling from survival to stress in rapid-onset neurodegeneration. J Neurosci 29:9903–9917

Puehringer D, Orel N, Luningschror P, Subramanian N, Herrmann T, Chao MV, Sendtner M (2013) EGF transactivation of Trk receptors regulates the migration of newborn cortical neurons. Nat Neurosci 16:407–415

Rajagopal R, Chen ZY, Lee FS, Chao MV (2004) Transactivation of Trk neurotrophin receptors by G-protein-coupled receptor ligands occurs on intracellular membranes. J Neurosci 24:6650–6658

Reichardt LF (2006) Neurotrophin-regulated signalling pathways. Philos Trans R Soc Lond B Biol Sci 361:1545–1564

Riccio A, Pierchala BA, Ciarallo CL, Ginty DD (1997) An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons. Science 277:1097–1100

Riccio A, Ahn S, Davenport CM, Blendy JA, Ginty DD (1999) Mediation by a CREB family transcription factor of NGF-dependent survival of sympathetic neurons. Science 286:2358–2361

Shi Y, Mantuano E, Inoue G, Campana WM, Gonias SL (2009) Ligand binding to LRP1 transactivates Trk receptors by a Src family kinase-dependent pathway. Sci Signal 2:ra18

Silos-Santiago I, Fagan AM, Garber M, Fritzsch B, Barbacid M (1997) Severe sensory deficits but normal CNS development in newborn mice lacking TrkB and TrkC tyrosine protein kinase receptors. Eur J Neurosci 9:2045–2056

Smeyne RJ, Klein R, Schnapp A, Long LK, Bryant S, Lewin A, Lira SA, Barbacid M (1994) Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature 368:246–249

Snider WD (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell 77:627–638

Sommerfeld MT, Schweigreiter R, Barde YA, Hoppe E (2000) Down-regulation of the neurotrophin receptor TrkB following ligand binding. Evidence for an involvement of the proteasome and differential regulation of TrkA and TrkB. J Biol Chem 275:8982–8990

Stockel K, Schwab M, Thoenen H (1975) Comparison between the retrograde axonal transport of nerve growth factor and tetanus toxin in motor, sensory and adrenergic neurons. Brain Res 99:1–16

Strohmaier C, Carter BD, Urfer R, Barde YA, Dechant G (1996) A splice variant of the neurotrophin receptor trkB with increased specificity for brain-derived neurotrophic factor. EMBO J 15:3332–3337

Tessarollo L, Tsoulfas P, Donovan MJ, Palko ME, Blair-Flynn J, Hempstead BL, Parada LF (1997) Targeted deletion of all isoforms of the trkC gene suggests the use of alternate receptors by its ligand neurotrophin-3 in neuronal development and implicates trkC in normal cardiogenesis. Proc Natl Acad Sci USA 94:14776–14781

Thoenen H, Sendtner M (2002) Neurotrophins: from enthusiastic expectations through sobering experiences to rational therapeutic approaches. Nat Neurosci 5(Suppl):1046–1050

Tuszynski MH (2007) Nerve growth factor gene therapy in Alzheimer disease. Alzheimer Dis Assoc Disord 21:179–189

Ultsch MH, Wiesmann C, Simmons LC, Henrich J, Yang M, Reilly D, Bass SH, de Vos AM (1999) Crystal structures of the neurotrophin-binding domain of TrkA, TrkB and TrkC. J Mol Biol 290:149–159

Wagner N, Wagner KD, Theres H, Englert C, Schedl A, Scholz H (2005) Coronary vessel development requires activation of the TrkB neurotrophin receptor by the Wilms' tumor transcription factor Wt1. Genes Dev 19:2631–2642

Wiese S, Jablonka S, Holtmann B, Orel N, Rajagopal R, Chao MV, Sendtner M (2007) Adenosine receptor A2A-R contributes to motoneuron survival by transactivating the tyrosine kinase receptor TrkB. Proc Natl Acad Sci USA 104:17210–17215

Wiesmann C, Ultsch MH, Bass SH, de Vos AM (1999) Crystal structure of nerve growth factor in complex with the ligand-binding domain of the TrkA receptor. Nature 401:184–188

Wu SH, Arevalo JC, Sarti F, Tessarollo L, Gan WB, Chao MV (2009) Ankyrin Repeat-rich Membrane Spanning/Kidins220 protein regulates dendritic branching and spine stability in vivo. Dev Neurobiol 69:547–557

Wu SH, Arevalo JC, Neubrand VE, Zhang H, Arancio O, Chao MV (2010) The ankyrin repeat-rich membrane spanning (ARMS)/Kidins220 scaffold protein is regulated by activity-dependent calpain proteolysis and modulates synpatic plasticity. J Biol Chem 285:40472–40478

Yano H, Ninan I, Zhang H, Milner TA, Arancio O, Chao MV (2006) BDNF-mediated neurotransmission relies upon a myosin VI motor complex. Nat Neurosci 9:1009–1018

Yu H, Wang DD, Wang Y, Liu T, Lee FS, Chen ZY (2012) Variant brain-derived neurotrophic factor Val66Met polymorphism alters vulnerability to stress and response to antidepressants. J Neurosci 32:4092–4101

Zheng J, Shen WH, Lu TJ, Zhou Y, Chen Q, Wang Z, Xiang T, Zhu YC, Zhang C, Duan S et al (2008) Clathrin-dependent endocytosis is required for TrkB-dependent Akt-mediated neuronal protection and dendritic growth. J Biol Chem 283:13280–13288

Zuccato C, Cattaneo E (2009) Brain-derived neurotrophic factor in neurodegenerative diseases. Nat Rev Neurol 5:311–322

The Biological Functions and Signaling Mechanisms of the p75 Neurotrophin Receptor

B.R. Kraemer, S.O. Yoon, and B.D. Carter

Abstract

The p75 neurotrophin receptor ($p75^{NTR}$) regulates a wide range of cellular functions, including programmed cell death, axonal growth and degeneration, cell proliferation, myelination, and synaptic plasticity. The multiplicity of cellular functions governed by the receptor arises from the variety of ligands and co-receptors which associate with $p75^{NTR}$ and regulate its signaling. $P75^{NTR}$ promotes survival through interactions with Trk receptors, inhibits axonal regeneration via partnerships with Nogo receptor (Nogo-R) and Lingo-1, and promotes apoptosis through association with Sortilin. Signals downstream of these interactions are further modulated through regulated intramembrane proteolysis (RIP) of $p75^{NTR}$ and by interactions with numerous cytosolic partners. In this chapter, we discuss the intricate signaling mechanisms of $p75^{NTR}$, emphasizing how these signals are differentially regulated to mediate these diverse cellular functions.

Keywords

NGF • BDNF • NT3 • NRIF • TRAF • NRAGE • NADE • Necdin • Trk • Sortilin • Nogo • p75NTR

B.R. Kraemer • B.D. Carter (✉)
Department of Biochemistry, Vanderbilt University School of Medicine, 625 Light Hall, Nashville, TN 37232, USA

Vanderbilt Brain Institute, Nashville, TN, USA
e-mail: Bruce.carter@vanderbilt.edu

S.O. Yoon
Department Molecular and Cellular Biochemistry, The Ohio State University, Columbus, OH 43210, USA

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_6,

1 Introduction

The discovery of nerve growth factor (NGF) as the factor released by the target to promote survival and differentiation quickly led to the hunt for the receptor involved in mediating its actions. Early studies characterizing radiolabeled NGF binding to peripheral neurons revealed that NGF bound its receptors in a complex manner that likely involved multiple sites (Frazier et al. 1974a, b). Subsequently, two distinct NGF binding sites, one with high affinity and one with low affinity, were demonstrated on sensory neurons (Sutter et al. 1979). Cross-linking studies confirmed two receptor components in sympathetic neurons (Massague et al. 1981) and PC12 cells (Grob et al. 1983; Massague et al. 1982) of approximately 140–200 kDa and 70–100 kDa, with the lower molecular weight species being most abundant. These binding studies set the stage for the expression cloning of the NGF receptor by Chao et al. (1986) and, independently, by the Shooter lab (Radeke et al. 1987). Both groups identified the cDNA for the lower molecular weight species; it was termed the p75 receptor, which proved to be the low affinity binding site. Eventually, the proto-oncogene tropomyosin-related kinase A, or TrkA, was recognized as another NGF receptor accounting for the higher molecular weight component (Kaplan et al. 1991; Klein et al. 1991), and a complex of both p75 and TrkA was shown to comprise the high affinity binding site (Hempstead et al. 1991).

The cloning of the p75 receptor was a major achievement in the field; however, it opened a Pandora's box of puzzles and paradoxes. Prior to the cloning of p75, it had been suggested that only the high-affinity binding component was able to mediate the neurite growth-promoting effects of NGF (Sonnenfeld and Ishii 1982; Stach and Wagner 1982), raising the question of the role of the low affinity component. Once TrkA was identified as another receptor for NGF, it was quickly established as the primary mediator of NGF's survival and differentiation effects, being a tyrosine kinase with potent signaling capability (Ibanez et al. 1992; Loeb et al. 1991). For several years the p75 receptor was thought to simply function as a binding partner for TrkA or acting to increase the local concentration of NGF to facilitate activation of TrkA (Chao and Hempstead 1995). However, there were a number of observations that piqued the interest of researchers in the field, causing them to further explore the role of p75; for example, the receptor is expressed widely in the developing nervous system, with expression in peripheral neurons, within the spinal cord, and throughout the brain (Ernfors et al. 1991). It is expressed by many neuronal cell types, as well as neural stem cells, some astrocytes, oligodendrocyte precursors, Schwann cells, and olfactory ensheathing glia (Cragnolini and Friedman 2008). Several nonneural tissues also express the receptor during some stage of development, such as kidney and muscle (Ernfors et al. 1991; Wheeler and Bothwell 1992). In contrast, the Trk receptors exhibit a much more restricted expression pattern. In addition, the p75 receptor is strongly upregulated in many neurons and glial cells following injury, suggesting that it has a functional role in such conditions (discussed below). Finally, there are portions of the intracellular domain, where signaling would initiate, that are highly conserved across species, from chicken to human. These findings prompted further study of the p75 receptor,

and in the 25 years since its initial cloning, it has been shown to regulate an amazing array of cellular responses, including cell survival, cell cycle, neurite outgrowth, synaptic function, and myelination. This chapter will discuss our current understanding of the molecular mechanisms by which the p75 receptor mediates these diverse signals.

2 Structure

After the cloning of p75 it was quickly recognized that it not only bound NGF but also brain-derived neurotrophic factor (BDNF) (Rodriguez-Tebar et al. 1990), neurotrophin-3 (NT3) (Rodriguez-Tebar et al. 1992), and neurotrophin-4 (NT4) (Ryden et al. 1995), with similar affinity, although with somewhat different kinetics (Rodriguez-Tebar et al. 1992). The ability of the receptor to bind all neurotrophins led to its designation as the p75 neurotrophin receptor ($p75^{NTR}$) as opposed to the p75 NGF receptor. The $p75^{NTR}$ interacts with the neurotrophins through the four cysteine-rich domains in its extracellular domain (Baldwin and Shooter 1995). The initial X-ray crystallography structural analysis of the extracellular domain of $p75^{NTR}$ bound to NGF indicated that the receptor monomer binds NGF in an asymmetrical fashion, resulting in a 1:2 ratio (He and Garcia 2004). However, considerable biochemical data indicated that $p75^{NTR}$ associates with neurotrophins in a 2:2 ratio. Binding analyses using cross-linkers to attach neurotrophins to the receptor indicated a dimer of $p75^{NTR}$ bound to a neurotrophin dimer (Grob et al. 1985). Further crystallographic analyses support a 2:2 complex between neurotrophins and $p75^{NTR}$ (Feng et al. 2010; Gong et al. 2008), and it has been suggested that the 1:2 asymmetrical binding may represent an intermediate in the formation of the 2:2 complex (Feng et al. 2010).

At least a fraction of $p75^{NTR}$ has been shown to preexist as a disulfide-linked dimer (Grob et al. 1985; Ross et al. 1984), and a highly conserved cysteine (257) in the transmembrane domain responsible for linking the monomers was recently identified, although non-covalent dimerization still occurred even when cysteine 257 was mutated (Vilar et al. 2009). Further analysis revealed that a conserved $AxxxG^{266}$ sequence in the transmembrane region, which is often found in self-associating transmembrane proteins, is required for the formation of dimers. Through their studies, the authors elucidated an interesting aspect of the receptor's structural dynamics that provided a mechanism by which $p75^{NTR}$ transduces its signal upon ligand binding: the disulfide in the transmembrane domain acts as a pivot point, such that when the extracellular domain clamps down on a neurotrophin, the intracellular domains separate. The parting of the dimerized intracellular portions of the receptor facilitates binding of signaling molecules necessary for $p75^{NTR}$-mediated cell death (Vilar et al. 2009).

The intracellular domain (ICD) of $p75^{NTR}$ contains a region similar to the Tumor necrosis factor receptor (TNFR) and the Fas antigen (Chapman 1995; Chapman and Kuntz 1995; Feinstein et al. 1995). Since TNFR and Fas mediate apoptotic signals, this portion of their ICD was termed the "death domain." The 3-D structure of

$p75^{NTR}$'s death domain was determined by NMR and was similar to the structure of the Fas death domain, although there were a few differences. In particular, the death domain of Fas and TNFR self-assemble, while that of $p75^{NTR}$ does not (Liepinsh et al. 1997). This result is in agreement with the ability of TNFR and Fas to signal by recruiting other death domain-containing proteins while the intracellular interactors of $p75^{NTR}$ so far identified do not contain a death domain.

In addition to the full length form of $p75^{NTR}$, a splice variant was reported lacking exon III, which encodes the cysteine-rich domains 2, 3, and 4 that are required for neurotrophin binding (von Schack et al. 2001). The original $p75^{NTR}$ knockout mouse was created by deleting exon III (Lee et al. 1992); thus the short form of $p75^{NTR}$ (s-$p75^{NTR}$) could still be detected in these mice, in principle. The existence of s-$p75^{NTR}$, however, remains rather controversial, and its function is not known. Nevertheless, an alternative mutant mouse was created lacking exon IV, such that both splice isoforms of $p75^{NTR}$ are deleted (von Schack et al. 2001). These mice exhibit a number of neurological and vascular defects similar to the exon III knockout mice, but with a more severe phenotype. However, understanding the phenotype of the exon IV mutants is complicated by the fact that the targeting strategy created a cryptic truncated protein encoding an extracellular stalk with the entire transmembrane and intracellular domains of the receptor (Paul et al. 2004). Since expression of the intracellular domain of the receptor can initiate signaling independent of ligand (Majdan et al. 1997), some phenotypic characteristics of this mouse may be due to the expression of this fragment. Clearly, results from using either of these genetically altered mice need to be interpreted with caution, and further study is needed to understand the role of s-$p75^{NTR}$.

3 Apoptotic Signaling

Although $p75^{NTR}$ was first discovered for its ability to bind NGF, which promotes neuronal survival, the most investigated function of the receptor is, ironically, its ability to induce programmed cell death. One of the earliest indications of this function was revealed in a study by Bredesen's group demonstrating that ectopic expression of $p75^{NTR}$ in an immortalized neural cell line increased apoptosis after serum withdrawal (Rabizadeh et al. 1993). These results proved challenging to reproduce in primary cells with the endogenous receptor; however, the groups of Barde and Chao found that activation of endogenous $p75^{NTR}$ by NGF could induce apoptosis in early retinal neurons in the chick (Frade et al. 1996) and oligodendrocytes in rat (Casaccia-Bonnefil et al. 1996), respectively. The ability of $p75^{NTR}$ to induce programmed cell death in response to ligand binding has now been observed in a wide variety of neuronal and non-neuronal cell types, including sympathetic (Bamji et al. 1998; Linggi et al. 2005; Teng et al. 2005), motor (Sedel et al. 1999), and hippocampal neurons (Volosin et al. 2008); photoreceptor cells (Srinivasan et al. 2004); oligodendrocytes (Casaccia-Bonnefil et al. 1996); Schwann cells (Khursigara et al. 2001; Syroid et al. 2000); and other cells (Bunone et al. 1997; Volosin et al. 2006; Wang et al. 2000). These in vitro studies together

with the analysis of $p75^{NTR}-/-$ mice have established this receptor as a critical regulator of developmental apoptosis, promoting the naturally occurring elimination of neurons within the developing basal forebrain (Naumann et al. 2002), trigeminal ganglia (Agerman et al. 2000), retina (Frade et al. 1996), superior cervical ganglion (Bamji et al. 1998), and spinal cord (Frade and Barde 1999). This developmental role of $p75^{NTR}$ has been particularly well characterized in sympathetic neurons. These neurons express TrkA and $p75^{NTR}$, which together mediate a survival signal in response to NGF (discussed below); however, Miller and colleagues demonstrated that selective activation of $p75^{NTR}$ by BDNF led to apoptosis (Bamji et al. 1998). Furthermore, deletion of the receptor resulted in an increase in the number of neurons during the development of the superior cervical ganglia, suggesting that $p75^{NTR}$ mediates normal developmental death in this population. Ginty's group later demonstrated that these neurons produce BDNF in response to NGF and suggested a model in which neurons receiving robust trophic support through NGF-induced activation of TrkA produce BDNF, thereby promoting $p75^{NTR}$-dependent death of neighboring neurons receiving insufficient NGF signal (Deppmann et al. 2008). Their computer simulations based on this model quite accurately predicted the normal developmental kinetics of cell death in the superior cervical ganglia.

3.1 Activation of the Mitochondrial Cascade

Over the past decade, significant progress has been made in understanding the cellular mechanisms through which $p75^{NTR}$ promotes apoptosis, although many facets of the receptor's signaling remain enigmatic. Members of the TNF receptor superfamily can activate two pathways that regulate cell survival. Through their death domain, they recruit other death domain-containing adaptors, such as TRADD and FADD, leading to caspase-8 activation and induction of a terminal caspase cascade (Dempsey et al. 2003). Despite attempts to detect activation of caspase-8 (Gu et al. 1999; Troy et al. 2002), no evidence supports $p75^{NTR}$ utilizing this pathway, which agrees with the structural divergence of $p75^{NTR}$'s death domain from that of TNFR and Fas. The second pathway initiated by many members of the TNF receptor family involves stimulation of the stress kinase c-Jun N-terminal kinase (JNK) and of the transcription factor NF-κB (Dempsey et al. 2003). JNK activation causes cell death by inducing phosphorylation of the transcription factor c-Jun and the tumor suppressors p53 and p73 (Dhanasekaran and Reddy 2008), resulting in transcriptional upregulation of an array of pro-apoptotic genes, including Bax (Miyashita and Reed 1995), PUMA (Nakano and Vousden 2001), Bak (Bogoyevitch and Kobe 2006), and Caspase-6 (MacLachlan and El-Deiry 2002), among others (Wu 2004). In addition, JNK directly phosphorylates several Bcl-2 family proteins, causing inhibition of pro-survival members such as Bcl-2 (Yamamoto et al. 1999) and activation of pro-death members such as Bim (Lei and Davis 2003) and Bad (Donovan

et al. 2002). These events ultimately lead to the release of cytochrome c from mitochondria and caspase-dependent apoptosis (Bogoyevitch and Kobe 2006).

An accumulation of evidence has indicated that $p75^{NTR}$-induced apoptosis occurs via this mitochondrial cascade. Activation of JNK in response to ligand binding to endogenous $p75^{NTR}$ has been demonstrated in oligodendocytes (Casaccia-Bonnefil et al. 1996), sympathetic neurons (Bamji et al. 1998), and hippocampal neurons (Friedman 2000), and inhibition of the kinase prevented the induction of apoptosis (Friedman 2000; Harrington et al. 2002; Kenchappa et al. 2010; Yeiser et al. 2004; Yoon et al. 1998). Overexpressing $p75^{NTR}$ in cortical neurons also resulted in activation of JNK (Bhakar et al. 2003). In mammals, there are three genes encoding the JNK family, JNK1–3. While JNK1 and JNK2 are ubiquitously expressed, JNK3 is selectively expressed in the nervous system and heart (Gupta et al. 1996; Kuan et al. 2003; Mohit et al. 1995) and has been suggested to be the primary isoform mediating neuronal death in response to a variety of ligands and insults (Dhanasekaran and Reddy 2008). Of these three JNK isoforms, JNK3 was selectively activated following ligand binding to $p75^{NTR}$ in oligodendrocytes (Harrington et al. 2002) and sympathetic neurons (Kenchappa et al. 2010), and gene deletion of JNK3 prevented receptor-mediated apoptosis both in vitro and in vivo (Kenchappa et al. 2010; Li et al. 2007).

Further support for $p75^{NTR}$ activating a JNK-p53 apoptotic pathway comes from the fact that cell death mediated by the receptor is associated with upregulation of p53 (Aloyz et al. 1998; Linggi et al. 2005). Induction of apoptosis by $p75^{NTR}$ has also been linked to phosphorylation of Bim (Becker et al. 2004) and Bad (Bhakar et al. 2003), cytochrome c release (Bhakar et al. 2003), and cleavage of procaspase-3, -6, -7, or -9 (Bhakar et al. 2003; Tabassum et al. 2003). Curiously, however, the receptor does not require c-Jun for killing sympathetic neurons (Palmada et al. 2002).

3.2 Cytosolic Factors Linking $p75^{NTR}$ to JNK

Like many other receptors of the tumor necrosis factor (TNF) receptor superfamily, $p75^{NTR}$ promotes downstream signaling via association with a number of cytosolic interactors (Fig. 1). One group of $p75^{NTR}$ interactors that contributes to activation of JNK is the family of TNF receptor-associated factors (TRAFs). TRAF family proteins are distinguished by a conserved C-terminal domain that is responsible for their oligomerization and interactions with the cytoplasmic domains of TNF receptor family members (Zotti et al. 2012). With the exception of TRAF1, all TRAF family members also feature an N-terminal domain containing RING and zinc finger structures that are critical for their signaling function. The RING finger domain in the TRAFs acts as an E3 ubiquitin ligase, but instead of targeting proteins for proteasomal degradation, the TRAFs form a ubiquitin chain through Lys 63 linkages, which serve as protein–protein interaction motifs (Ha et al. 2009; Hacker et al. 2011). TRAF1–6 have been reported to associate with $p75^{NTR}$, with TRAF2, 4, and 6 shown to modulate $p75^{NTR}$-induced cell death via interactions

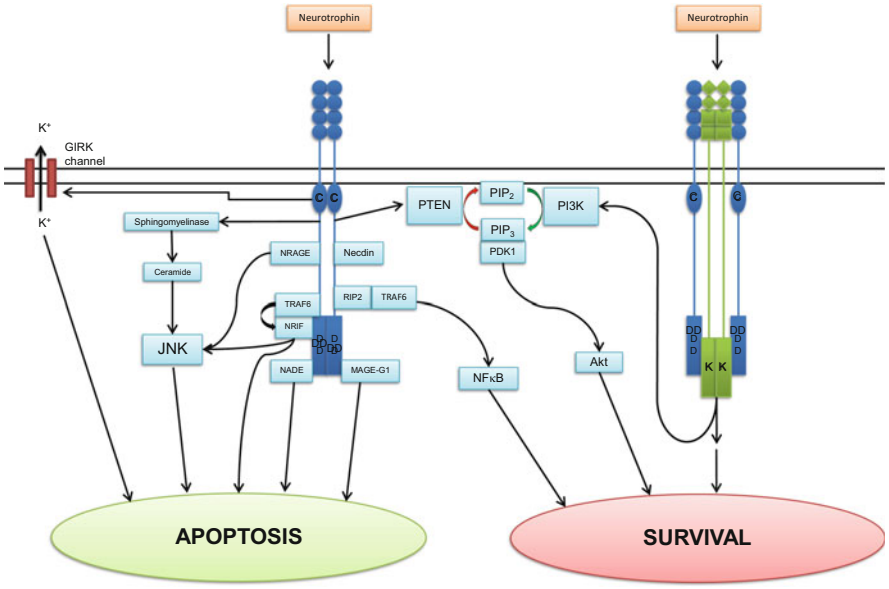

Fig. 1 Signaling pathways mediated by $p75^{NTR}$ that regulate cell survival and apoptosis. In response to neurotrophin binding, $p75^{NTR}$ promotes JNK activation via interactions with NRAGE, TRAF6, and NRIF, thus leading to apoptosis. Activation of JNK by $p75^{NTR}$ also occurs through induction of sphingomyelinases. The chopper domain of $p75^{NTR}$ promotes apoptosis by facilitating depletion of internal K^+ through GIRK channels. Other cytosolic interactors contribute to $p75^{NTR}$-mediated cell death, including NADE, MAGE-G1, and Necdin. In response to pro-neurotrophins, $p75^{NTR}$ inhibits Trk-mediated survival signaling via induction of PTEN and the resultant inhibition of PI3K-Akt survival signaling. Promotion of cell survival by $p75^{NTR}$ is facilitated by its interactions with Trk receptors which enhance Trk-mediated PI3K-Akt survival signaling, as well as other Trk-mediated survival pathways. $P75^{NTR}$ may also promote survival via activation of NFκB, possibly through associations between RIP2 and TRAF6 (abbreviations: *DD* $p75^{NTR}$ death domain, *C* $p75^{NTR}$ chopper domain, *K* Trk receptor tyrosine kinase domain)

with the ICD of the receptor (Khursigara et al. 1999; Ye et al. 1999). However, the role of TRAF6 in $p75^{NTR}$ signaling has been the most thoroughly studied. TRAF6 associates with $p75^{NTR}$ in a ligand-dependent manner (Khursigara et al. 1999) and mediates signaling from the receptor to both JNK and NF-κB (Khursigara et al. 1999; Yeiser et al. 2004). Sympathetic neurons from *traf6*−/− mice fail to activate JNK in response to BDNF binding to $p75^{NTR}$ and fail to undergo apoptosis (Yeiser et al. 2004). Furthermore, there is reduced developmental cell death in the superior cervical ganglia in *traf6*−/− mice relative to the wild type, indicating that TRAF6 is essential for $p75^{NTR}$-mediated apoptotic signaling in vivo.

TRAF6 also associates with the neurotrophin receptor-interacting factor (NRIF) to promote JNK activation (Gentry et al. 2004; Linggi et al. 2005). NRIF is a zinc-finger protein that was first identified in a yeast 2-hybrid screen for proteins interacting with the ICD of $p75^{NTR}$ (Casademunt et al. 1999). NRIF and TRAF6 can directly interact, and overexpression of NRIF together with TRAF6 enhanced

TRAF6-mediated JNK activation (Gentry et al. 2004). Furthermore, BDNF-induced JNK activation and cell death were significantly attenuated in *nrif*−/− sympathetic neurons (Linggi et al. 2005). Gene deletion revealed that NRIF was required for developmental apoptosis in the retina (Casademunt et al. 1999), which is a $p75^{NTR}$-dependent process (Frade et al. 1996). Thus, interaction of NRIF with TRAF6 and $p75^{NTR}$ appears to be critical for $p75^{NTR}$-mediated JNK activation and apoptosis. However, expression of NRIF alone in mouse embryonic fibroblasts was not sufficient to activate the kinase, although it did induce cell death (Linggi et al. 2005). Exactly how NRIF contributes to the activation of JNK is not clear, but it may facilitate oligomerization of TRAF6, which is necessary for it to mediate its biological actions (Yin et al. 2009).

Another intracellular binding partner of $p75^{NTR}$ that is linked to JNK activation is the neurotrophin receptor-interacting MAGE homolog, NRAGE (also known as Maged1 and dlxin) (Salehi et al. 2002). NRAGE contains a melanoma-associated antigen (MAGE) domain, which is a region of homology defining the MAGE family of proteins. The function of the MAGE proteins is poorly understood, but many have been implicated in the regulation of cell cycle and apoptosis (Sang et al. 2011). Ectopic expression of NRAGE along with $p75^{NTR}$ in a sympathetic precursor cell line enabled NGF-dependent cell death, thereby implicating this interactor in the apoptotic pathway activated by $p75^{NTR}$ (Salehi et al. 2000). Overexpression of NRAGE in PC12 cells led to potent activation of JNK, release of cytochrome c from mitochondria, and the induction of caspases -3, -6, and -9, ultimately resulting in cell death (Salehi et al. 2002). These results suggested that NRAGE could be involved in $p75^{NTR}$-mediated stimulation of JNK. Corroborating evidence came from analysis of *nrage*−/− mice: $p75^{NTR}$-induced JNK activation in *nrage*−/− sympathetic neurons was significantly reduced compared to wild-type neurons (Bertrand et al. 2008). Furthermore, the null animals have an increased number of neurons in their superior cervical ganglia, like $p75^{NTR}$−/− mice, and sympathetic neurons isolated from *nrage*−/− mice were resistant to $p75^{NTR}$-mediated apoptosis (Bertrand et al. 2008). These results suggest a function for NRAGE as an adaptor protein, linking the receptor to JNK activation and apoptosis. Whether NRAGE, TRAF6, and NRIF form a complex or function independently to regulate the kinase remains an open question; however, they may function at different stages of the cascade to affect the kinetics of JNK activity (discussed below). It should be noted that sequestering the anti-apoptotic factor XIAP (Jordan et al. 2001; Kendall et al. 2005) and promoting degradation of the anti-apoptotic transcription factor Che1 (Di Certo et al. 2007) have also been suggested as mechanisms through which NRAGE affects cell survival, though these interactions have not been studied in the context of $p75^{NTR}$ signaling.

Another mechanism through which $p75^{NTR}$ has been suggested to regulate JNK involves production of the lipid signaling molecule ceramide (Fig. 1). When the field was searching for evidence of signaling by $p75^{NTR}$, a NGF-mediated increase in ceramide levels through activation of neutral sphingomyelinase in T9 glioma cells was one of the first signals detected (Dobrowsky et al. 1994). Multiple reports have since confirmed the ability of $p75^{NTR}$ to stimulate ceramide production in

other cell types, including in oligodendrocytes (Casaccia-Bonnefil et al. 1996), hippocampal neurons (Brann et al. 2002), Schwann cells (Hirata et al. 2001), and mesencephalic neurons (Blochl and Sirrenberg 1996). One known downstream effect of elevated ceramide is activation of JNK (Westwick et al. 1995), and thus ceramide may couple $p75^{NTR}$ to JNK phosphorylation. Indeed, in cultured hippocampal neurons activation of $p75^{NTR}$ resulted in upregulation of ceramide, stimulation of JNK, and cell death (Brann et al. 2002). Furthermore, inhibition of sphingomyelinase in these neurons prevented ceramide accumulation, JNK activation, and the induction of apoptosis. However, increasing ceramide levels does not always result in cell death. In fact, $p75^{NTR}$-mediated ceramide production has also been linked to promotion of cell survival (DeFreitas et al. 2001; McCollum and Estus 2004). Understanding this lipid signaling pathway is complicated by the fact that ceramide is a central intermediate in sphingolipid metabolism and can have a variety of effects depending on the specific fatty acid chain attached and its cellular concentration and localization (Horres and Hannun 2012). Further studies are needed to elucidate the mechanisms by which $p75^{NTR}$ activates sphingomyelinase and to reveal how ceramide elicits its effects in various cellular contexts.

3.3 Other Factors Involved in $p75^{NTR}$ Mediated Apoptosis

Apart from TRAF6, NRIF, and NRAGE, several other cytosolic proteins have been shown to associate with $p75^{NTR}$ and suggested to regulate its apoptotic signaling. For example, $p75^{NTR}$-associated cell death executor (NADE), a novel protein isolated in a two-hybrid screening for proteins binding to the ICD of the receptor, was reported to associate with endogenous $p75^{NTR}$ in PC12 cells (Mukai et al. 2000). Overexpression of NADE together with $p75^{NTR}$ in HEK 293 cells induced apoptosis (Mukai et al. 2000) and expression of a fragment of NADE lacking the region identified as necessary for promoting apoptosis blocked receptor-mediated cell death in oligodendrocytes (Mukai et al. 2002). Currently, though, how NADE contributes to $p75^{NTR}$-mediated apoptotic signaling is unknown. In addition, MAGE-G1, MAGE-H1 and the MAGE-related protein, Necdin, have also been shown to interact with $p75^{NTR}$ (Kuwako et al. 2004; Tcherpakov et al. 2002). Both Necdin and MAGE-G1 associate with E2F1, a transcription factor that is important for G1/S transition in the cell cycle and that can induce apoptosis in postmitotic cells (Ginsberg 2002). When the ICD of $p75^{NTR}$ was overexpressed in a neuroblastoma cell line, Necdin and MAGE-G1 bound to the receptor ICD, thereby releasing E2F1 and triggering apoptosis (Kuwako et al. 2004; Lopez-Sanchez et al. 2007). Additional studies are needed to determine whether Necdin and MAGE-G1 regulate ligand-mediated cell death in primary cells. The $p75^{NTR}$ has also been reported to promote apoptosis through upregulation of the sugar binding protein Galectin-1 (Plachta et al. 2007). Embryonic stem (ES) cells engineered to express $p75^{NTR}$ degenerated when they were induced to differentiate into neurons. This degeneration correlated with expression of Galectin-1, which promoted death of the ES cells as well as cortical neurons (Plachta et al. 2007). Furthermore, mice

lacking Galectin-1 were resistant to neuronal apoptosis caused by pilocarpine-induced seizures (Bischoff et al. 2012), which was demonstrated to be a $p75^{NTR}$-dependent process (Roux et al. 1999; Volosin et al. 2008). The mechanisms by which this lectin causes cell death remain to be determined.

3.4 Regulated Intramembrane Proteolysis of $p75^{NTR}$

In a manner similar to Notch and amyloid precursor protein (APP), $p75^{NTR}$ undergoes regulated intramembrane proteolysis (RIP). Proteolysis of $p75^{NTR}$ was first described as a response to phorbol esters in HEK293 cells transfected with the receptor (Jung et al. 2003; Kanning et al. 2003). The extracellular region of $p75^{NTR}$ is first cleaved by the metalloproteinase TNFα-converting enzyme (TACE, also known as ADAM17), thereby producing a 24 kDa membrane-bound C-terminal fragment ($p75^{NTR}$-CTF) (Weskamp et al. 2004). This cleavage event appears to be quite promiscuous in terms of the amino acid sequence; however, deletion analysis revealed that at least 15 residues extracellular to the transmembrane domain are required (Zampieri et al. 2005). Following release of the soluble ectodomain, the $p75^{NTR}$-CTF is then further cleaved within its transmembrane region by the γ-secretase complex, thereby releasing the 19 kDa intracellular domain of the receptor ($p75^{NTR}$-ICD). Similar to proteolysis by TACE, cleavage by γ-secretase is quite permissive for various amino acids; nevertheless, there must be some sequence specificity for both enzymes since substituting the transmembrane domain of Fas for that of $p75^{NTR}$ blocked cutting by γ-secretase and replacing the 15 amino acid juxtamembrane sequence with the Fas sequence blocked $p75^{NTR}$ proteolysis by TACE (Zampieri et al. 2005). The order of the two cleavage reactions is also invariant, with TACE acting on the receptor prior to γ-secretase. This was determined by studies in which cleavage of $p75^{NTR}$ by γ-secretase was prevented by TACE inhibition, but inhibition of γ-secretase did not affect TACE activity, thus indicating that release of the extracellular domain is required for further proteolysis of the receptor within the transmembrane domain (Kenchappa et al. 2010; Zampieri et al. 2005). Since the initial finding of RIP of $p75^{NTR}$ in response to phorbol esters, a number of reports have demonstrated that proteolysis of $p75^{NTR}$ occurs through a ligand-dependent mechanism; for example, treatment of sympathetic neurons with BDNF (Kenchappa et al. 2006, 2010), Schwann cells with NGF (Frade 2005), and cerebellar neurons with myelin-associated glycoprotein (Domeniconi et al. 2005) (this ligand is discussed below) resulted in RIP. It is unclear, however, whether ligand activated $p75^{NTR}$ always results in RIP.

One functional role of $p75^{NTR}$ cleavage, like for many γ-secretase substrates, is to facilitate signaling to the nucleus. Release of the $p75^{NTR}$-ICD may facilitate nuclear translocation of associated factors such as NRIF. Although NRIF was shown to be required for $p75^{NTR}$-mediated apoptotic signaling based on analyses of *nrif−/−* mice (Casademunt et al. 1999; Linggi et al. 2005), exactly how it contributed to the cell death was not clear. NRIF contains a classic C2H2 zinc-finger motif (Casademunt et al. 1999), which is typically found among DNA

binding transcription factors (Wolfe et al. 2000), suggesting that in addition to facilitating JNK activation, NRIF could bind DNA and regulate transcription. The recognition of $p75^{NTR}$ proteolysis by γ-secretase revealed a possible mechanism by which NRIF could be translocated from the surface-bound ICD of the receptor to the nucleus. Indeed, it was demonstrated that BDNF-induced cleavage of the receptor in sympathetic neurons facilitated nuclear localization of NRIF and, subsequently, apoptosis (Kenchappa et al. 2006). Blocking receptor cleavage prevented both localization of NRIF to the nucleus and cell death. A similar signaling cascade has been detected in hippocampal neurons, where neuronal death due to pilocarpine-induced seizures was associated with $p75^{NTR}$ proteolysis and NRIF nuclear translocation. Moreover, the number of apoptotic neurons after seizure was significantly reduced in $p75^{NTR}-/-$ (Troy et al. 2002) and in *nrif*−/− mice (Volosin et al. 2008).

The mechanism of NRIF nuclear translocation also depends on TRAF6-mediated ubiquitylation. TRAF6 was shown to ubiquitylate NRIF following ligand binding to $p75^{NTR}$, and blocking this event by mutating the ubiquitin-attachment site of NRIF prevented its nuclear translocation and inhibited $p75^{NTR}$-mediated apoptosis (Geetha et al. 2005). The ubiquitylation of NRIF required $p75^{NTR}$ cleavage (Kenchappa et al. 2006), suggesting that receptor proteolysis facilitates an interaction between NRIF and TRAF6, enabling ubiquitylation of NRIF, which is needed for it to enter the nucleus, and oligomerization of TRAF6, which promotes the activation of JNK (Fig. 2).

The cleavage of $p75^{NTR}$ and the activation of JNK were recently shown to occur through interdependent pathways. In sympathetic neurons, JNK activation was required for ligand-induced proteolysis of the receptor by both TACE and γ-secretase (Kenchappa et al. 2010), as blocking JNK activity or deleting JNK3 prevented receptor cleavage by both proteases. The activation of JNK facilitated the transcriptional upregulation of TACE and, through an unknown mechanism, stimulated both TACE and γ-secretase, thereby inducing $p75^{NTR}$ processing. Interestingly, the release of the receptor's ICD, along with NRIF and TRAF6, was necessary for prolonged JNK stimulation by the receptor. Expression of a non-cleavable mutant $p75^{NTR}$ prevented JNK activation at 24 h, yet the kinase was still activated for the first hour after ligand binding (Kenchappa et al. 2010). Hence, there appears to be a biphasic activation of JNK by $p75^{NTR}$, with an early signal, perhaps initiated through NRAGE, inducing proteolytic processing of the receptor, which allows NRIF and TRAF6 to promote long-term stimulation of the kinase as well as nuclear signaling, ultimately resulting in cell death (Fig. 2).

In contrast to the evidence that proteolytic processing of $p75^{NTR}$ induces apoptosis by releasing the $p75^{NTR}$-ICD, in certain cellular contexts programmed cell death may be activated by the $p75^{NTR}$-CTF alone. Coulson et al. found that overexpression of the $p75^{NTR}$-CTF was sufficient to promote the apoptosis of dorsal root ganglion (DRG) neurons and that the death domain was not necessary (Coulson et al. 2000; Underwood et al. 2008). This function of the $p75^{NTR}$-CTF required a 29 amino acid sequence in the cytoplasmic juxtamembrane region of the receptor termed the "chopper domain" (Coulson et al. 2000). Coulson and

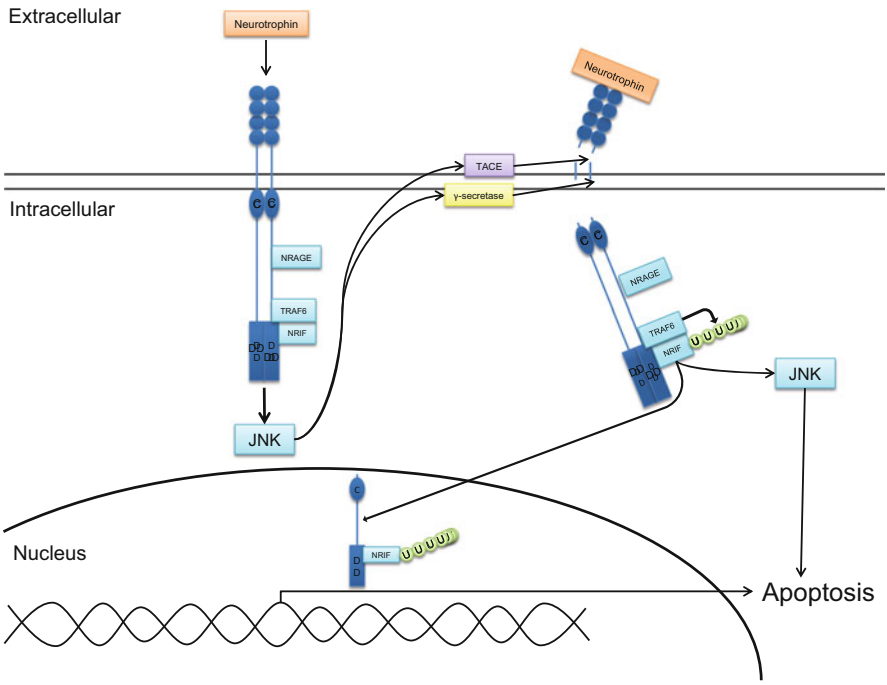

Fig. 2 Cell death signaling initiated by regulated intramembrane proteolysis (RIP) of $p75^{NTR}$. Stimulation of $p75^{NTR}$ by neurotrophins promotes an early phase of JNK activation, occurring within 30 min of ligand binding. Through a mechanism currently unknown, JNK induces sequential proteolytic cleavage of $p75^{NTR}$ by TACE and γ-secretase. Release of the $p75^{NTR}$ intracellular domain promotes TRAF6-dependent ubiquitylation and nuclear translocation of NRIF, as well as persistent JNK activation, ultimately leading to induction of programmed cell death (abbreviations: *DD* death domain, *C* chopper domain, *U* ubiquitin)

colleagues demonstrated that ectopic expression of membrane-associated fragments of $p75^{NTR}$ containing the chopper domain promoted apoptosis by inducing a Rac-dependent increase in phosphatidylinositol 4,5-bisphosphate (PIP_2). In turn, PIP_2 stimulated G-protein-coupled inwardly rectifying potassium (GIRK) channels, causing a depletion of internal potassium that ultimately activated an apoptotic protease activating factor 1 (APAF-1)-dependent cell death pathway (Coulson et al. 2004, 2008; Skeldal et al. 2011). It should be cautioned, however, that these studies relied on overexpression of the CTF; thus, further studies are needed to determine how the various fragments of the receptor regulate cell death under different physiological conditions.

3.5 Proneurotrophins and Sortilin

The initial discovery that $p75^{NTR}$ can induce programmed cell death was somewhat puzzling, as in vitro studies indicated that relatively high concentrations of

neurotrophins were needed to induce apoptosis, and in certain cell types, cross-reactivity of neurotrophins with Trk receptors could potentially promote an opposing, pro-survival signal. An answer was found, at least in part, by Hempstead's group, who discovered that precursor forms of neurotrophins are biologically active, selective ligands for $p75^{NTR}$. Like most secreted proteins, neurotrophins are initially synthesized as larger precursors, which are enzymatically cleaved to generate the mature form of the protein (Edwards et al. 1988; Suter et al. 1991). Proneurotrophins have an amino-terminal pro-domain that assists in their proper folding and dimerization (Heymach and Shooter 1995; Rattenholl et al. 2001a, b). The pro-domain can be proteolytically removed by furin and pro-protein convertases in the endoplasmic reticulum and Golgi apparatus (Seidah et al. 1996). Alternatively, the cleavage of the pro-domain can also be mediated by plasmin and matrix metalloproteases following secretion of the proneurotrophin into the extracellular milieu (Lee et al. 2001). While it was originally thought that mature neurotrophins are the only physiologically active ligands for $p75^{NTR}$, it is now well established that endogenous proneurotrophins can be secreted to function as potent activators of $p75^{NTR}$ signaling (Beattie et al. 2002; Harrington et al. 2004; Lebrun-Julien et al. 2010; Lee et al. 2001; Teng et al. 2005).

Proneurotrophins do not activate Trk receptors (Boutilier et al. 2008; Lee et al. 2001) and have been demonstrated to induce significant $p75^{NTR}$-mediated cell death at sub-nanomolar concentrations (Lee et al. 2001). Thus, proteolytic processing determines the functional fate of nascent neurotrophins, with uncleaved forms selectively triggering $p75^{NTR}$-mediated cell death and mature forms activating either $p75^{NTR}$ or Trk receptors, depending upon the cellular context. Proneurotrophins induce programmed cell death by binding to a high affinity protein complex containing $p75^{NTR}$ and its co-receptor Sortilin, a member of the Vps10p-domain receptor family (Nykjaer et al. 2004; Teng et al. 2005). Mammalian members of the Vps10p family, which consists of Sortilin, SorLA, and SorCS-1, -2, and -3, are type I transmembrane receptors with multifunctional roles that include the modulation of protein sorting and trafficking, as well as regulation of signal transduction (Willnow et al. 2008). Proneurotrophins bind to Sortilin via their pro-domain and to $p75^{NTR}$ by their mature domain, thus facilitating the association of these two receptors to initiate programmed cell death (Nykjaer et al. 2004, 2005; Teng et al. 2005). Following initial reports that Sortilin mediates neurotrophin-induced cell death in vitro (Nykjaer et al. 2004; Teng et al. 2005), studies have indicated that Sortilin is required for developmental $p75^{NTR}$-mediated cell death in vivo. For example, mice lacking Sortilin have a reduction in the developmental apoptosis of retinal ganglion cells that is indistinguishable from that of $p75^{NTR}$-deficient mice (Jansen et al. 2007). However, Sortilin may not be required for all $p75^{NTR}$-mediated cell death, as these mice did not have defects in the apoptosis of sympathetic neurons during the developmental time period in which $p75^{NTR}$-mediated death is known to occur (Jansen et al. 2007). Loss of Sortilin did, however, impair age-related degeneration of these neurons, suggesting that proneurotrophins may not have been involved in the early development of the sympathetic neurons, but do have a role in their loss during aging.

3.6 Apoptotic Role of p75^{NTR} in Pathology

In addition to its critical role during neurodevelopment, p75^{NTR} is a stress-activated receptor that stimulates the death of cells within injured tissue. Though the receptor is downregulated in most regions of the nervous system after early postnatal development, reexpression of p75^{NTR} occurs in response to many forms of cellular damage. For example, increases in p75^{NTR} expression have been reported following neuronal axotomy (Ernfors et al. 1989; Giehl et al. 2001; Harrington et al. 2004; Koliatsos et al. 1991; Taniuchi et al. 1986), mechanical damage (Beattie et al. 2002; Brunello et al. 1990; Rende et al. 1993), elevated intraocular pressure (Wei et al. 2007), seizures (Roux et al. 1999; Volosin et al. 2008), and focal ischemia (Kokaia et al. 1998). Beyond measuring increases in expression of the receptor, multiple studies have more definitively demonstrated that p75^{NTR} signaling is responsible for injury-induced cell death in vivo. In one such study, unilateral administration of kainic acid to the basal forebrain resulted in reexpression of p75^{NTR}in the degenerating cholinergic neurons, which correlated with their apoptosis. Administration of a function-blocking p75^{NTR} antibody prevented this cell death, thereby indicating that p75^{NTR} signaling contributes to excitotoxin-induced death of basal forebrain neurons (Oh et al. 2000). Similarly, expression of p75^{NTR} was induced and associated with programmed cell death caused by axotomy of corticospinal neurons, and antibodies to p75^{NTR} prevented this apoptosis (Giehl et al. 2001). Although these two studies indicated that p75^{NTR} promotes neuronal death after injury, whether proneurotrophins contribute to the death caused by these injuries was not known. In a later report, injury to the spinal cord was found to induce production of proNGF and to stimulate p75^{NTR}-dependent apoptosis of spinal cord oligodendrocytes (Beattie et al. 2002). ProNGF extracted from the injured region elicited apoptosis of cultured oligodendrocytes expressing p75^{NTR} but not of *p75^{NTR}*−/− oligodendrocytes. Thus, this work suggested that proNGF functions to promote the elimination of damaged cells by activating p75^{NTR} after spinal cord injury (Beattie et al. 2002). A subsequent study by Yoon and colleagues demonstrated that axotomy of corticospinal neurons also resulted in apoptosis of the neurons through a proNGF–p75^{NTR}-dependent mechanism (Harrington et al. 2004). Following lesion of the internal capsule, proNGF was detected in cerebral spinal fluid, indicating that proNGF is produced and secreted in vivo after brain injury. In the cortex of lesioned animals, an interaction between proNGF and p75^{NTR} was detected in vivo, and disruption of this interaction by infusion of an antibody specific for proNGF prevented the apoptosis caused by the injury (Harrington et al. 2004). These experiments provided the first conclusive evidence that proNGF is a pathophysiological ligand that induces apoptosis in response to neuronal damage. Since then, a growing body of evidence has linked proneurotrophins to cell death induced by various types of injury. For example, hippocampal seizures stimulated the upregulation and secretion of proNGF in vivo, and antibodies specific for proNGF prevented seizure-induced apoptosis of neurons within the dentate gyrus (Volosin et al. 2008). In another study, increases in proNGF, along with p75^{NTR} and Sortilin, were reported in the retina after exposure

of albino mice to intense light, and blockade of Sortilin with the pro-domain of proNGF attenuated light-induced retinal cell death (Santos et al. 2012). The ability of proneurotrophins to induce cell death in response to cellular damage is likely not specific to proNGF, as proBDNF has also been demonstrated to promote apoptosis in a number of cell culture models (Fan et al. 2008; Taylor et al. 2012; Teng et al. 2005), and upregulation of proBDNF has been detected in in vivo injury models, such as in an animal model of cochlear damage (Tan and Shepherd 2006). ProBDNF has also been implicated in apoptosis occurring due to neuronal axotomy, as infusion of a proBDNF antibody prevented the death of sensory neurons induced by lesion of the sciatic nerve in vivo (Fan et al. 2008).

While the signaling mechanisms responsible for the induction of $p75^{NTR}$ and the proneurotrophins after injury are not well understood, several studies have provided some clues. One possibility is that cellular damage prevents the proteolytic processing of neurotrophins, thus increasing the release of death inducing proneurotrophins. A recent study by Friedman and colleagues has revealed that following kainic acid-induced seizures, the proneurotrophin-processing enzyme matrix metalloproteinase-7 (MMP-7) and its inhibitor tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) were regulated in a manner that would hinder cleavage of proneurotrophins and lead to increased release of proNGF (Le and Friedman 2012). Decreased MMP-7 production has also been observed in samples from human patients and animal models with diabetic retinopathy (Ali et al. 2011). These findings suggest that regulation of proteolytic processing of proneurotrophins is one mechanism by which the levels of these factors are modulated, though a greater understanding of the pathways regulating their release in the unprocessed versus the mature form is needed. Along with increases in the levels of proneurotrophins, upregulation of $p75^{NTR}$ after injury may occur due to inflammatory signals released in response to tissue damage. The inflammatory cytokines interleukin-12 (IL-12), tumor necrosis factor alpha (TNFα), and interleukin-1β have been demonstrated to increase $p75^{NTR}$ expression in a variety of in vitro systems, such as in cultured hippocampal neurons (Choi and Friedman 2009), natural killer cells (Rogers et al. 2010), or astrocytes (Choi and Friedman 2009). Interestingly, a recent report indicated that trauma-induced upregulation of $p75^{NTR}$ could also result from calcium influx. Within axotomized hippocampal neurons, cellular responses to GABA change from hyperpolarizing to depolarizing, leading to increased intracellular calcium and the subsequent activation of Rho kinase (ROCK). The activation of ROCK resulted in upregulation of $p75^{NTR}$, ultimately leading to neuronal death (Shulga et al. 2012). Thus, multiple signals may contribute to trauma-induced upregulation of the receptor. However, the mechanisms by which these signals increase $p75^{NTR}$ transcription are still poorly understood. The ubiquitous transcription factor Sp1 has been linked to $p75^{NTR}$ basal expression (Poukka et al. 1996) and upregulation following hypo-osmotic stress (Kommaddi et al. 2011a; Ramos et al. 2007), but whether this factor is involved in other forms of injury is not known. It should also be added that long-term treatment of SH-SY5Y

cell lines with IGF1 resulted in a significant upregulation of $p75^{NTR}$ levels (Costantini et al. 2006), suggesting that a factor that responds to IGF1 signaling may also be involved.

In addition to regulating cell death following injury, $p75^{NTR}$ signaling has been suggested to contribute to neurodegeneration caused by a number of diseases. Among these disorders, the link between $p75^{NTR}$ and Alzheimer's disease (AD) has been most studied. Besides Purkinje neurons in the cerebellum, $p75^{NTR}$ is expressed at high levels in cholinergic neurons of the adult basal forebrain, a population of neurons that undergoes severe degeneration early in the progression of AD pathology. Additionally, several in vitro studies have indicated that amyloid beta 1–42 (Aβ), the main component of plaques commonly found within brains of AD patients, is a pro-apoptotic ligand for $p75^{NTR}$ (Costantini et al. 2005; Hashimoto et al. 2004; Yaar et al. 1997). These findings have led to the hypothesis that activation of $p75^{NTR}$ by Aβ contributes to neurodegeneration caused by AD. This idea has remained controversial, however, due to other reports indicating that expression of $p75^{NTR}$ is protective against Aβ-induced toxicity (Bengoechea et al. 2009; Zhang et al. 2003). Nonetheless, a role for $p75^{NTR}$ in Aβ-induced neurotoxicity was recently strengthened by an in vivo finding that deletion of $p75^{NTR}$ prevented the degeneration of cholinergic basal forebrain neurons in vivo following Aβ injection into the hippocampus (Sotthibundhu et al. 2008). Furthermore, when $p75^{NTR}-/-$ mice were crossed with the Thy1-$hAPP^{Lond/Swe}$ mouse model of AD, the degeneration of hippocampal and forebrain cholinergic fibers was dramatically rescued (Knowles et al. 2009). Just as for the in vitro studies, however, these in vivo studies were also challenged by a recent study by Wang et al, which indicated that $p75^{NTR}$ signaling induces production of Aβ, since deletion of the $p75^{NTR}$ gene in the APPswe/PS1dE mouse model of AD resulted in decreased production of Aβ within cortical neurons (Wang et al. 2011). Despite some differences, these findings together suggest that $p75^{NTR}$ signaling by Aβ peptides contributes to overall AD pathology. Apart from Aβ-induced apoptosis, studies have also implicated proNGF in AD pathology. Increased expression of proNGF has been detected in human brains affected by AD (Pedraza et al. 2005; Peng et al. 2004), and proNGF isolated from these brain samples induced $p75^{NTR}$-mediated death of cultured sympathetic neurons (Pedraza et al. 2005; Podlesniy et al. 2006). Thus, in addition to activation of $p75^{NTR}$ by Aβ, enhanced production of proNGF may contribute to neurodegeneration within the AD brain. While these studies provide multiple links between $p75^{NTR}$ signaling and AD-induced neurodegeneration, collective evidence suggests that the degeneration of neurons in AD occurs near the end-stages of the disease (Jack et al. 2010). Hence, understanding whether $p75^{NTR}$ plays a critical role in the onset and early progression of AD remains essential.

While the majority of studies related to $p75^{NTR}$ and neurodegenerative disease have focused on the contributions of the receptor to AD, it is perhaps not surprising that $p75^{NTR}$ has been linked to a number of other disorders. For example, $p75^{NTR}$ may contribute to degeneration of motor neurons during the progression of amyotrophic lateral sclerosis (ALS). Though $p75^{NTR}$ is downregulated in motor

neurons of the spinal cord during the perinatal period, reexpression of the receptor was detected in spinal motorneurons of an ALS mouse model (Copray et al. 2003; Lowry et al. 2001), as well as in spinal cord samples from human patients with ALS (Lowry et al. 2001; Seeburger et al. 1993). Furthermore, the receptor was implicated in ALS-associated motoneuron death by a study in which knockdown of $p75^{NTR}$ delayed locomotor impairment and mortality in the SOD1G93A mouse model of ALS (Turner et al. 2003). However, when the SOD1G93A mice were crossed with the $p75^{NTR}-/-$ mice, prolonged survival was only detected in the female mice, and this improvement did not correlate with increased motorneuron survival, but with reduced astrocytosis (Kust et al. 2003). Nevertheless, the SOD mutation represents a very small fraction of ALS patients, thus further study into the role of the receptor in this disease is warranted.

Degeneration of dopaminergic neurons in Parkinson's disease (PD) could also involve $p75^{NTR}$. A study by Simon and colleagues demonstrated that loss of the *Engrailed* transcription factors results in increased expression of $p75^{NTR}$ in the ventral midbrain (Alavian et al. 2009). This finding has implications for Parkinson's disease because mice deficient in Engrailed-1 and Engrailed-2 exhibit progressive loss of mesencephalic dopaminergic neurons and have PD-like motor deficiencies (Alavian et al. 2009). Importantly, knocking down $p75^{NTR}$ or addition of a receptor-blocking antibody prevented the apoptosis of mesencephalic dopaminergic neurons in cultures from the *engrailed 1, 2* double knockout mice. Upregulation of $p75^{NTR}$ in dopaminergic nigro-striatal neurons has also been reported following kainic-acid treatment (Wang et al. 2008). However, direct evidence for $p75^{NTR}$ expression in nigral dopaminergic neurons in PD and causal evidence linking expression of $p75^{NTR}$ to PD-associated nigral neurodegeneration in vivo is still missing.

In addition to these neurodegenerative conditions, evidence continues to grow implicating $p75^{NTR}$ in the pathology of other neurological diseases. For example, $p75^{NTR}$ has been suggested as having a role in spongiform encephalomyelopathy (Stoica et al. 2008), diabetes-related impairment of neovascularization (Caporali et al. 2008), and psoriasis (Truzzi et al. 2011), among others. The abundance of links between $p75^{NTR}$ and such a variety of diseases indicates that the receptor may function in a broader sense as a stress-induced apoptotic signal that is activated by a mechanism common to all of these pathological conditions. Thus, further elucidation of the mechanisms by which $p75^{NTR}$ is upregulated and activated during these pathological conditions and of the contributions of the receptor to the resulting neurodegeneration may be of critical therapeutic importance.

4 Promotion of Cell Survival

Despite its currently known role in eliciting programmed cell death, early studies of $p75^{NTR}$ demonstrated that in a variety of cellular contexts the receptor has the opposite function: to promote cell survival. Though $p75^{NTR}$-induced apoptosis has been more widely studied, the receptor has been demonstrated to promote cell survival in a wide variety of cell types. One of the first indications that the receptor

can promote neuronal survival came from analysis of *p75^{NTR}*−/− mice, which revealed significant loss of sensory innervation of limbs (Lee et al. 1992). Subsequently, the number of neurons in the dorsal root ganglia (DRG) was reported to be reduced by 50–75 % in the knockout mice (Murray et al. 1999). Although the DRG is a very heterogeneous population of neurons, a decrease in virtually all types of neurons was detected, based on morphological criteria (Bergmann et al. 1997; Gjerstad et al. 2002) or expression of various markers (Jiang et al. 2004). Since then, numerous reports have suggested that p75^{NTR} promotes survival in a wide range of cell types, with the majority suggesting that this is through cooperation with the Trk family, leading to a high affinity receptor complex or enhanced Trk signaling.

4.1 P75^{NTR} Interactions with the Trks

Shortly after TrkA was identified as a receptor for NGF, Chao and colleagues demonstrated that p75^{NTR} interacts with TrkA to form a high-affinity binding complex (Hempstead et al. 1991). While TrkA alone was found to bind NGF with nanomolar affinity, co-expression with p75^{NTR} was discovered to increase this interaction by 100-fold (Esposito et al. 2001; Hempstead et al. 1991). Thus, p75^{NTR} can augment Trk-mediated survival by increasing its interaction with neurotrophins. Given that neurotrophins are typically present in limiting amounts in the target tissues, the presence of high-affinity receptors is an obvious advantage. The requirement for p75^{NTR} in forming the high-affinity complex was initially offered as an explanation for the sensory neuron loss in the animals lacking the receptor. Indeed, neurotrophin dose–response curves revealed that higher doses of NGF were needed to promote survival of sensory and sympathetic neurons from *p75^{NTR}*−/− mice (Davies et al. 1993; Lee et al. 1992). However, a critical element unanswered by this interpretation of the data relates to the fact that, unlike the loss of neurons in the DRG, *p75^{NTR}*−/− mice actually have excess sympathetic neurons (Bamji et al. 1998; Deppmann et al. 2008; Jansen et al. 2007). As discussed above, p75^{NTR} contributes to normal, developmental apoptosis of sympathetic neurons, which could explain the increased neuronal number in the knockout mice, yet why the receptor functions differently in sensory neurons has yet to be resolved.

Although functional interaction between p75^{NTR} and Trk receptors is clear, the molecular details are not fully understood. Surprisingly, the transmembrane and intracellular domains of p75^{NTR}, but not the neurotrophin-binding portion of the extracellular domain, are required for the high-affinity complex (Esposito et al. 2001). Furthermore, structure analysis by X-ray crystallography and complementation assays (using fragments of beta-galactosidase) indicated that complexes of each receptor bind NGF independently and that there is no direct interaction between p75^{NTR} and TrkA (Wehrman et al. 2007). The structural analysis disagrees with many early cross-linking experiments (discussed above) and co-immunoprecipitation studies in HEK293 cells (e.g., Bibel et al. 1999) that

indicate the presence of a complex of both receptors. Clearly, further study is required to resolve the nature of the high affinity complex.

It is also important to note that a $p75^{NTR}$ homolog, neurotrophin receptor homolog 2 (NRH2), was recently identified (Kanning et al. 2003). Like $p75^{NTR}$, NRH2 can also undergo cleavage by TACE and γ-secretase (Kanning et al. 2003), associate with Sortilin (Kim and Hempstead 2009), and form a high-affinity NGF receptor with TrkA (Murray et al. 2004). NRH2 is co-expressed with $p75^{NTR}$ in multiple neuronal subtypes. Thus, understanding how NRH2 and $p75^{NTR}$ function together with their co-receptors is necessary to interpret the phenotype of $p75^{NTR}-/-$ mice.

Remarkably, Trk–$p75^{NTR}$ interactions not only facilitate the formation of a high-affinity receptor complex but also regulate the neurotrophin selectivity of the tyrosine kinase receptor. For example, in the absence of $p75^{NTR}$, TrkA can respond to both NT3 and NGF; however, the Trk–$p75^{NTR}$ complex is highly selective for NGF (Benedetti et al. 1993; Clary and Reichardt 1994). During the development of sympathetic neurons, NT3–TrkA interaction is necessary for neuronal survival (Ernfors et al. 1994; Farinas et al. 1994; Francis et al. 1999). Ginty and colleagues demonstrated that intermediate targets, such as blood vessels, produce NT3 and promote axon growth, but not survival, through TrkA. However, as the neurons innervate their NGF-secreting targets, $p75^{NTR}$ is upregulated, causing TrkA to become selective for NGF over NT3. The NGF–Trk–$p75^{NTR}$ complex is then retrogradely transported to promote survival (Kuruvilla et al. 2004). Hence, $p75^{NTR}$ can function as a switch factor, allowing differential TrkA responses. Similar selectivity is observed with TrkB ligands; co-expression of $p75^{NTR}$ with TrkB increased its selectivity for BDNF over NT3 and NT4 (Bibel et al. 1999).

Beyond regulating the affinity and selectivity of Trks for neurotrophins, $p75^{NTR}$ also potentiates Trk survival signaling. Prevention of neurotrophin binding to $p75^{NTR}$ attenuated TrkA signaling in several in vitro systems (Barker and Shooter 1994; Lachance et al. 1997; Ryden et al. 1997; Verdi et al. 1994). The mechanism by which $p75^{NTR}$ enhances Trk signaling remains poorly understood. Barker and colleagues demonstrated that co-expression of $p75^{NTR}$ with TrkA attenuated TrkA ubiquitylation and delayed the NGF-dependent internalization and degradation of the receptor (Makkerh et al. 2005). Therefore, one mechanism utilized by $p75^{NTR}$ to augment Trk-mediated survival signaling is prolonging cell surface expression of the Trk receptor. Chao and colleagues identified a large transmembrane protein, Ankyrin repeat-rich membrane spanning (ARMS/Kidins220), that interacts with both $p75^{NTR}$ and Trk (Kong et al. 2001). ARMS is tyrosine phosphorylated following neurotrophin treatment and is expressed in many of the neuronal populations that receive neurotrophin stimulation (Kong et al. 2001). While these data suggest that ARMS may serve as a link between $p75^{NTR}$ and Trk receptors, expression of ARMS was discovered to decrease association of TrkA with $p75^{NTR}$ (Chang et al. 2004), and the functional role of the protein has yet to be determined. Recently, however, analysis of mice lacking ARMS revealed substantial apoptosis of sensory neurons (Cesca et al. 2011), a phenotype similar to that observed in $p75^{NTR}-/-$ animals (Murray et al. 1999), thus further highlighting the potential

importance of this interactor in regulation of cell survival through $p75^{NTR}$–Trk interaction.

In addition to $p75^{NTR}$ regulating Trk function at the cell surface, there is evidence that intracellular signaling pathways are modulated by co-activation of the receptors. One such signaling event particularly affected by this interaction is activation of the pro-survival kinase Akt. Treatment of PC12 cells with NGF in the presence of an antibody that blocked binding to $p75^{NTR}$ inhibited the activation of Akt (Bui et al. 2002). Similarly, silencing of $P75^{NTR}$ in PC12 cells or cerebellar granule neurons reduced neurotrophin-induced activation of the kinase (Ceni et al. 2010). These authors also reported that activation of Akt required proteolysis of $p75^{NTR}$. In contrast to what was observed for $p75^{NTR}$ in sympathetic neurons (Kenchappa et al. 2006), Ceni et al. reported that the cleavage of $p75^{NTR}$ was induced by Trk activation. They have since extended these findings, demonstrating that Trk activation promoted phosphorylation of TACE, which activated the protease and lead to cleavage of $p75^{NTR}$, which was necessary for potentiation of neurotrophin-induced survival signaling (Kommaddi et al. 2011b).

Although $p75^{NTR}$ can function in cooperation with Trks to promote survival signals, Friedman and colleagues found that in basal forebrain neurons, $p75^{NTR}$ reduced Akt signaling by increasing the levels of active PTEN (phosphatase and tensin homolog deleted on chromosome 10), an inhibitor of the PI3 kinase–Akt pathway (Song et al. 2010). ProNGF binding to $p75^{NTR}$ upregulated PTEN, which resulted in apoptosis of the neurons, even if TrkB was activated by BDNF. Since proNGF signals through binding a complex of p75NTR and Sortilin, the effects of $p75^{NTR}$ on Akt activity appears to depend on its co-receptor.

4.2 $P75^{NTR}$ Activation of NFκB

Apart from enhancing Trk survival signaling, there is evidence that $p75^{NTR}$ can activate an independent pro-survival signal; for example, selective activation of $p75^{NTR}$ prevented the death of certain neuroblastoma (Cortazzo et al. 1996) and breast cancer cells (Verbeke et al. 2010), of hippocampal neurons treated with NMDA (Bui et al. 2002), and of both sensory neurons (Longo et al. 1997) and cortical subplate neurons deprived of trophic support (DeFreitas et al. 2001). In addition, the receptor appears to play a protective role after certain injuries; e.g., $p75^{NTR}-/-$ mice have increased death of primary auditory neurons following acoustic trauma (Tan et al. 2010). The pro-survival effects of $p75^{NTR}$ appear to be more of a modulatory signal, as they are not as potent as the effects of tyrosine kinases like the Trks. The molecular mechanisms by which $p75^{NTR}$ promotes survival independent of the Trk receptors are not fully understood; however, one downstream pathway that has been identified involves the transcription factor nuclear factor kappa B (NFκB). NFκB is best characterized for its role in the immune system, where it is activated by many cytokine and Toll-like receptors, leading to upregulation of other cytokines and pro-survival genes (Baldwin 2012). The activation of NFκB by $p75^{NTR}$ was first reported in Schwann cells

(Carter et al. 1996) and has since been demonstrated in a variety of cell types, including Schwannoma cells (Gentry et al. 2000), primary Schwann cells (Khursigara et al. 2001), trigeminal neurons (Hamanoue et al. 1999), and hippocampal neurons (Culmsee et al. 2002). NFκB exists as a dimer, held in the cytosol through binding to its inhibitor IκB. The transcription factor is activated through phosphorylation of IκB by the IκB kinase (IKK) complex, leading to proteasomal degradation of the inhibitor and release of the NFκB dimer to translocate into the nucleus (Baldwin 2012). As mentioned above, neurotrophin binding to $p75^{NTR}$ can recruit members of the TRAF family, which activate the IKK complex (Ha et al. 2009; Hacker et al. 2011); specifically, TRAF6 was shown to mediate activation of NFκB, as Schwann cells from *traf6−/−* mice did not respond to $p75^{NTR}$ activation (Yeiser et al. 2004). Since TRAF6 promotes both NFκB and JNK activation, it was recognized as a potential nodal point for determining survival vs. apoptotic signaling. How TRAF6 selectively promotes one pathway over the other remains to be fully elucidated; however, the finding that the adaptor protein receptor-interacting protein 2 (RIP2) directly associates with the death domain of $p75^{NTR}$ provided an important clue. Chao and colleagues demonstrated that expression of RIP2 in Schwann cells conferred NGF-dependent activation of NFκB through interaction with TRAF6. Expression of RIP2 in these cells also reduced JNK activation and the subsequent apoptosis (Khursigara et al. 2001). Thus, RIP2 expression may serve as the key toggle, switching TRAF6 signaling to NFκB from JNK (Fig. 1).

Another well-established activator of NFκB is Akt. Although evidence suggests that $p75^{NTR}$ enhances Trk activation of Akt, as discussed above, $p75^{NTR}$ has also been reported to promote Akt activation in a manner that was independent of Trk signaling in hippocampal neurons (Arevalo and Rodriguez-Tebar 2006), melanoma cells, and mutant PC12 cells lacking TrkA (Roux et al. 2001). Therefore, NFκB may be among the downstream pro-survival signals activated by $p75^{NTR}$ in some contexts.

It is also notable that the $p75^{NTR}$ was recently shown to regulate the stability of HIF1a, a transcription factor induced by oxidative stress that controls the expression of a wide variety of genes involved in protection from reactive oxygen species and, importantly, promoting cell survival (Hu et al. 2003). Le Moan et al. (2011) reported that the ICD of the receptor can bind the E3 ubiquitin ligase Siah2, which targets HIF1a for degradation. The interaction between $p75^{NTR}$-ICD and Siah2 lead to upregulation of HIF1a and increased expression of vascular endothelial growth factor, which promoted angiogenesis after retinal hypoxia. While the authors did not address a potential role in regulating survival, given that many target genes of HIF1a are pro-survival, it will be interesting to determine whether this pathway has a role in promoting neuronal survival in response to activation of the receptor.

Because of the ability of $p75^{NTR}$ to augment Trk-mediated survival signaling and ligand selectivity, the overall effect of $p75^{NTR}$ on cell survival is quite variable by cell type and highly dependent upon the presence or absence of the Trk receptor. In general, though not always, simultaneous activation of both Trk receptors and $p75^{NTR}$ by mature neurotrophins results in cell survival. However, selective activation of $p75^{NTR}$ by neurotrophins in the absence of Trk-receptor activation more

often promotes cell death than survival. For example, NGF treatment of sympathetic neurons, which express both $p75^{NTR}$ and TrkA, promotes neuronal survival. Stimulation of these neurons with BDNF, however, results in apoptosis, as these neurons do not express TrkB (Bamji et al. 1998). The proliferative state of the cell also may influence the effects of $p75^{NTR}$ signaling, as the majority of reports describing $p75^{NTR}$ mediated cell death have involved post-mitotic neurons, while studies of $p75^{NTR}$ in proliferative cells have revealed more variable survival outcomes (Skeldal et al. 2011).

5 Regulation of the Cell Cycle

One of the first effects described for selective activation of $p75^{NTR}$ in the absence of Trks was cell cycle arrest in a glioma cell line treated with NGF (Dobrowsky et al. 1994). The effects of $p75^{NTR}$ on cell cycle have since been linked to several receptor-interacting proteins, including SC-1 (Chittka et al. 2004), NRIF (Benzel et al. 2001), and NRAGE (Salehi et al. 2000). Like NRIF, SC-1 is a C2H2 zinc finger-containing protein, and translocation of SC-1 to the nucleus was demonstrated in response to NGF binding to $p75^{NTR}$ in transfected COS cells (Chittka and Chao 1999). Expression of SC-1 in these cells blocked cell proliferation through a mechanism involving repression of cyclin E transcription (Chittka et al. 2004). Chao and colleagues have also demonstrated that expression of $p75^{NTR}$-ICD inhibited cyclin E mRNA production in HeLa cells, and the endogenous $p75^{NTR}$-ICD in PC12 cells could be localized to the cyclin E promoter by chromatin immunoprecipitation following NGF treatment (Parkhurst et al. 2010). These results suggest that the ICD of $p75^{NTR}$ translocates to the nucleus along with SC-1 to modulate genes involved in the cell cycle. Whether SC-1 has a role in the receptor's apoptotic signal remains an open question.

NRIF (Benzel et al. 2001) and NRAGE (Salehi et al. 2000) have also been implicated in the regulation of cell cycle based on the observation that ectopic expression of either factor in HEK 293 cells induced cell cycle arrest. It is interesting that these two proteins inhibit proliferation in immortalized fibroblasts but cause apoptosis when expressed in neurons, Schwann cells (Linggi et al. 2005), or neural precursors (Salehi et al. 2000, 2002). Perhaps the differential effects relate to the presence of the tumor suppressor p53, which is mutated or inhibited in many immortalized cells, including HEK 293s. Cell death induced by ectopic expression of NRIF is dependent on p53 (Linggi et al. 2005) and over-expression of NRAGE in breast cancer cells upregulated p53 (Du et al. 2009). One interpretation of these findings is that in response to $p75^{NTR}$ activation, NRIF and NRAGE may alter expression of key cell cycle genes, thereby triggering an increase in p53 expression, ultimately resulting in cell death, but in cells with p53 mutated or blocked, the effects are limited to inhibiting proliferation.

6 Regulation of Synaptic Plasticity

In addition to regulating neuronal survival, the balance of signaling between the Trks and $p75^{NTR}$ plays an important role in modulating synaptic efficacy. BDNF, acting through TrkB, is essential for the strengthening of synaptic function, referred to as long-term potentiation (LTP) (Figurov et al. 1996; Kang et al. 1997; Korte et al. 1998; Patterson et al. 2001). In contrast, $p75^{NTR}$ has a critical role in the weakening of synaptic connections, a process called long-term depression (LTD). Analysis of $p75^{NTR}$ knockout mice revealed a deficiency in the ability to induce LTD in hippocampal slices, although LTP was not impaired (Rosch et al. 2005; Woo et al. 2005). The mechanism by which $p75^{NTR}$ regulates synaptic function has yet to be fully resolved; however, glutamate receptor expression appears to be altered in the null animals. Lu and colleagues found reduced levels of the NMDA receptor subunit NR2B in $p75^{NTR}-/-$ hippocampal lysates, and NMDA currents measured in slices from the null mice were not affected by the NR2B antagonist ifenprodil, while in wild types they were blocked (Woo et al. 2005). In addition, Korte's group found impaired AMPA receptor function and decreased levels of the AMPA receptor subunits GluR2 and 3 in the hippocampus of $p75^{NTR}-/-$ mice (Rosch et al. 2005). Glutamatergic signaling is essential for the development of LTD, and both NMDA and AMPA receptors have been implicated as contributing to the synaptic changes, depending on the mechanism of induction (Hunt and Castillo 2012). Therefore, the altered expression of these receptors in $p75^{NTR}-/-$ mice likely contributes to their inability to induce LTD. Furthermore, these changes in glutamatergic signaling may underlie the impairments in learning, inhibitory avoidance, and habituation that have been observed in the $p75^{NTR}$ null mice (Peterson et al. 1999).

$P75^{NTR}$ may also regulate synaptic plasticity through modulating dendritic structure. No gross morphological changes in the structure of the hippocampus have been detected in the $p75^{NTR}-/-$ animals; however, careful measurements of dendritic spines revealed an increase in their density and complexity (Zagrebelsky et al. 2005). Moreover, immunoelectron microscopy revealed that $p75^{NTR}$ is expressed on dendritic shafts and spines in the hippocampus (Woo et al. 2005). These results suggest that the receptor may be important for normal pruning and refining of these postsynaptic structures. Correspondingly, overexpression of $p75^{NTR}$ in hippocampal slices resulted in reduced spine density and complexity. Decreases in spine size and number are associated with LTD (Okamoto et al. 2004; Zhou et al. 2004) and increases in size and number correlate with LTP (Desmond and Levy 1986; Fifkova and Anderson 1981; Van Harreveld and Fifkova 1975); therefore, it is interesting to speculate that during LTD induction, $p75^{NTR}$ may cause retraction of dendritic spines. As described below, the receptor can activate the Rho family of GTPases, which regulate the actin-cytoskeleton (Yamashita et al. 1999). Thus, local dendritic activation of the receptor may result in collapse or shrinkage of a spine through activation of Rho or inhibition of Rac, ultimately resulting in reduced synaptic efficacy. However, how morphological changes in dendritic spines affect synaptic plasticity remains an open question.

The ligand responsible for activating $p75^{NTR}$ during LTD has been suggested to be proBDNF; however, this remains somewhat controversial. Pang et al. (2004) reported that proBDNF cleavage by the extracellular protease plasmin to produce mature BDNF was required for the induction of LTP. Previous studies demonstrated that tissue plasmingen activator (tPA), which activates plasmin from plasminogen, is secreted from axon terminals (Krystosek and Seeds 1981) and is required for LTP (Baranes et al. 1998; Frey et al. 1996; Huang et al. 1996), but what role the protease played in the process was not clear. The finding that tPA targets proBDNF provided a relevant substrate. Moreover, the fact that tPA/plasmin is acting extracellularly suggested that proBDNF is released into the synaptic cleft, where it could activate $p75^{NTR}$ if it's not cleaved. Indeed, Woo et al. (2005) found that perfusion of hippocampal slices with proBDNF enhanced LTD, but slices from $p75^{NTR}-/-$ mice were insensitive. More recently, this group also demonstrated that synaptic competition is regulated by a balance between pro- and mature-BDNF. Using cocultures of *Xenopus* myocytes and motor neurons, they demonstrated that when two neurons innervate one myocyte, the active terminal promotes cleavage of proBDNF to mature BDNF, which stabilizes the synapse through activation of TrkB. In contrast, at the less active terminal proBDNF is not cleaved and causes axon retraction through binding to $p75^{NTR}$ (Je et al. 2012). Such activity-dependent synaptic competition is a common principle in many areas of the developing nervous system, although the underlying molecular mechanisms are not known. The finding that pro-/mature BDNF levels can be dynamically regulated to act as the punishment and reward signal provides some important clues as to the mechanisms that could underlie this competition. Nevertheless, despite these elegant studies, results from the Barde group cast doubt on a role for proBDNF in synaptic plasticity. They reported that proBDNF was not secreted by hippocampal neurons in culture and that the induction of LTD was unaffected by conditional deletion of BDNF (both the pro- and mature-form of the neurotrophin) in neurons (Matsumoto et al. 2008). Clearly, additional studies are needed to fully understand the role of $p75^{NTR}$ in synaptic plasticity.

7 Promotion of Peripheral Myelination

All cells of the neural crest lineage express $p75^{NTR}$ during development, including those that become Schwann cells in the sciatic nerve. Schwann cells continue to express $p75^{NTR}$ until myelination, at which time it is downregulated. This reduction in its expression was attributed to axonal signals, whose identity still remains unknown (Lemke and Chao 1988). Due to its regulated expression in the sciatic nerve, questions have persisted as to whether $p75^{NTR}$ plays a role in some aspects of the myelination process by Schwann cells. Anton et al. (1994) first reported that $p75^{NTR}$ is involved in migration of Schwann cells: Schwann cell migration out of DRGs onto sciatic nerve explants was enhanced by NGF treatment, but REX, a $p75^{NTR}$ antibody, blocked its effect. The effect was, however, observed only with explants from denervated sciatic nerves and not with intact sciatic nerves,

suggesting that $p75^{NTR}$ may play a role only after injury. When Schwann cell migration was examined during embryogenesis in trigeminal ganglia, however, $p75^{NTR}$ did have an effect: the extent of Schwann cell migration to the axon tips was significantly reduced in $p75^{NTR}$ knockout mice compared to littermates (Bentley and Lee 2000). Since trigeminal ganglion neurons also express $p75^{NTR}$, Bentley and Lee examined Schwann cell migration in vitro using sciatic nerve explants. The extent of Schwann cell migration was reduced in cultures from $p75^{NTR}$ knockout mice compared to that in the wild-type counterparts, although NGF addition had no effect. These results suggest that $p75^{NTR}$ expression specifically in Schwann cells regulates their migration both during development and after injury.

Schwann cells have to migrate along the axon before they form myelin sheaths around axons. The fact that $p75^{NTR}$ plays a role in Schwann cell migration suggests that the receptor could regulate the myelination process. This question was first addressed by Eric Shooter's group in 2002. Cosgaya et al. (2002) reported that blocking $p75^{NTR}$ signaling resulted in inhibition of myelin formation both in DRG-Schwann cell cocultures as well as in developing sciatic nerves. In particular, EM analysis of sciatic nerves that were injected with REX antibody at P0, before the onset of myelination, revealed that the myelin sheath became thinner 4 days later, without affecting the number of myelinated axons, suggesting that $p75^{NTR}$ promotes Schwann cell myelination in vivo. As for the ligand that activates $p75^{NTR}$ in Schwann cells, BDNF, which the group had previously reported to promote Schwann cell myelination (Chan et al. 2001), was shown to be responsible. Attributing the effect of BDNF to $p75^{NTR}$ in Schwann cells was indirect, however, because $p75^{NTR}$ is expressed both in axons and Schwann cells at the neonate stage in the sciatic nerve. For instance, Cosgaya et al. (2002) illustrated that while the full-length TrkB levels were barely detectable, $p75^{NTR}$ levels were very high in Schwann cells. While modulating BDNF signaling by either injecting excess BDNF or scavenging the neurotrophin with TrkB-Fc affected myelination in wild type mice, no effect on myelination was observed in $p75^{NTR}$ knockout mice. Since $p75^{NTR}$ is the only receptor in Schwann cells that could elicit downstream signaling to promote myelination, the lack of effect was attributed to BDNF acting through $p75^{NTR}$ in Schwann cells.

The idea that BDNF promotes myelination through $p75^{NTR}$ in Schwann cells was challenged by the Murray group (Xiao et al. 2009). In their study, a DRG-Schwann cell coculture system was utilized, wherein $p75^{NTR}$ expression was regulated both in DRG and Schwann cells independently. To delete $p75^{NTR}$ from DRG neurons, DRG neurons were isolated from $p75^{NTR}$ knockout mice, maintained in NGF, and subsequently seeded with rat Schwann cells. To knock-down $p75^{NTR}$ in Schwann cells, they transduced rat Schwann cells with $p75^{NTR}$-shRNA prior to seeding them onto NGF-dependent DRG neurons. Surprisingly, adding BDNF to the NGF-dependent DRG neurons from $p75^{NTR}$ knockout mice failed to promote myelination, while in wild-type counterparts BDNF increased myelin protein levels by 1.5–2-fold. When $p75^{NTR}$ expression was knocked down in Schwann cells before they were seeded onto NGF-dependent DRG neurons, however, there was little effect, regardless of whether BDNF was present or not.

This study thus demonstrated that it is $p75^{NTR}$ in DRG neurons and not in Schwann cells that influences Schwann cell myelination.

Tep et al., on the other hand, reported opposite results by using the same DRG-Schwann cell coculture system, wherein $p75^{NTR}$ expression was knocked down only in Schwann cells before they were seeded onto NGF-dependent DRG neurons (Tep et al. 2012): the number of myelinated nerves were reduced by $\geq$50 %. As for the reason why the two studies differ, Tep et al. stated that while Xiao et al. isolated DRG neurons at P2 and cultured them in the presence of NGF for 2–3 weeks, Tep et al. cultured DRG neurons from embryonic day 15 animals and cultured them for a week before Schwann cells were seeded. It is not clear whether the age of DRG neurons subjected to myelination in culture is responsible for the opposite outcome, since the studies were conducted independently. Since $p75^{NTR}$ was shown to affect the extent of remyelination after injury (Song et al. 2006), it is possible that under injury conditions in the adult PNS, $p75^{NTR}$ expressed in DRG neurons controls aspects of remyelination. What now awaits is the analysis of conditional $p75^{NTR}$ knockout mice in Schwann cells and neurons, individually, to resolve the issue.

Regardless of where $p75^{NTR}$ exerts its effect, the question remained as to what signaling pathway $p75^{NTR}$ employs to promote myelination in Schwann cells. $P75^{NTR}$ was shown to regulate small GTPases that are critical for cytoskeletal reorganization, such as RhoA (Domeniconi et al. 2005; Harrington et al. 2008; Passino et al. 2007; Wang et al. 2002; Yamashita et al. 1999, 2002; Yamashita and Tohyama 2003; Yamauchi et al. 2004) and Rac1 (Deinhardt et al. 2011; Harrington et al. 2002; Tep et al. 2012) in a variety of tissues both in and outside of the nervous system. During Schwann cell myelination, $p75^{NTR}$-mediated Rac1 activation appears to be important, as partitioning-defective 3 (Par3) was shown to bind $p75^{NTR}$ through its PDZ-binding tri-peptide motifs in Schwann cells (Chan et al. 2006). Par3 is a member of the polarization complex that includes Par6 and protein kinase C, regulating activation of Rac1 and Cdc42 (Goldstein and Macara 2007). In Schwann cells, Par3 was colocalized with $p75^{NTR}$ at axon–glial interfaces in response to BDNF (Chan et al. 2006) and was shown to mediate Rac1 activation by BDNF (Tep et al. 2012). Rac1 activation was also significantly reduced in neonate sciatic nerves from $p75^{NTR}$ knockout mice, supporting the notion that $p75^{NTR}$ activates Rac1 as it forms a complex with Par3 at the axon–glial interface (Tep et al. 2012). Thus, Rac1 activation appears to be a pathway induced by $p75^{NTR}$ that regulates Schwann cell myelination.

8 Regulation of Neurite Growth and Axonal Degeneration

Although $p75^{NTR}$ was shown to activate members of the Rac/Rho family in Schwann cells, thereby affecting myelination, the role of $p75^{NTR}$-dependent RhoA activation has been best studied in the context of neurite outgrowth. The regulation of RhoA by $p75^{NTR}$ was first demonstrated by Yamashita et al, who identified RhoA as a receptor-interacting factor in a yeast two-hybrid screen. They

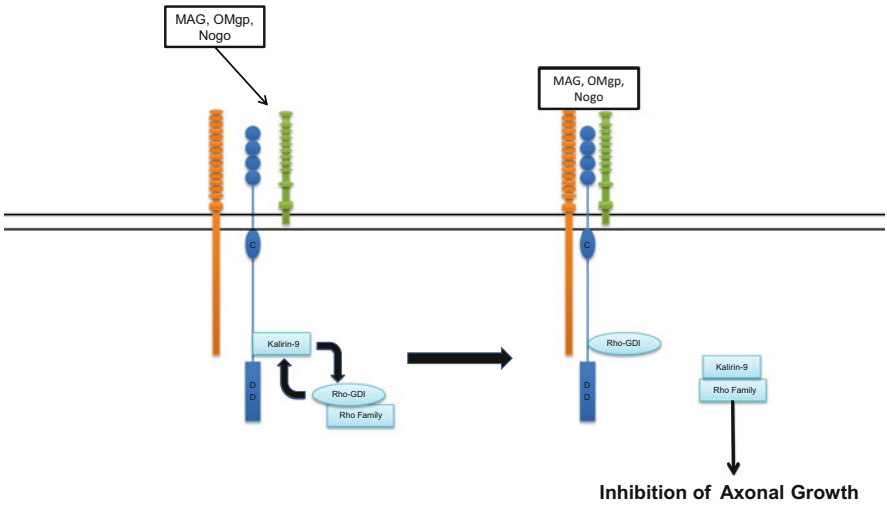

Fig. 3 Regulation of axonal growth by $p75^{NTR}$. A complex comprised of $p75^{NTR}$, NogoR, and Lingo-1 is formed in response to myelin-derived inhibitors such as MAG, OMgp, and Nogo. In response to stimulation by these ligands, Rho-GDI is recruited to the intracellular domain of $p75^{NTR}$. Concurrently, association of Kalirin-9 with the $p75^{NTR}$ intracellular domain is decreased, thus resulting in enhanced activation of Rho family members by Kalirin-9 and inhibition of axonal growth

found that unliganded $p75^{NTR}$ promoted RhoA activation and neurotrophin binding prevented this activation, thereby promoting neurite outgrowth (Yamashita et al. 1999). Soon after this finding, $p75^{NTR}$ was shown to regulate RhoA in response to a group of myelin proteins, including myelin associated glycoprotein (MAG), Oligodendrocyte myelin glycoprotein (OMgp), and Nogo (Wang et al. 2002; Wong et al. 2002; Yamashita et al. 2002). These myelin proteins are expressed by oligodendrocytes and are known to inhibit neurite outgrowth, which has made them a major focus for studies aimed at axonal regeneration after spinal cord injury (Akbik et al. 2012). All three of these inhibitors bind to a glycosylphosphatidylinositol (gpi)-linked receptor, the Nogo receptor (NgR). The NgR lacks an intracellular domain, but it can associate with $p75^{NTR}$ and another transmembrane protein, Lingo-1, to regulate RhoA (Mi et al. 2004) (Fig. 3).

The ability of $p75^{NTR}$ to regulate Rho family members depends on a number of intracellular interacting factors. The activation of RhoA by myelin proteins occurs through the recruitment of the Rho inhibitor RhoGDI to $p75^{NTR}$, thereby releasing RhoA (Yamashita and Tohyama 2003). RhoGDI competes with the guanine nucleotide exchange factor (GEF), Kalirin 9, for $p75^{NTR}$ binding (Harrington et al. 2008), such that upon receptor activation, Kalirin 9 is released, allowing binding of RhoGDI. Once RhoA is freed from RhoGDI, it is then activated by Kalirin 9 (Fig. 3). It should be added that RhoA activation by MAG in cerebellar granule neurons required cleavage of $p75^{NTR}$ (Domeniconi et al. 2005). It is not known

how receptor cleavage facilitates the stimulation of Rho or whether other myelin inhibitory molecules that bind the NgR–$p75^{NTR}$–Lingo-1 complex also induce $p75^{NTR}$ cleavage. In addition to regulating RhoA, Hempstead's group recently demonstrated that proneurotrophin binding to $p75^{NTR}$ caused a decrease in Rac activity. Rac typically counterbalances Rho, such that decreasing Rac has a similar effect to activating Rho. The reduction in Rac activation after proneurotrophin binding was linked to dissociation of the Rac GEF Trio from a complex of $p75^{NTR}$ with another Sortilin family member, SorCS2, suggesting that the release of Trio reduces basal Rac activity (Deinhardt et al. 2011). Hence, there may be multiple mechanisms by which $p75^{NTR}$ regulates members of the Rho family, depending on the ligand and the co-receptors present.

As might be expected, evidence suggests that $p75^{NTR}$ can regulate axonal growth and retraction at the tip of the growing axon in the growth cone. Treatment of *Xenopus* spinal neurons with MAG caused $p75^{NTR}$-dependent repulsion of the growth cone (Wong et al. 2002). Similarly, proneurotrophins were recently reported to induce growth cone collapse in cortical neurons (Sun et al. 2012) and in hippocampal neurons (Deinhardt et al. 2011). In contrast to the inhibitory effects of proneurotrophins and myelin proteins on growth cones, mature neurotrophin binding to $p75^{NTR}$ increased filopodial length in the growth cone of subplate neurons (McQuillen et al. 2002), as well as in retinal ganglion and dorsal root ganglion cells, where it was demonstrated that the receptor promoted filopodia growth through inhibition of RhoA (Gehler et al. 2004).

The recognition of $p75^{NTR}$ as a signal transducer for MAG, OMgp and Nogo created a considerable amount of interest in targeting the receptor as a means of promoting regeneration following CNS injury; however, the regulation of axonal regeneration is very complex, and the role of $p75^{NTR}$ is not well understood. Despite the fact that $p75^{NTR}$ null neurons failed to activate RhoA and respond to myelin inhibitors in culture (Wang et al. 2002; Yamashita et al. 2002; Yamashita and Tohyama 2003; Zheng et al. 2005) and after spinal cord injury (Dubreuil et al. 2003), there was no effect on regeneration of corticospinal tract after spinal cord injury in the $p75^{NTR}$ knockout mice (Song et al. 2004; Zheng et al. 2005). The reason why $p75^{NTR}$ does not appear to regulate regeneration after spinal cord injury is not clear, but this may be due to the fact that $p75^{NTR}$ expression is upregulated in oligodendrocytes as well as denervated axons following spinal cord injury (Beattie et al. 2002; Tep et al. 2013), potentially confounding its effect on regeneration. It is also possible that the NgR can partner with other receptors in addition to $p75^{NTR}$; for example, another member of the TNF receptor superfamily, Troy, was shown to function as a signal transducer for the NgR (Park et al. 2005; Shao et al. 2005). In addition, another receptor, PirB, was identified that also binds myelin inhibitory molecules (Atwal et al. 2008), and Fujita et al. (2011b) reported that myelin inhibitory molecules induced interaction between the PirB and $p75^{NTR}$ in cerebellar granule neurons. In that study, the authors demonstrated that optic nerve regeneration was significantly improved after injury in $p75^{NTR}$ knockout mice, suggesting that $p75^{NTR}$ does play a role in regeneration at least in the optic system, which is less complex than spinal cord injury in terms of affected cell types and the cellular

responses to injury. The question remains, however, whether this effect is mediated through the interaction of $p75^{NTR}$ with PirB, since PirB knockout mice had no effect on optic nerve regeneration (Fujita et al. 2011a). The ultimate outcome of whether $p75^{NTR}$ promotes axon regrowth or retraction after an injury may depend, in part, on the type of injury and the different ligands produced that bind to $p75^{NTR}$ and its signaling partners: $p75^{NTR}$ interacts with Lingo-1 and NgR or PirB in response to myelin inhibitory molecules (Mi et al. 2004; Wang et al. 2002), Sortilin in response to proNGF (Nykjaer et al. 2004), EphA in response to Ephrin-As (Lim et al. 2008), in addition to TrkA, TrkB, and TrkC in response to NGF, BDNF, and NT3.

Does myelin play a similarly inhibitory role in intact brains in the absence of any injury? Freda Miller's group addressed this question by examining axonal sprouting of $p75^{NTR}$-expressing cholinergic septal neurons into the corpus callosum in the adult brain (Park et al. 2010). Surprisingly, there were many more cholinergic fibers entering the corpus callosum in *$p75^{NTR}$* knockout than in the wild type mice, suggesting that $p75^{NTR}$ normally prevents misrouting of these fibers in vivo. $P75^{NTR}$ appeared to have induced degeneration of the fibers that entered the white matter tract, since cholinergic axons degenerated when plated on myelin. Similar axonal breakdown was observed in cultured sympathetic neurons when BDNF was applied selectively to axons while the soma was maintained in NGF (Singh et al. 2008). The axonal degeneration induced by $p75^{NTR}$ in vitro and in vivo extended beyond the collapse of growth cones, as there was overt breakdown of axonal fibers; however, there was no apoptosis of the neurons. The signaling mechanisms involved in the degeneration appear to involve both Rho and caspase-6, as activation of both enzymes was detected in axons following stimulation of $p75^{NTR}$, and inhibition of either Rho or caspase-6 blocked the axonal breakdown (Park et al. 2010). Local activation of caspases in distal axons during axonal degeneration has been increasingly reported; for example, degeneration due to withdrawal of NGF selectively from distal axons of DRG neurons grown in compartmentalized chambers was dependent on both caspase-3 and -6 (Simon et al. 2012). These caspases were not activated in the soma; thus the neurons did not undergo apoptosis. How such degenerative/apoptotic signaling is restricted to the axon is not known. The caspase inhibitor XIAP is also upregulated in mature sympathetic neurons, making them resistant to injection of cytochrome c (Potts et al. 2003). Thus, NGF acting at the soma may prevent the spread of caspase activation through upregulation of such inhibitors. Additional studies are needed to understand the spatial control of such signaling pathways.

Conclusion

Since the initial cloning of $p75^{NTR}$, the field has undergone a remarkable transformation in its understanding of this puzzling receptor. Initially, $p75^{NTR}$ was viewed as a non-signaling, auxiliary binding component for members of the Trk family that simply enabled neurotrophins to bind with high affinity to the Trks. Now, $p75^{NTR}$ is considered a key signaling component for multiple ligands through the formation of a variety of receptor complexes that regulate a wide

array of biological responses, from cell survival to neurite growth, axon degeneration, or myelin formation. Many questions remain to be answered regarding the mechanisms utilized by $p75^{NTR}$ to mediate its responses, such as how the receptor can selectively activate one pathway over another, some of which can be in direct opposition (e.g., survival vs. apoptosis). The genes regulated by receptor proteolysis and the ICD-binding proteins that translocate to the nucleus are also yet to be identified. What role Sortilin and other Vps10p family members play in $p75^{NTR}$ signaling beyond binding to the pro-domain of proneurotrophins remains to be determined. Even the molecular nature of the high affinity complex between $p75^{NTR}$ and the Trks is still poorly understood. Beyond these questions regarding receptor signaling, it will be equally important to explore the role of the receptor in the many injuries and diseases where it is upregulated. Given that $p75^{NTR}$ has the potential to signal both survival and degeneration/apoptosis, it is enticing to consider the possibility of developing novel therapeutics that switch the receptor's degeneration signal to one promoting survival. Certainly, further understanding of how the receptor functions will provide additional insights for developing novel therapeutics for many neuropathologies.

In this chapter, we attempted to cover the many aspects of signaling through the $p75^{NTR}$ receptor and the biological outcomes; nevertheless, we could not fully encompass the many contributions made by the large number of researchers who have studied this complex system. We apologize for those omissions.

Acknowledgments This work was supported by grants NS038220 (B.D.C. and B.K.) and NS039472 and NS050585 (S.O.Y.) from the National Institutes of Health.

References

Agerman K, Baudet C, Fundin B, Willson C, Ernfors P (2000) Attenuation of a caspase-3 dependent cell death in NT4- and p75-deficient embryonic sensory neurons. Mol Cell Neurosci 16:258–268

Akbik F, Cafferty WB, Strittmatter SM (2012) Myelin associated inhibitors: a link between injury-induced and experience-dependent plasticity. Exp Neurol 235:43–52

Alavian KN, Sgado P, Alberi L, Subramaniam S, Simon HH (2009) Elevated P75NTR expression causes death of engrailed-deficient midbrain dopaminergic neurons by Erk1/2 suppression. Neural Dev 4:11

Ali TK, Al-Gayyar MM, Matragoon S, Pillai BA, Abdelsaid MA et al (2011) Diabetes-induced peroxynitrite impairs the balance of pro-nerve growth factor and nerve growth factor, and causes neurovascular injury. Diabetologia 54:657–668

Aloyz RS, Bamji SX, Pozniak CD, Toma JG, Atwal J et al (1998) p53 is essential for developmental neuron death as regulated by the TrkA and p75 neurotrophin receptors. J Cell Biol 143:1691–1703

Anton ES, Weskamp G, Reichardt LF, Matthew WD (1994) Nerve growth factor and its low-affinity receptor promote Schwann cell migration. Proc Natl Acad Sci U S A 91:2795–2799

Arevalo MA, Rodriguez-Tebar A (2006) Activation of casein kinase II and inhibition of phosphatase and tensin homologue deleted on chromosome 10 phosphatase by nerve growth factor/p75NTR inhibit glycogen synthase kinase-3beta and stimulate axonal growth. Mol Biol Cell 17:3369–3377

Atwal JK, Pinkston-Gosse J, Syken J, Stawicki S, Wu Y et al (2008) PirB is a functional receptor for myelin inhibitors of axonal regeneration. Science 322:967–970

Baldwin AS (2012) Regulation of cell death and autophagy by IKK and NF-kB: critical mechanisms in immune function and cancer. Immunol Rev 246:327–345

Baldwin AN, Shooter EM (1995) Zone mapping of the binding domain of the rat low affinity nerve growth factor receptor by the introduction of novel N-glycosylation sites. J Biol Chem 270:4594–4602

Bamji SX, Majdan M, Pozniak CD, Belliveau DJ, Aloyz R et al (1998) The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. J Cell Biol 140:911–923

Baranes D, Lederfein D, Huang YY, Chen M, Bailey CH, Kandel ER (1998) Tissue plasminogen activator contributes to the late phase of LTP and to synaptic growth in the hippocampal mossy fiber pathway. Neuron 21:813–825

Barker PA, Shooter EM (1994) Disruption of NGF binding to the low affinity neurotrophin receptor p75LNTR reduces NGF binding to TrkA on PC12 cells. Neuron 13:203–215

Beattie MS, Harrington AW, Lee R, Kim JY, Boyce SL et al (2002) ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. Neuron 36:375–386

Becker EB, Howell J, Kodama Y, Barker PA, Bonni A (2004) Characterization of the c-Jun N-terminal kinase-BimEL signaling pathway in neuronal apoptosis. J Neurosci 24:8762–8770

Benedetti M, Levi A, Chao MV (1993) Differential expression of nerve growth factor receptors leads to altered binding affinity and neurotrophin responsiveness. Proc Natl Acad Sci U S A 90:7859–7863

Bengoechea TG, Chen Z, O'Leary DA, Masliah E, Lee KF (2009) p75 reduces beta-amyloid-induced sympathetic innervation deficits in an Alzheimer's disease mouse model. Proc Natl Acad Sci U S A 106:7870–7875

Bentley CA, Lee KF (2000) p75 is important for axon growth and schwann cell migration during development. J Neurosci 20:7706–7715

Benzel I, Barde YA, Casademunt E (2001) Strain-specific complementation between NRIF1 and NRIF2, two zinc finger proteins sharing structural and biochemical properties. Gene 281:19–30

Bergmann I, Priestley JV, McMahon SB, Brocker EB, Toyka KV, Koltzenburg M (1997) Analysis of cutaneous sensory neurons in transgenic mice lacking the low affinity neurotrophin receptor p75. Eur J Neurosci 9:18–28

Bertrand MJ, Kenchappa RS, Andrieu D, Leclercq-Smekens M, Nguyen HN et al (2008) NRAGE, a p75NTR adaptor protein, is required for developmental apoptosis in vivo. Cell Death Differ 15:1921–1929

Bhakar AL, Howell JL, Paul CE, Salehi AH, Becker EB et al (2003) Apoptosis induced by p75NTR overexpression requires Jun kinase-dependent phosphorylation of Bad. J Neurosci 23:11373–11381

Bibel M, Hoppe E, Barde YA (1999) Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR. EMBO J 18:616–622

Bischoff V, Deogracias R, Poirier F, Barde YA (2012) Seizure-induced neuronal death is suppressed in the absence of the endogenous lectin Galectin-1. J Neurosci 32:15590–15600

Blochl A, Sirrenberg C (1996) Neurotrophins stimulate the release of dopamine from rat mesencephalic neurons via Trk and p75Lntr receptors. J Biol Chem 271:21100–21107

Bogoyevitch MA, Kobe B (2006) Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. Microbiol Mol Biol Rev 70:1061–1095

Boutilier J, Ceni C, Pagdala PC, Forgie A, Neet KE, Barker PA (2008) Proneurotrophins require endocytosis and intracellular proteolysis to induce TrkA activation. J Biol Chem 283:12709–12716

Brann AB, Tcherpakov M, Williams IM, Futerman AH, Fainzilber M (2002) Nerve growth factor-induced p75-mediated death of cultured hippocampal neurons is age-dependent and transduced through ceramide generated by neutral sphingomyelinase. J Biol Chem 277:9812–9818

Brunello N, Reynolds M, Wrathall JR, Mocchetti I (1990) Increased nerve growth factor receptor mRNA in contused rat spinal cord. Neurosci Lett 118:238–240

Bui NT, Konig HG, Culmsee C, Bauerbach E, Poppe M et al (2002) p75 neurotrophin receptor is required for constitutive and NGF-induced survival signalling in PC12 cells and rat hippocampal neurones. J Neurochem 81:594–605

Bunone G, Mariotti A, Compagni A, Morandi E, Della Valle G (1997) Induction of apoptosis by p75 neurotrophin receptor in human neuroblastoma cells. Oncogene 14:1463–1470

Caporali A, Pani E, Horrevoets AJ, Kraenkel N, Oikawa A et al (2008) Neurotrophin p75 receptor (p75NTR) promotes endothelial cell apoptosis and inhibits angiogenesis: implications for diabetes-induced impaired neovascularization in ischemic limb muscles. Circ Res 103: e15–e26

Carter BD, Kaltschmidt C, Kaltschmidt B, Offenhauser N, Bohm-Matthaei R et al (1996) Selective activation of NF-kappa B by nerve growth factor through the neurotrophin receptor p75. Science 272:542–545

Casaccia-Bonnefil P, Carter BD, Dobrowsky RT, Chao MV (1996) Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. Nature 383:716–719

Casademunt E, Carter BD, Benzel I, Frade JM, Dechant G, Barde YA (1999) The zinc finger protein NRIF interacts with the neurotrophin receptor p75(NTR) and participates in programmed cell death. EMBO J 18:6050–6061

Ceni C, Kommaddi RP, Thomas R, Vereker E, Liu X et al (2010) The p75NTR intracellular domain generated by neurotrophin-induced receptor cleavage potentiates Trk signaling. J Cell Sci 123:2299–2307

Cesca F, Yabe A, Spencer-Dene B, Arrigoni A, Al-Qatari M et al (2011) Kidins220/ARMS is an essential modulator of cardiovascular and nervous system development. Cell Death Dis 2:e226

Chan JR, Cosgaya JM, Wu YJ, Shooter EM (2001) Neurotrophins are key mediators of the myelination program in the peripheral nervous system. Proc Natl Acad Sci U S A 98:14661–14668

Chan JR, Jolicoeur C, Yamauchi J, Elliott J, Fawcett JP et al (2006) The polarity protein Par-3 directly interacts with p75NTR to regulate myelination. Science 314:832–836

Chang MS, Arevalo JC, Chao MV (2004) Ternary complex with Trk, p75, and an ankyrin-rich membrane spanning protein. J Neurosci Res 78:186–192

Chao MV, Hempstead BL (1995) p75 and Trk: a two-receptor system. Trends Neurosci 18:321–326

Chao MV, Bothwell MA, Ross AH, Koprowski H, Lanahan AA et al (1986) Gene transfer and molecular cloning of the human NGF receptor. Science 232:518–521

Chapman BS (1995) A region of the 75 kDa neurotrophin receptor homologous to the death domains of TNFR-I and Fas. FEBS Lett 374:216–220

Chapman BS, Kuntz ID (1995) Modeled structure of the 75-kDa neurotrophin receptor. Protein Sci 4:1696–1707

Chittka A, Chao MV (1999) Identification of a zinc finger protein whose subcellular distribution is regulated by serum and nerve growth factor. Proc Natl Acad Sci U S A 96:10705–10710

Chittka A, Arevalo JC, Rodriguez-Guzman M, Perez P, Chao MV, Sendtner M (2004) The p75NTR-interacting protein SC1 inhibits cell cycle progression by transcriptional repression of cyclin E. J Cell Biol 164:985–996

Choi S, Friedman WJ (2009) Inflammatory cytokines IL-1beta and TNF-alpha regulate p75NTR expression in CNS neurons and astrocytes by distinct cell-type-specific signalling mechanisms. ASN Neuro 1(2):e00010

Clary DO, Reichardt LF (1994) An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. Proc Natl Acad Sci U S A 91:11133–11137

Copray JC, Jaarsma D, Kust BM, Bruggeman RW, Mantingh I et al (2003) Expression of the low affinity neurotrophin receptor p75 in spinal motoneurons in a transgenic mouse model for amyotrophic lateral sclerosis. Neuroscience 116:685–694

Cortazzo MH, Kassis ES, Sproul KA, Schor NF (1996) Nerve growth factor (NGF)-mediated protection of neural crest cells from antimitotic agent-induced apoptosis: the role of the low-affinity NGF receptor. J Neurosci 16:3895–3899

Cosgaya JM, Chan JR, Shooter EM (2002) The neurotrophin receptor p75NTR as a positive modulator of myelination. Science 298:1245–1248

Costantini C, Rossi F, Formaggio E, Bernardoni R, Cecconi D, Della-Bianca V (2005) Characterization of the signaling pathway downstream p75 neurotrophin receptor involved in beta-amyloid peptide-dependent cell death. J Mol Neurosci 25:141–156

Costantini C, Scrable H, Puglielli L (2006) An aging pathway controls the TrkA to p75NTR receptor switch and amyloid beta-peptide generation. EMBO J 25:1997–2006

Coulson EJ, Reid K, Baca M, Shipham KA, Hulett SM et al (2000) Chopper, a new death domain of the p75 neurotrophin receptor that mediates rapid neuronal cell death. J Biol Chem 275:30537–30545

Coulson EJ, Reid K, Shipham KM, Morley S, Kilpatrick TJ, Bartlett PF (2004) The role of neurotransmission and the Chopper domain in p75 neurotrophin receptor death signaling. Prog Brain Res 146:41–62

Coulson EJ, May LM, Osborne SL, Reid K, Underwood CK et al (2008) p75 neurotrophin receptor mediates neuronal cell death by activating GIRK channels through phosphatidylinositol 4,5-bisphosphate. J Neurosci 28:315–324

Cragnolini AB, Friedman WJ (2008) The function of p75NTR in glia. Trends Neurosci 31:99–104

Culmsee C, Gerling N, Lehmann M, Nikolova-Karakashian M, Prehn JH et al (2002) Nerve growth factor survival signaling in cultured hippocampal neurons is mediated through TrkA and requires the common neurotrophin receptor P75. Neuroscience 115:1089–1108

Davies AM, Lee KF, Jaenisch R (1993) p75-deficient trigeminal sensory neurons have an altered response to NGF but not to other neurotrophins. Neuron 11:565–574

DeFreitas MF, McQuillen PS, Shatz CJ (2001) A novel p75NTR signaling pathway promotes survival, not death, of immunopurified neocortical subplate neurons. J Neurosci 21:5121–5129

Deinhardt K, Kim T, Spellman DS, Mains RE, Eipper BA et al (2011) Neuronal growth cone retraction relies on proneurotrophin receptor signaling through Rac. Sci Signal 4:ra82

Dempsey PW, Doyle SE, He JQ, Cheng G (2003) The signaling adaptors and pathways activated by TNF superfamily. Cytokine Growth Factor Rev 14:193–209

Deppmann CD, Mihalas S, Sharma N, Lonze BE, Niebur E, Ginty DD (2008) A model for neuronal competition during development. Science 320:369–373

Desmond NL, Levy WB (1986) Changes in the postsynaptic density with long-term potentiation in the dentate gyrus. J Comp Neurol 253:476–482

Dhanasekaran DN, Reddy EP (2008) JNK signaling in apoptosis. Oncogene 27:6245–6251

Di Certo MG, Corbi N, Bruno T, Iezzi S, De Nicola F et al (2007) NRAGE associates with the anti-apoptotic factor Che-1 and regulates its degradation to induce cell death. J Cell Sci 120:1852–1858

Dobrowsky RT, Werner MH, Castellino AM, Chao MV, Hannun YA (1994) Activation of the sphingomyelin cycle through the low-affinity neurotrophin receptor. Science 265:1596–1599

Domeniconi M, Zampieri N, Spencer T, Hilaire M, Mellado W et al (2005) MAG induces regulated intramembrane proteolysis of the p75 neurotrophin receptor to inhibit neurite outgrowth. Neuron 46:849–855

Donovan N, Becker EB, Konishi Y, Bonni A (2002) JNK phosphorylation and activation of BAD couples the stress-activated signaling pathway to the cell death machinery. J Biol Chem 277:40944–40949

Du Q, Zhang Y, Tian XX, Li Y, Fang WG (2009) MAGE-D1 inhibits proliferation, migration and invasion of human breast cancer cells. Oncol Rep 22:659–665

Dubreuil CI, Winton MJ, McKerracher L (2003) Rho activation patterns after spinal cord injury and the role of activated Rho in apoptosis in the central nervous system. J Cell Biol 162:233–243

Edwards RH, Selby MJ, Garcia PD, Rutter WJ (1988) Processing of the native nerve growth factor precursor to form biologically active nerve growth factor. J Biol Chem 263:6810–6815

Ernfors P, Henschen A, Olson L, Persson H (1989) Expression of nerve growth factor receptor mRNA is developmentally regulated and increased after axotomy in rat spinal cord motoneurons. Neuron 2:1605–1613

Ernfors P, Wetmore C, Eriksdotter-Nilsson M, Bygdeman M, Stromberg I et al (1991) The nerve growth factor receptor gene is expressed in both neuronal and non-neuronal tissues in the human fetus. Int J Dev Neurosci 9:57–66

Ernfors P, Lee KF, Kucera J, Jaenisch R (1994) Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. Cell 77:503–512

Esposito D, Patel P, Stephens RM, Perez P, Chao MV et al (2001) The cytoplasmic and transmembrane domains of the p75 and Trk A receptors regulate high affinity binding to nerve growth factor. J Biol Chem 276:32687–32695

Fan YJ, Wu LL, Li HY, Wang YJ, Zhou XF (2008) Differential effects of pro-BDNF on sensory neurons after sciatic nerve transection in neonatal rats. Eur J Neurosci 27:2380–2390

Farinas I, Jones KR, Backus C, Wang XY, Reichardt LF (1994) Severe sensory and sympathetic deficits in mice lacking neurotrophin-3. Nature 369:658–661

Feinstein E, Kimchi A, Wallach D, Boldin M, Varfolomeev E (1995) The death domain: a module shared by proteins with diverse cellular functions. Trends Biochem Sci 20:342–344

Feng D, Kim T, Ozkan E, Light M, Torkin R et al (2010) Molecular and structural insight into proNGF engagement of p75NTR and sortilin. J Mol Biol 396:967–984

Fifkova E, Anderson CL (1981) Stimulation-induced changes in dimensions of stalks of dendritic spines in the dentate molecular layer. Exp Neurol 74:621–627

Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B (1996) Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature 381:706–709

Frade JM (2005) Nuclear translocation of the p75 neurotrophin receptor cytoplasmic domain in response to neurotrophin binding. J Neurosci 25:1407–1411

Frade JM, Barde YA (1999) Genetic evidence for cell death mediated by nerve growth factor and the neurotrophin receptor p75 in the developing mouse retina and spinal cord. Development 126:683–690

Frade JM, Rodriguez-Tebar A, Barde YA (1996) Induction of cell death by endogenous nerve growth factor through its p75 receptor. Nature 383:166–168

Francis N, Farinas I, Brennan C, Rivas-Plata K, Backus C et al (1999) NT-3, like NGF, is required for survival of sympathetic neurons, but not their precursors. Dev Biol 210:411–427

Frazier WA, Boyd LF, Bradshaw RA (1974a) Properties of the specific binding of 125I-nerve growth factor to responsive peripheral neurons. J Biol Chem 249:5513–5519

Frazier WA, Boyd LF, Szutowicz A, Pulliam MW, Bradshaw RA (1974b) Specific binding sites for 125I-nerve growth factor in peripheral tissues and brain. Biochem Biophys Res Commun 57:1096–1103

Frey U, Muller M, Kuhl D (1996) A different form of long-lasting potentiation revealed in tissue plasminogen activator mutant mice. J Neurosci 16:2057–2063

Friedman WJ (2000) Neurotrophins induce death of hippocampal neurons via the p75 receptor. J Neurosci 20:6340–6346

Fujita Y, Endo S, Takai T, Yamashita T (2011a) Myelin suppresses axon regeneration by PIR-B/SHP-mediated inhibition of Trk activity. EMBO J 30:1389–1401

Fujita Y, Takashima R, Endo S, Takai T, Yamashita T (2011b) The p75 receptor mediates axon growth inhibition through an association with PIR-B. Cell Death Dis 2:e198

Geetha T, Kenchappa RS, Wooten MW, Carter BD (2005) TRAF6-mediated ubiquitination regulates nuclear translocation of NRIF, the p75 receptor interactor. EMBO J 24:3859–3868

Gehler S, Shaw AE, Sarmiere PD, Bamburg JR, Letourneau PC (2004) Brain-derived neurotrophic factor regulation of retinal growth cone filopodial dynamics is mediated through actin depolymerizing factor/cofilin. J Neurosci 24:10741–10749

Gentry JJ, Casaccia-Bonnefil P, Carter BD (2000) Nerve growth factor activation of nuclear factor kappaB through its p75 receptor is an anti-apoptotic signal in RN22 schwannoma cells. J Biol Chem 275:7558–7565

Gentry JJ, Rutkoski NJ, Burke TL, Carter BD (2004) A functional interaction between the p75 neurotrophin receptor interacting factors, TRAF6 and NRIF. J Biol Chem 279:16646–16656

Giehl KM, Rohrig S, Bonatz H, Gutjahr M, Leiner B et al (2001) Endogenous brain-derived neurotrophic factor and neurotrophin-3 antagonistically regulate survival of axotomized corticospinal neurons in vivo. J Neurosci 21:3492–3502

Ginsberg D (2002) E2F1 pathways to apoptosis. FEBS Lett 529:122–125

Gjerstad MD, Tandrup T, Koltzenburg M, Jakobsen J (2002) Predominant neuronal B-cell loss in L5 DRG of p75 receptor-deficient mice. J Anat 200:81–87

Goldstein B, Macara IG (2007) The PAR proteins: fundamental players in animal cell polarization. Dev Cell 13:609–622

Gong Y, Cao P, Yu HJ, Jiang T (2008) Crystal structure of the neurotrophin-3 and p75NTR symmetrical complex. Nature 454:789–793

Grob PM, Berlot CH, Bothwell MA (1983) Affinity labeling and partial purification of nerve growth factor receptors from rat pheochromocytoma and human melanoma cells. Proc Natl Acad Sci U S A 80:6819–6823

Grob PM, Ross AH, Koprowski H, Bothwell M (1985) Characterization of the human melanoma nerve growth factor receptor. J Biol Chem 260:8044–8049

Gu C, Casaccia-Bonnefil P, Srinivasan A, Chao MV (1999) Oligodendrocyte apoptosis mediated by caspase activation. J Neurosci 19:3043–3049

Gupta S, Barrett T, Whitmarsh AJ, Cavanagh J, Sluss HK et al (1996) Selective interaction of JNK protein kinase isoforms with transcription factors. EMBO J 15:2760–2770

Ha H, Han D, Choi Y (2009) TRAF-mediated TNFR-family signaling. Curr Protoc Immunol Chapter 11:Unit11 9D

Hacker H, Tseng PH, Karin M (2011) Expanding TRAF function: TRAF3 as a tri-faced immune regulator. Nat Rev Immunol 11:457–468

Hamanoue M, Middleton G, Wyatt S, Jaffray E, Hay RT, Davies AM (1999) p75-mediated NF-kappaB activation enhances the survival response of developing sensory neurons to nerve growth factor. Mol Cell Neurosci 14:28–40

Harrington AW, Kim JY, Yoon SO (2002) Activation of Rac GTPase by p75 Is Necessary for c-jun N-Terminal Kinase-Mediated Apoptosis. J Neurosci 22:156–166

Harrington AW, Leiner B, Blechschmitt C, Arevalo JC, Lee R et al (2004) Secreted proNGF is a pathophysiological death-inducing ligand after adult CNS injury. Proc Natl Acad Sci U S A 101:6226–6230

Harrington AW, Li QM, Tep C, Park JB, He Z, Yoon SO (2008) The role of Kalirin9 in p75/nogo receptor-mediated RhoA activation in cerebellar granule neurons. J Biol Chem 283:24690–24697

Hashimoto Y, Kaneko Y, Tsukamoto E, Frankowski H, Kouyama K et al (2004) Molecular characterization of neurohybrid cell death induced by Alzheimer's amyloid-beta peptides via p75NTR/PLAIDD. J Neurochem 90:549–558

He XL, Garcia KC (2004) Structure of nerve growth factor complexed with the shared neurotrophin receptor p75. Science 304:870–875

Hempstead BL, Martin-Zanca D, Kaplan DR, Parada LF, Chao MV (1991) High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. Nature 350:678–683

Heymach JV Jr, Shooter EM (1995) The biosynthesis of neurotrophin heterodimers by transfected mammalian cells. J Biol Chem 270:12297–12304

Hirata H, Hibasami H, Yoshida T, Ogawa M, Matsumoto M et al (2001) Nerve growth factor signaling of p75 induces differentiation and ceramide-mediated apoptosis in Schwann cells cultured from degenerating nerves. Glia 36:245–258

Horres CR, Hannun YA (2012) The roles of neutral sphingomyelinases in neurological pathologies. Neurochem Res 37:1137–1149

Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC (2003) Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. Mol Cell Biol 23:9361–9374

Huang YY, Bach ME, Lipp HP, Zhuo M, Wolfer DP et al (1996) Mice lacking the gene encoding tissue-type plasminogen activator show a selective interference with late-phase long-term potentiation in both Schaffer collateral and mossy fiber pathways. Proc Natl Acad Sci U S A 93:8699–8704

Hunt DL, Castillo PE (2012) Synaptic plasticity of NMDA receptors: mechanisms and functional implications. Curr Opin Neurobiol 22:496–508

Ibanez CF, Ebendal T, Barbany G, Murray-Rust J, Blundell TL, Persson H (1992) Disruption of the low affinity receptor-binding site in NGF allows neuronal survival and differentiation by binding to the trk gene product. Cell 69:329–341

Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS et al (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 9:119–128

Jansen P, Giehl K, Nyengaard JR, Teng K, Lioubinski O et al (2007) Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. Nat Neurosci 10:1449–1457

Je HS, Yang F, Ji Y, Nagappan G, Hempstead BL, Lu B (2012) Role of pro-brain-derived neurotrophic factor (proBDNF) to mature BDNF conversion in activity-dependent competition at developing neuromuscular synapses. Proc Natl Acad Sci U S A 109:15924–15929

Jiang Y, Nyengaard JR, Zhang JS, Jakobsen J (2004) Selective loss of calcitonin gene-related Peptide-expressing primary sensory neurons of the a-cell phenotype in early experimental diabetes. Diabetes 53:2669–2675

Jordan BW, Dinev D, LeMellay V, Troppmair J, Gotz R et al (2001) Neurotrophin receptor-interacting mage homologue is an inducible inhibitor of apoptosis protein-interacting protein that augments cell death. J Biol Chem 276:39985–39989

Jung KM, Tan S, Landman N, Petrova K, Murray S et al (2003) Regulated intramembrane proteolysis of the p75 neurotrophin receptor modulates its association with the TrkA receptor. J Biol Chem 278:42161–42169

Kang H, Welcher AA, Shelton D, Schuman EM (1997) Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. Neuron 19:653–664

Kanning KC, Hudson M, Amieux PS, Wiley JC, Bothwell M, Schecterson LC (2003) Proteolytic processing of the p75 neurotrophin receptor and two homologs generates C-terminal fragments with signaling capability. J Neurosci 23:5425–5436

Kaplan DR, Martin-Zanca D, Parada LF (1991) Tyrosine phosphorylation and tyrosine kinase activity of the trk proto-oncogene product induced by NGF. Nature 350:158–160

Kenchappa RS, Zampieri N, Chao MV, Barker PA, Teng HK et al (2006) Ligand-dependent cleavage of the P75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. Neuron 50:219–232

Kenchappa RS, Tep C, Korade Z, Urra S, Bronfman FC et al (2010) p75 neurotrophin receptor-mediated apoptosis in sympathetic neurons involves a biphasic activation of JNK and up-regulation of tumor necrosis factor-alpha-converting enzyme/ADAM17. J Biol Chem 285:20358–20368

Kendall SE, Battelli C, Irwin S, Mitchell JG, Glackin CA, Verdi JM (2005) NRAGE mediates p38 activation and neural progenitor apoptosis via the bone morphogenetic protein signaling cascade. Mol Cell Biol 25:7711–7724

Khursigara G, Orlinick JR, Chao MV (1999) Association of the p75 neurotrophin receptor with TRAF6. J Biol Chem 274:2597–2600

Khursigara G, Bertin J, Yano H, Moffett H, DiStefano PS, Chao MV (2001) A prosurvival function for the p75 receptor death domain mediated via the caspase recruitment domain receptor-interacting protein 2. J Neurosci 21:5854–5863

Kim T, Hempstead BL (2009) NRH2 is a trafficking switch to regulate sortilin localization and permit proneurotrophin-induced cell death. EMBO J 28:1612–1623

Klein R, Jing SQ, Nanduri V, O'Rourke E, Barbacid M (1991) The trk proto-oncogene encodes a receptor for nerve growth factor. Cell 65:189–197

Knowles JK, Rajadas J, Nguyen TV, Yang T, LeMieux MC et al (2009) The p75 neurotrophin receptor promotes amyloid-beta(1–42)-induced neuritic dystrophy in vitro and in vivo. J Neurosci 29:10627–10637

Kokaia Z, Andsberg G, Martinez-Serrano A, Lindvall O (1998) Focal cerebral ischemia in rats induces expression of P75 neurotrophin receptor in resistant striatal cholinergic neurons. Neuroscience 84:1113–1125

Koliatsos VE, Crawford TO, Price DL (1991) Axotomy induces nerve growth factor receptor immunoreactivity in spinal motor neurons. Brain Res 549:297–304

Kommaddi RP, Dickson KM, Barker PA (2011a) Stress-induced expression of the p75 neurotrophin receptor is regulated by O-GlcNAcylation of the Sp1 transcription factor. J Neurochem 116:396–405

Kommaddi RP, Thomas R, Ceni C, Daigneault K, Barker PA (2011b) Trk-dependent ADAM17 activation facilitates neurotrophin survival signaling. FASEB J 25:2061–2070

Kong H, Boulter J, Weber JL, Lai C, Chao MV (2001) An evolutionarily conserved transmembrane protein that is a novel downstream target of neurotrophin and ephrin receptors. J Neurosci 21:176–185

Korte M, Kang H, Bonhoeffer T, Schuman E (1998) A role for BDNF in the late-phase of hippocampal long-term potentiation. Neuropharmacology 37:553–559

Krystosek A, Seeds NW (1981) Plasminogen activator release at the neuronal growth cone. Science 213:1532–1534

Kuan CY, Whitmarsh AJ, Yang DD, Liao G, Schloemer AJ et al (2003) A critical role of neural-specific JNK3 for ischemic apoptosis. Proc Natl Acad Sci U S A 100:15184–15189

Kuruvilla R, Zweifel LS, Glebova NO, Lonze BE, Valdez G et al (2004) A neurotrophin signaling cascade coordinates sympathetic neuron development through differential control of TrkA trafficking and retrograde signaling. Cell 118:243–255

Kust BM, Brouwer N, Mantingh IJ, Boddeke HW, Copray JC (2003) Reduced p75NTR expression delays disease onset only in female mice of a transgenic model of familial amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 4:100–105

Kuwako K, Taniura H, Yoshikawa K (2004) Necdin-related MAGE proteins differentially interact with the E2F1 transcription factor and the p75 neurotrophin receptor. J Biol Chem 279:1703–1712

Lachance C, Belliveau DJ, Barker PA (1997) Blocking nerve growth factor binding to the p75 neurotrophin receptor on sympathetic neurons transiently reduces trkA activation but does not affect neuronal survival. Neuroscience 81:861–871

Le Moan N, Houslay DM, Christian F, Houslay MD, Akassoglou K (2011) Oxygen-dependent cleavage of the p75 neurotrophin receptor triggers stabilization of HIF-1alpha. Mol Cell 44:476–490

Le AP, Friedman WJ (2012) Matrix metalloproteinase-7 regulates cleavage of pro-nerve growth factor and is neuroprotective following kainic acid-induced seizures. J Neurosci 32:703–712

Lebrun-Julien F, Bertrand MJ, De Backer O, Stellwagen D, Morales CR et al (2010) ProNGF induces TNFalpha-dependent death of retinal ganglion cells through a p75NTR non-cell-autonomous signaling pathway. Proc Natl Acad Sci U S A 107:3817–3822

Lee KF, Li E, Huber LJ, Landis SC, Sharpe AH et al (1992) Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. Cell 69:737–749

Lee R, Kermani P, Teng KK, Hempstead BL (2001) Regulation of cell survival by secreted proneurotrophins. Science 294:1945–1948

Lei K, Davis RJ (2003) JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. Proc Natl Acad Sci U S A 100:2432–2437

Lemke G, Chao M (1988) Axons regulate Schwann cell expression of the major myelin and NGF receptor genes. Development 102:499–504

Li QM, Tep C, Yune TY, Zhou XZ, Uchida T et al (2007) Opposite regulation of oligodendrocyte apoptosis by JNK3 and Pin1 after spinal cord injury. J Neurosci 27:8395–8404

Liepinsh E, Ilag LL, Otting G, Ibanez CF (1997) NMR structure of the death domain of the p75 neurotrophin receptor. EMBO J 16:4999–5005

Lim YS, McLaughlin T, Sung TC, Santiago A, Lee KF, O'Leary DD (2008) p75(NTR) mediates ephrin-A reverse signaling required for axon repulsion and mapping. Neuron 59:746–758

Linggi MS, Burke TL, Williams BB, Harrington A, Kraemer R et al (2005) Neurotrophin receptor interacting factor (NRIF) is an essential mediator of apoptotic signaling by the p75 neurotrophin receptor. J Biol Chem 280:13801–13808

Loeb DM, Maragos J, Martin-Zanca D, Chao MV, Parada LF, Greene LA (1991) The trk proto-oncogene rescues NGF responsiveness in mutant NGF-nonresponsive PC12 cell lines. Cell 66:961–966

Longo FM, Manthorpe M, Xie YM, Varon S (1997) Synthetic NGF peptide derivatives prevent neuronal death via a p75 receptor-dependent mechanism. J Neurosci Res 48:1–17

Lopez-Sanchez N, Gonzalez-Fernandez Z, Niinobe M, Yoshikawa K, Frade JM (2007) Single mage gene in the chicken genome encodes CMage, a protein with functional similarities to mammalian type II Mage proteins. Physiol Genomics 30:156–171

Lowry KS, Murray SS, McLean CA, Talman P, Mathers S et al (2001) A potential role for the p75 low-affinity neurotrophin receptor in spinal motor neuron degeneration in murine and human amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2:127–134

MacLachlan TK, El-Deiry WS (2002) Apoptotic threshold is lowered by p53 transactivation of caspase-6. Proc Natl Acad Sci U S A 99:9492–9497

Majdan M, Lachance C, Gloster A, Aloyz R, Zeindler C et al (1997) Transgenic mice expressing the intracellular domain of the p75 neurotrophin receptor undergo neuronal apoptosis. J Neurosci 17:6988–6998

Makkerh JP, Ceni C, Auld DS, Vaillancourt F, Dorval G, Barker PA (2005) p75 neurotrophin receptor reduces ligand-induced Trk receptor ubiquitination and delays Trk receptor internalization and degradation. EMBO Rep 6:936–941

Massague J, Guillette BJ, Czech MP, Morgan CJ, Bradshaw RA (1981) Identification of a nerve growth factor receptor protein in sympathetic ganglia membranes by affinity labeling. J Biol Chem 256:9419–9424

Massague J, Buxser S, Johnson GL, Czech MP (1982) Affinity labeling of a nerve growth factor receptor component on rat pheochromocytoma (PC12) cells. Biochim Biophys Acta 693:205–212

Matsumoto T, Rauskolb S, Polack M, Klose J, Kolbeck R et al (2008) Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat Neurosci 11:131–133

McCollum AT, Estus S (2004) NGF acts via p75 low-affinity neurotrophin receptor and calpain inhibition to reduce UV neurotoxicity. J Neurosci Res 77:552–564

McQuillen PS, DeFreitas MF, Zada G, Shatz CJ (2002) A novel role for p75NTR in subplate growth cone complexity and visual thalamocortical innervation. J Neurosci 22:3580–3593

Mi S, Lee X, Shao Z, Thill G, Ji B et al (2004) LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat Neurosci 7:221–228

Miyashita T, Reed JC (1995) Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 80:293–299

Mohit AA, Martin JH, Miller CA (1995) p493F12 kinase: a novel MAP kinase expressed in a subset of neurons in the human nervous system. Neuron 14:67–78

Mukai J, Hachiya T, Shoji-Hoshino S, Kimura MT, Nadano D et al (2000) NADE, a p75NTR-associated cell death executor, is involved in signal transduction mediated by the common neurotrophin receptor p75NTR. J Biol Chem 275:17566–17570

Mukai J, Shoji S, Kimura MT, Okubo S, Sano H et al (2002) Structure-function analysis of NADE: identification of regions that mediate nerve growth factor-induced apoptosis. J Biol Chem 277:13973–13982

Murray SS, Bartlett PF, Cheema SS (1999) Differential loss of spinal sensory but not motor neurons in the p75NTR knockout mouse. Neurosci Lett 267:45–48

Murray SS, Perez P, Lee R, Hempstead BL, Chao MV (2004) A novel p75 neurotrophin receptor-related protein, NRH2, regulates nerve growth factor binding to the TrkA receptor. J Neurosci 24:2742–2749

Nakano K, Vousden KH (2001) PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 7:683–694

Naumann T, Casademunt E, Hollerbach E, Hofmann J, Dechant G et al (2002) Complete deletion of the neurotrophin receptor p75NTR leads to long-lasting increases in the number of basal forebrain cholinergic neurons. J Neurosci 22:2409–2418

Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P et al (2004) Sortilin is essential for proNGF-induced neuronal cell death. Nature 427:843–848

Nykjaer A, Willnow TE, Petersen CM (2005) p75NTR – live or let die. Curr Opin Neurobiol 15:49–57

Oh JD, Chartisathian K, Chase TN, Butcher LL (2000) Overexpression of neurotrophin receptor p75 contributes to the excitotoxin-induced cholinergic neuronal death in rat basal forebrain. Brain Res 853:174–185

Okamoto K, Nagai T, Miyawaki A, Hayashi Y (2004) Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat Neurosci 7:1104–1112

Palmada M, Kanwal S, Rutkoski NJ, Gustafson-Brown C, Johnson RS et al (2002) c-jun is essential for sympathetic neuronal death induced by NGF withdrawal but not by p75 activation. J Cell Biol 158:453–461

Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K et al (2004) Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 306:487–491

Park JB, Yiu G, Kaneko S, Wang J, Chang J et al (2005) A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. Neuron 45:345–351

Park KJ, Grosso CA, Aubert I, Kaplan DR, Miller FD (2010) p75NTR-dependent, myelin-mediated axonal degeneration regulates neural connectivity in the adult brain. Nat Neurosci 13:559–566

Parkhurst CN, Zampieri N, Chao MV (2010) Nuclear localization of the p75 neurotrophin receptor intracellular domain. J Biol Chem 285:5361–5368

Passino MA, Adams RA, Sikorski SL, Akassoglou K (2007) Regulation of hepatic stellate cell differentiation by the neurotrophin receptor p75NTR. Science 315:1853–1856

Patterson SL, Pittenger C, Morozov A, Martin KC, Scanlin H et al (2001) Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. Neuron 32:123–140

Paul CE, Vereker E, Dickson KM, Barker PA (2004) A pro-apoptotic fragment of the p75 neurotrophin receptor is expressed in p75NTRExonIV null mice. J Neurosci 24:1917–1923

Pedraza CE, Podlesniy P, Vidal N, Arevalo JC, Lee R et al (2005) Pro-NGF isolated from the human brain affected by Alzheimer's disease induces neuronal apoptosis mediated by p75NTR. Am J Pathol 166:533–543

Peng S, Wuu J, Mufson EJ, Fahnestock M (2004) Increased proNGF levels in subjects with mild cognitive impairment and mild Alzheimer disease. J Neuropathol Exp Neurol 63:641–649

Peterson DA, Dickinson-Anson HA, Leppert JT, Lee KF, Gage FH (1999) Central neuronal loss and behavioral impairment in mice lacking neurotrophin receptor p75. J Comp Neurol 404:1–20

Plachta N, Annaheim C, Bissiere S, Lin S, Ruegg M et al (2007) Identification of a lectin causing the degeneration of neuronal processes using engineered embryonic stem cells. Nat Neurosci 10:712–719

Podlesniy P, Kichev A, Pedraza C, Saurat J, Encinas M et al (2006) Pro-NGF from Alzheimer's disease and normal human brain displays distinctive abilities to induce processing and nuclear translocation of intracellular domain of p75NTR and apoptosis. Am J Pathol 169:119–131

Poukka H, Kallio PJ, Janne OA, Palvimo JJ (1996) Regulation of the rat p75 neurotrophin receptor promoter by GC element binding proteins. Biochem Biophys Res Commun 229:565–570

Potts PR, Singh S, Knezek M, Thompson CB, Deshmukh M (2003) Critical function of endogenous XIAP in regulating caspase activation during sympathetic neuronal apoptosis. J Cell Biol 163:789–799.

Rabizadeh S, Oh J, Zhong LT, Yang J, Bitler CM et al (1993) Induction of apoptosis by the low-affinity NGF receptor. Science 261:345–348

Radeke MJ, Misko TP, Hsu C, Herzenberg LA, Shooter EM (1987) Gene transfer and molecular cloning of the rat nerve growth factor receptor. Nature 325:593–597

Ramos A, Ho WC, Forte S, Dickson K, Boutilier J et al (2007) Hypo-osmolar stress induces p75NTR expression by activating Sp1-dependent transcription. J Neurosci 27:1498–1506

Rattenholl A, Lilie H, Grossmann A, Stern A, Schwarz E, Rudolph R (2001a) The pro-sequence facilitates folding of human nerve growth factor from Escherichia coli inclusion bodies. Eur J Biochem 268:3296–3303

Rattenholl A, Ruoppolo M, Flagiello A, Monti M, Vinci F et al (2001b) Pro-sequence assisted folding and disulfide bond formation of human nerve growth factor. J Mol Biol 305:523–533

Rende M, Provenzano C, Tonali P (1993) Modulation of low-affinity nerve growth factor receptor in injured adult rat spinal cord motoneurons. J Comp Neurol 338:560–574

Rodriguez-Tebar A, Dechant G, Barde YA (1990) Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. Neuron 4:487–492

Rodriguez-Tebar A, Dechant G, Gotz R, Barde YA (1992) Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. EMBO J 11:917–922

Rogers ML, Bailey S, Matusica D, Nicholson I, Muyderman H et al (2010) ProNGF mediates death of Natural Killer cells through activation of the p75NTR-sortilin complex. J Neuroimmunol 226:93–103

Rosch H, Schweigreiter R, Bonhoeffer T, Barde YA, Korte M (2005) The neurotrophin receptor p75NTR modulates long-term depression and regulates the expression of AMPA receptor subunits in the hippocampus. Proc Natl Acad Sci U S A 102:7362–7367

Ross AH, Grob P, Bothwell M, Elder DE, Ernst CS et al (1984) Characterization of nerve growth factor receptor in neural crest tumors using monoclonal antibodies. Proc Natl Acad Sci U S A 81:6681–6685

Roux PP, Colicos MA, Barker PA, Kennedy TE (1999) p75 neurotrophin receptor expression is induced in apoptotic neurons after seizure. J Neurosci 19:6887–6896

Roux PP, Bhakar AL, Kennedy TE, Barker PA (2001) The p75 neurotrophin receptor activates Akt (protein kinase B) through a phosphatidylinositol 3-kinase-dependent pathway. J Biol Chem 276:23097–23104

Ryden M, Murray-Rust J, Glass D, Ilag LL, Trupp M et al (1995) Functional analysis of mutant neurotrophins deficient in low-affinity binding reveals a role for p75LNGFR in NT-4 signalling. EMBO J 14:1979–1990

Ryden M, Hempstead B, Ibanez CF (1997) Differential modulation of neuron survival during development by nerve growth factor binding to the p75 neurotrophin receptor. J Biol Chem 272:16322–16328

Salehi AH, Roux PP, Kubu CJ, Zeindler C, Bhakar A et al (2000) NRAGE, a novel MAGE protein, interacts with the p75 neurotrophin receptor and facilitates nerve growth factor-dependent apoptosis. Neuron 27:279–288

Salehi AH, Xanthoudakis S, Barker PA (2002) NRAGE, a p75 neurotrophin receptor-interacting protein, induces caspase activation and cell death through a JNK-dependent mitochondrial pathway. J Biol Chem 277:48043–48050

Sang M, Wang L, Ding C, Zhou X, Wang B et al (2011) Melanoma-associated antigen genes – an update. Cancer Lett 302:85–90

Santos AM, Lopez-Sanchez N, Martin-Oliva D, de la Villa P, Cuadros MA, Frade JM (2012) Sortilin participates in light-dependent photoreceptor degeneration in vivo. PLoS One 7: e36243

Sedel F, Bechade C, Triller A (1999) Nerve growth factor (NGF) induces motoneuron apoptosis in rat embryonic spinal cord in vitro. Eur J Neurosci 11:3904–3912

Seeburger JL, Tarras S, Natter H, Springer JE (1993) Spinal cord motoneurons express p75NGFR and p145trkB mRNA in amyotrophic lateral sclerosis. Brain Res 621:111–115

Seidah NG, Benjannet S, Pareek S, Savaria D, Hamelin J et al (1996) Cellular processing of the nerve growth factor precursor by the mammalian pro-protein convertases. Biochem J 314 (Pt 3):951–960

Shao Z, Browning JL, Lee X, Scott ML, Shulga-Morskaya S et al (2005) TAJ/TROY, an orphan TNF receptor family member, binds Nogo-66 receptor 1 and regulates axonal regeneration. Neuron 45:353–359

Shulga A, Magalhaes AC, Autio H, Plantman S, di Lieto A et al (2012) The loop diuretic bumetanide blocks posttraumatic p75NTR upregulation and rescues injured neurons. J Neurosci 32:1757–1770

Simon DJ, Weimer RM, McLaughlin T, Kallop D, Stanger K et al (2012) A caspase cascade regulating developmental axon degeneration. J Neurosci 32:17540–17553

Singh KK, Park KJ, Hong EJ, Kramer BM, Greenberg ME et al (2008) Developmental axon pruning mediated by BDNF-p75NTR-dependent axon degeneration. Nat Neurosci 11:649–658

Skeldal S, Matusica D, Nykjaer A, Coulson EJ (2011) Proteolytic processing of the p75 neurotrophin receptor: a prerequisite for signalling?: Neuronal life, growth and death signalling are crucially regulated by intra-membrane proteolysis and trafficking of p75(NTR). Bioessays 33:614–625

Song XY, Zhong JH, Wang X, Zhou XF (2004) Suppression of p75NTR does not promote regeneration of injured spinal cord in mice. J Neurosci 24:542–546

Song XY, Zhou FH, Zhong JH, Wu LL, Zhou XF (2006) Knockout of p75(NTR) impairs re-myelination of injured sciatic nerve in mice. J Neurochem 96:833–842

Song W, Volosin M, Cragnolini AB, Hempstead BL, Friedman WJ (2010) ProNGF induces PTEN via p75NTR to suppress Trk-mediated survival signaling in brain neurons. J Neurosci 30:15608–15615

Sonnenfeld KH, Ishii DN (1982) Nerve growth factor effects and receptors in cultured human neuroblastoma cell lines. J Neurosci Res 8:375–391

Sotthibundhu A, Sykes AM, Fox B, Underwood CK, Thangnipon W, Coulson EJ (2008) Beta-amyloid(1–42) induces neuronal death through the p75 neurotrophin receptor. J Neurosci 28:3941–3946

Srinivasan B, Roque CH, Hempstead BL, Al-Ubaidi MR, Roque RS (2004) Microglia-derived pronerve growth factor promotes photoreceptor cell death via p75 neurotrophin receptor. J Biol Chem 279:41839–41845

Stach RW, Wagner BJ (1982) Decrease in the number of lower affinity (type II) nerve growth factor receptors on embryonic sensory neurons does not affect fiber outgrowth. J Neurosci Res 7:103–110

Stoica G, Lungu G, Kim HT, Wong PK (2008) Up-regulation of pro-nerve growth factor, neurotrophin receptor p75, and sortilin is associated with retrovirus-induced spongiform encephalomyelopathy. Brain Res 1208:204–216

Sun Y, Lim Y, Li F, Liu S, Lu JJ et al (2012) ProBDNF collapses neurite outgrowth of primary neurons by activating RhoA. PLoS One [Electronic Resource] 7:e35883

Suter U, Heymach JV Jr, Shooter EM (1991) Two conserved domains in the NGF propeptide are necessary and sufficient for the biosynthesis of correctly processed and biologically active NGF. EMBO J 10:2395–2400

Sutter A, Riopelle RJ, Harris-Warrick RM, Shooter EM (1979) Nerve growth factor receptors. Characterization of two distinct classes of binding sites on chick embryo sensory ganglia cells. J Biol Chem 254:5972–5982

Syroid DE, Maycox PJ, Soilu-Hanninen M, Petratos S, Bucci T et al (2000) Induction of postnatal schwann cell death by the low-affinity neurotrophin receptor in vitro and after axotomy. J Neurosci 20:5741–5747

Tabassum A, Khwaja F, Djakiew D (2003) The p75(NTR) tumor suppressor induces caspase-mediated apoptosis in bladder tumor cells. Int J Cancer 105:47–52

Tan J, Shepherd RK (2006) Aminoglycoside-induced degeneration of adult spiral ganglion neurons involves differential modulation of tyrosine kinase B and p75 neurotrophin receptor signaling. Am J Pathol 169:528–543

Tan J, Clarke M, Barrett G, Millard R (2010) The p75 neurotrophin receptor protects primary auditory neurons against acoustic trauma in mice. Hear Res 268(1–2):46–59

Taniuchi M, Clark HB, Johnson EM Jr (1986) Induction of nerve growth factor receptor in Schwann cells after axotomy. Proc Natl Acad Sci U S A 83:4094–4098

Taylor AR, Gifondorwa DJ, Robinson MB, Strupe JL, Prevette D et al (2012) Motoneuron programmed cell death in response to proBDNF. Dev Neurobiol 72:699–712

Tcherpakov M, Bronfman FC, Conticello SG, Vaskovsky A, Levy Z et al (2002) The p75 neurotrophin receptor interacts with multiple MAGE proteins. J Biol Chem 277:49101–49104

Teng HK, Teng KK, Lee R, Wright S, Tevar S et al (2005) ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. J Neurosci 25:5455–5463

Tep C, Kim ML, Opincariu LI, Limpert AS, Chan JR et al (2012) Brain-derived neurotrophic factor (BDNF) induces polarized signaling of small GTPase (Rac1) protein at the onset of Schwann cell myelination through partitioning-defective 3 (Par3) protein. J Biol Chem 287:1600–1608

Tep C, Lim TH, Ko PO, Getahun S, Ryu JC et al (2013) Oral administration of a small molecule targeted to block proNGF binding to p75 promotes myelin sparing and functional recovery after spinal cord injury. J Neurosci 33:397–410

Troy CM, Friedman JE, Friedman WJ (2002) Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. J Biol Chem 277:34295–34302

Truzzi F, Marconi A, Atzei P, Panza MC, Lotti R et al (2011) p75 neurotrophin receptor mediates apoptosis in transit-amplifying cells and its overexpression restores cell death in psoriatic keratinocytes. Cell Death Differ 18:948–958

Turner BJ, Cheah IK, Macfarlane KJ, Lopes EC, Petratos S et al (2003) Antisense peptide nucleic acid-mediated knockdown of the p75 neurotrophin receptor delays motor neuron disease in mutant SOD1 transgenic mice. J Neurochem 87:752–763

Underwood CK, Reid K, May LM, Bartlett PF, Coulson EJ (2008) Palmitoylation of the C-terminal fragment of p75(NTR) regulates death signaling and is required for subsequent cleavage by gamma-secretase. Mol Cell Neurosci 37:346–358

Van Harreveld A, Fifkova E (1975) Swelling of dendritic spines in the fascia dentata after stimulation of the perforant fibers as a mechanism of post-tetanic potentiation. Exp Neurol 49:736–749

Verbeke S, Meignan S, Lagadec C, Germain E, Hondermarck H et al (2010) Overexpression of p75(NTR) increases survival of breast cancer cells through p21(waf1). Cell Signal 22:1864–1873

Verdi JM, Birren SJ, Ibanez CF, Persson H, Kaplan DR et al (1994) p75LNGFR regulates Trk signal transduction and NGF-induced neuronal differentiation in MAH cells. Neuron 12:733–745

Vilar M, Charalampopoulos I, Kenchappa RS, Simi A, Karaca E et al (2009) Activation of the p75 neurotrophin receptor through conformational rearrangement of disulphide-linked receptor dimers. Neuron 62:72–83

Volosin M, Song W, Almeida RD, Kaplan DR, Hempstead BL, Friedman WJ (2006) Interaction of survival and death signaling in basal forebrain neurons: roles of neurotrophins and proneurotrophins. J Neurosci 26:7756–7766

Volosin M, Trotter C, Cragnolini A, Kenchappa RS, Light M et al (2008) Induction of proneurotrophins and activation of p75NTR-mediated apoptosis via neurotrophin receptor-interacting factor in hippocampal neurons after seizures. J Neurosci 28:9870–9879

von Schack D, Casademunt E, Schweigreiter R, Meyer M, Bibel M, Dechant G (2001) Complete ablation of the neurotrophin receptor p75NTR causes defects both in the nervous and the vascular system. Nat Neurosci 4:977–978

Wang S, Bray P, McCaffrey T, March K, Hempstead BL, Kraemer R (2000) p75(NTR) mediates neurotrophin-induced apoptosis of vascular smooth muscle cells. Am J Pathol 157:1247–1258

Wang KC, Kim JA, Sivasankaran R, Segal R, He Z (2002) P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. Nature 420:74–78

Wang YQ, Bian GL, Bai Y, Cao R, Chen LW (2008) Identification and kainic acid-induced up-regulation of low-affinity p75 neurotrophin receptor (p75NTR) in the nigral dopamine neurons of adult rats. Neurochem Int 53:56–62

Wang YJ, Wang X, Lu JJ, Li QX, Gao CY et al (2011) p75NTR regulates Abeta deposition by increasing Abeta production but inhibiting Abeta aggregation with its extracellular domain. J Neurosci 31:2292–2304

Wehrman T, He X, Raab B, Dukipatti A, Blau H, Garcia KC (2007) Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors. Neuron 53:25–38

Wei Y, Wang N, Lu Q, Zhang N, Zheng D, Li J (2007) Enhanced protein expressions of sortilin and p75NTR in retina of rat following elevated intraocular pressure-induced retinal ischemia. Neurosci Lett 429:169–174

Weskamp G, Schlondorff J, Lum L, Becherer JD, Kim TW et al (2004) Evidence for a critical role of the tumor necrosis factor alpha convertase (TACE) in ectodomain shedding of the p75 neurotrophin receptor (p75NTR). J Biol Chem 279:4241–4249

Westwick JK, Bielawska AE, Dbaibo G, Hannun YA, Brenner DA (1995) Ceramide activates the stress-activated protein kinases. J Biol Chem 270:22689–22692

Wheeler EF, Bothwell M (1992) Spatiotemporal patterns of expression of NGF and the low-affinity NGF receptor in rat embryos suggest functional roles in tissue morphogenesis and myogenesis. J Neurosci 12:930–945

Willnow TE, Petersen CM, Nykjaer A (2008) VPS10P-domain receptors – regulators of neuronal viability and function. Nat Rev Neurosci 9:899–909

Wolfe SA, Nekludova L, Pabo CO (2000) DNA recognition by Cys2His2 zinc finger proteins. Annu Rev Biophys Biomol Struct 29:183–212

Wong ST, Henley JR, Kanning KC, Huang KH, Bothwell M, Poo MM (2002) A p75(NTR) and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. Nat Neurosci 5:1302–1308

Woo NH, Teng HK, Siao CJ, Chiaruttini C, Pang PT et al (2005) Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat Neurosci 8:1069–1077

Wu GS (2004) The functional interactions between the p53 and MAPK signaling pathways. Cancer Biol Ther 3:156–161

Xiao J, Wong AW, Willingham MM, Kaasinen SK, Hendry IA et al (2009) BDNF exerts contrasting effects on peripheral myelination of NGF-dependent and BDNF-dependent DRG neurons. J Neurosci 29:4016–4022

Yaar M, Zhai S, Pilch PF, Doyle SM, Eisenhauer PB et al (1997) Binding of beta-amyloid to the p75 neurotrophin receptor induces apoptosis. A possible mechanism for Alzheimer's disease. J Clin Invest 100:2333–2340

Yamamoto K, Ichijo H, Korsmeyer SJ (1999) BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. Mol Cell Biol 19:8469–8478

Yamashita T, Tohyama M (2003) The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. Nat Neurosci 6:461–467

Yamashita T, Tucker KL, Barde YA (1999) Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. Neuron 24:585–593

Yamashita T, Higuchi H, Tohyama M (2002) The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. J Cell Biol 157:565–570

Yamauchi J, Chan JR, Shooter EM (2004) Neurotrophins regulate Schwann cell migration by activating divergent signaling pathways dependent on Rho GTPases. Proc Natl Acad Sci U S A 101:8774–8779

Ye X, Mehlen P, Rabizadeh S, VanArsdale T, Zhang H et al (1999) TRAF family proteins interact with the common neurotrophin receptor and modulate apoptosis induction. J Biol Chem 274:30202–30208

Yeiser EC, Rutkoski NJ, Naito A, Inoue J, Carter BD (2004) Neurotrophin signaling through the p75 receptor is deficient in traf6−/− mice. J Neurosci 24:10521–10529

Yin Q, Lin SC, Lamothe B, Lu M, Lo YC et al (2009) E2 interaction and dimerization in the crystal structure of TRAF6. Nat Struct Mol Biol 16:658–666

Yoon SO, Casaccia-Bonnefil P, Carter B, Chao MV (1998) Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival. J Neurosci 18:3273–3281

Zagrebelsky M, Holz A, Dechant G, Barde YA, Bonhoeffer T, Korte M (2005) The p75 neurotrophin receptor negatively modulates dendrite complexity and spine density in hippocampal neurons. J Neurosci 25:9989–9999

Zampieri N, Xu CF, Neubert TA, Chao MV (2005) Cleavage of p75 neurotrophin receptor by alpha-secretase and gamma-secretase requires specific receptor domains. J Biol Chem 280:14563–14571

Zhang Y, Hong Y, Bounhar Y, Blacker M, Roucou X et al (2003) p75 neurotrophin receptor protects primary cultures of human neurons against extracellular amyloid beta peptide cytotoxicity. J Neurosci 23:7385–7394

Zheng B, Atwal J, Ho C, Case L, He XL et al (2005) Genetic deletion of the Nogo receptor does not reduce neurite inhibition in vitro or promote corticospinal tract regeneration in vivo. Proc Natl Acad Sci U S A 102:1205–1210

Zhou Q, Homma KJ, Poo MM (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron 44:749–757

Zotti T, Vito P, Stilo R (2012) The seventh ring: exploring TRAF7 functions. J Cell Physiol 227:1280–1284

Sortilins in Neurotrophic Factor Signaling

S. Glerup, A. Nykjaer, and C.B. Vaegter

Abstract

The sortilin family of Vps10p-domain receptors includes sortilin, SorLA, and SorCS1–3. These type-I transmembrane receptors predominate in distinct neuronal tissues, but expression is also present in certain specialized non-neuronal cell populations including hepatocytes and cells of the immune system. The biology of sortilins is complex as they participate in both cell signaling and in intracellular protein sorting. Sortilins function physiologically in signaling by pro- and mature neurotrophins in neuronal viability and functionality. Recent genome-wide association studies have linked members to neurological disorders such as Alzheimer's disease and bipolar disorder and outside the nervous system to development of coronary artery disease and type-2 diabetes. Particularly well described are the receptor functions in neuronal signaling by pro- (proNT) and mature (NT) neurotrophins and in the processing/metabolism of amyloid precursor protein (APP).

Keywords

Vps10p-domain receptors • Proneurotrophins • p75NTR • SorCS1 • SorCS2 • SorCS3 • Alzheimer's disease • Bipolar disorder • Cell death • Amyloid precursor protein • Protein sorting • Apoptosis

S. Glerup • A. Nykjaer • C.B. Vaegter (✉)
Danish Research Institute of Translational Neuroscience DANDRITE, Nordic EMBL Partnership, and The Lundbeck Foundation Research Center MIND, Department of Biomedicine, University of Aarhus, Ole Worms Allé 3, 8000 Aarhus C, Denmark
e-mail: cv@biokemi.au.dk

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_7,

1 The Vps10p Domain Receptor Family: Sortilins

Sortilins, also denoted Vps10p domain receptors, are emerging as critical regulators of neuronal survival and function (Fig. 1). They partake in a multitude of functions from anterograde and retrograde protein sorting to signal transduction induced by neurotrophic factors (Willnow et al. 2008). The mammalian sortilin family includes sortilin, SorLA, and SorCS1–3 and appears to have evolved with increasing demand for cellular complexity. The unifying structural Vps10p domain, short for vacuolar protein sorting 10 protein, was first identified in the yeast (Marcusson et al. 1994). Vps10p is a type I receptor with two copies of the domain in its extracellular part that participates in a mannose-6-phosphate receptor-independent pathway for sorting of proteins targeted for the yeast vacuole. Human sortilin and SorLA were first isolated from brain homogenates by receptor-associated protein (RAP) affinity chromatography in an attempt to discover novel low density lipoprotein receptor-related proteins (Jacobsen et al. 1996; Petersen et al. 1997). Sortilin is the prototype family member as its entire extracellular domain consists of a single copy of the ~700 amino acids Vps10p domain followed by a transmembrane domain and a short cytoplasmic tail. In contrast, SorLA is a mosaic receptor with a large extracellular part. In SorLA, the Vps10p domain is followed by an EGF precursor-type repeat, a cluster of 11 complement repeats, and six fibronectin type-III repeats (Fig. 1). Interestingly, SorLA appears to have originated in the first organism with a nervous system, the hydra, where it acts as a receptor for the neuropeptide head activator (Hampe et al. 2000).

The homologues SorCS1, SorCS2, and SorCS3 were identified by database mining (Hampe et al. 2000; Hermey 2009; Rezgaoui et al. 2001), and contain in their extracellular domains in addition to the Vps10p domain, a polycystic kidney disease (PKD) module, and a juxtamembrane leucine-rich region (Fig. 1). The global sequence identity between SorCS1 and SorCS3 proteins is 70 %, whereas their identity with SorCS2 is much lower ranging from 45 to 47 % with highest conservation found in the Vps10p domain and lowest identity in their propeptides and cytoplasmic tails.

The composition of the yeast Vps10p with two luminal copies of the domain is only found in fungi whereas a sortilin-like composition is conserved in protozoans, echinoderms, and vertebrates. Highly conserved orthologues of all the mammalian family members are found in birds and fish, but no Vps10p orthologues have been identified in species such as flies, nematodes, and plants (Hermey 2009).

In mammals, sortilins prevail in most regions of the developing and adult nervous system. However, receptors are also expressed in a dynamic manner in a number of non-neuronal cell types. Sortilin and SorLA, for example, are abundant in tissues such as embryonic lung, kidney, liver, and several developing glands. In the adult organism expression persists in most tissues and now also appears in cells of the immune system (Hermans-Borgmeyer et al. 1999; Sarret et al. 2003; Fauchais et al. 2008; Wahe et al. 2010; Kjolby et al. 2010). While expression of sortilin and SorLA mostly overlap, SorCS1, -2, and -3 show a much more restricted and complementary pattern of expression. For instance, SorCS1 and SorCS3 are highly expressed in CA1 of the hippocampus, whereas SorCS2 displays the highest

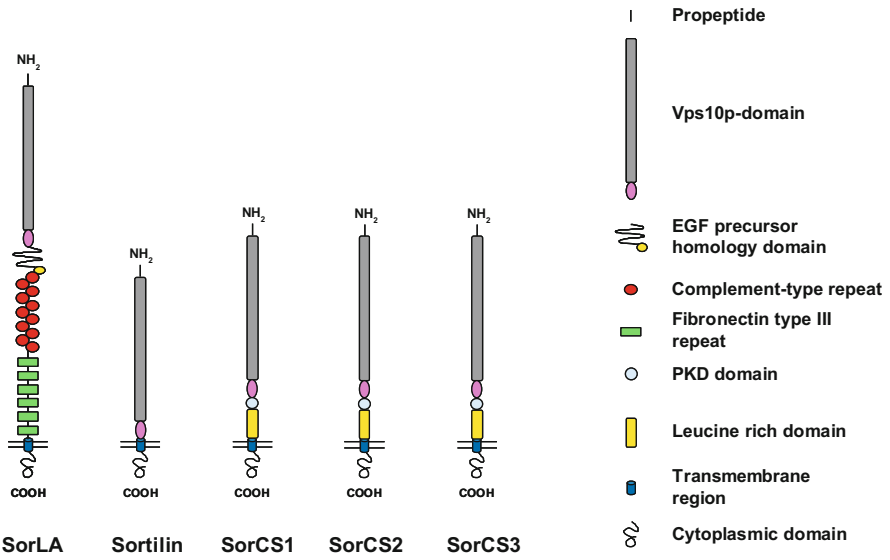

Fig. 1 The Vps10p domain receptor family. Sortilin represents the prototype family member containing an N-terminal propeptide followed by the Vps10p domain that constitutes the entire extracellular part of the mature receptor. The receptor also contains a transmembrane region and a short cytoplasmic domain containing several functional sorting motifs. The propeptide is cleaved off by furin during processing of the receptor in the Golgi. In the extracellular part of SorLA, the Vps10p domain is followed by an EGF precursor homology domain, a series of complement repeats originally known from the low-density lipoprotein receptor and a series of fibronection type III repeats. SorCS1-3 are global homologues and contain in addition to the Vps10p domain, a polycystic kidney disease (PKD) domain and a leucine-rich domain

expression in the CA2 and dentate gyrus. Of note, the hippocampal expression of SorCS1 and SorCS3 is dynamically regulated as both can be induced by neuronal activity, suggesting their potential participation in activity-dependent synaptic modifications (Hermey et al. 2004).

2 The Vps10p Domain Structure

Sortilins bind a wide variety of ligands through the Vps10p domain, ranging from transmembrane receptors to soluble proteins involved in processes as diverse as lipid metabolism and signaling by neurotrophic factors. Mammalian sortilin and yeast Vps10p show low amino acid sequence identity, yet they are both predicted to adopt a beta-propeller fold. The crystal structure of sortilin in complex with the small neuropeptide ligand neurotensin (Table 1) was recently solved and revealed a completely novel fold (Quistgaard et al. 2009) (Fig. 2). The domain forms an unusually large ten-bladed beta-propeller structure creating a large tunnel with multiple ligand-binding sites formed by loops protruding from the beta-strand ends into the tunnel cavity. The beta-propeller is followed C-terminally by a

Table 1 Known binding partners of sortilins

Ligands	Sortilin	SorLA	SorCS1	SorCS2	SorCS3	Function
APP		+				Golgi retention (Andersen et al. 2005)
ApoB100	+					VLDL particle assembly (Kjolby et al. 2010)
CLC/CLF-1	+					Signaling (Larsen et al. 2010)
CNTF	+					Signaling (Larsen et al. 2010)
LPL	+	+				Lysosomal sorting (Nielsen et al. 1999)
Neurotensin	+	+				Unknown (Mazella et al. 1998)
$P75^{NTR}$	+					Apoptosis (Nykjaer et al. 2004)
PDGFβ		+	+		+	Unknown (Hermey et al. 2006)
ProBDNF	+					Apoptosis (Teng et al. 2005)
Progranulin	+					Lysosomal sorting (Hu et al. 2010)
ProNGF	+				+	Apoptosis (Nykjaer et al. 2004)
ProNT3	+					Apoptosis (Yano et al. 2009; Tauris et al. 2011)
Prosaposin	+					Lysosomal sorting (Lefrancois et al. 2003)
RAP	+	+				Unknown (Petersen et al. 1997; Jacobsen et al. 1996)
SorLA propeptide	+	+				Inhibition of premature ligand binding (Jacobsen et al. 2001)
Sortilin propeptide	+	+				Inhibition of premature ligand binding (Munck Petersen et al. 1999)
TGFβ	+					Lysosomal sorting (Kwon and Christian 2011)
Thyroglobulin	+					Recycling (Botta et al. 2009)
TrkA, -B, -C	+					Sorting (Vaegter et al. 2011)

APP amyloid precursor protein, *CLC/CLF-1* cardiotrophin-like cytokine/receptor cytokine-like factor-1, *CNTF* ciliary neurotrophic factor, *LPL* lipoprotein lipase, *PDGFβ* platelet-derived growth factor-β, *proBDNF* pro-brain-derived neurotrophic factor, *proNGF* pro-nerve growth factor, *proNT3* pro-neurotrophin-3, *RAP* receptor-associated protein, *Trk* tropomyosin-related kinase

small domain designated 10CC. This domain has no secondary structure but comprises ten cysteine residues forming five disulfide bonds. 10CC interacts extensively with one side of the propeller and is believed to stabilize the tunnel while restricting the access of ligands to this side of the tunnel.

Neurotensin inhibits the binding of most sortilin ligands in a competitive manner, and the co-crystal structure showed that the neurotensin binding site resides inside the tunnel cavity. This suggests that different binding sites for soluble ligands are formed within the tunnel likely by differential combinations of the protruding loops. Most Vps10p domain ligands compete with each other for binding, but this ability most likely relies on steric hindrance rather than on identical binding sites. The ten blades of the Vps10p domain propeller results in a fourfold increase in the tunnel

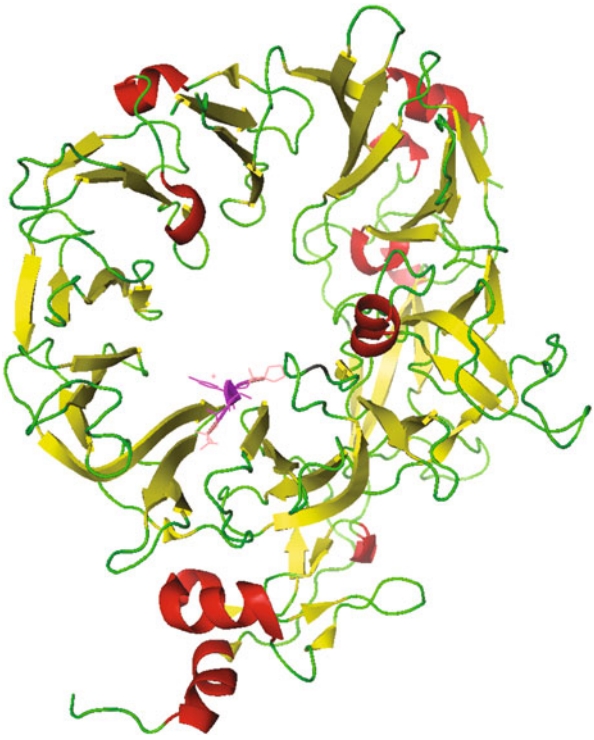

Fig. 2 Crystal structure of the sortilin Vps10p domain in complex with neurotensin. The structure is visualized using PyMOL

volume as compared to, for example, the eight-bladed propeller peptidase IV. Thus, unlike smaller beta propellers, the sortilin tunnel can accommodate large protein ligands and provide specific binding sites for an extended set of ligands. Also, confining binding sites to the tunnel, rather than being scattered on an exposed outer surface, ensures that sortilin accommodates only one ligand at a time.

Another interesting structural feature of the Vps10p domain is two protruding hydrophobic loops (Quistgaard et al. 2009). These loops have been proposed to interact directly with the cell membrane or with transmembrane receptor partners such as the p75 neurotrophin receptor (p75NTR) or the tropomyosin-related kinase (Trk) family of receptors (see below). Apart from the sortilin Vps10p domain, the solution structures of the second SorLA fibronectin type III domain (PDB code: 2DM4) in addition to SorCS2 PKD (PDB code: 1WGO) have been solved by NMR. The PKD structure related it to the immunoglobin superfamily.

3 Ligands of Sortilins

A number of both soluble and transmembrane ligands binding to the extracellular domains of sortilin family members have been described, and those shown to bind in a direct manner are listed in Table 1. Sortilin has the highest number of ligands

followed by SorLA with many overlaps. Only two ligands have been identified for SorCS1 and -3, whereas SorCS2 is so far an orphan receptor. The majority of sortilin ligands are related to lipid metabolism such as lipoprotein lipase (LPL) (Nielsen et al. 1999) and ApoB100 (Kjolby et al. 2010), or to neurotrophic factor signaling, notably, the neurotrophin system and their receptors (Nykjaer et al. 2004; Teng et al. 2005; Yano et al. 2009; Tauris et al. 2011; Vaegter et al. 2011) in addition to a subset of helical type I cytokines (Larsen et al. 2010). SorLA is unique in binding amyloid precursor protein (APP), and this interaction is considered important to avoid pathological amyloid plaque formation (Andersen et al. 2005) (see below).

4 Processing Conditions Sortilin and SorLA for Ligand Binding

An additional unifying feature of Vps10p domain receptors throughout species is an N-terminal propeptide containing an RXXR motif that defines the consensus cleavage site for furin and other proprotein convertases. Sortilin, SorLA, and SorCS1–3 are all synthesized as proproteins, and their ~50 amino acids propeptides are removed late in the trans-Golgi network (TGN), possibly following internalization and recycling of the newly synthesized receptor, as processing of mutated sortilin lacking the cytoplasmic tail is slowed dramatically (Munck Petersen et al. 1999; Nielsen et al. 2001). Furin cleavage results in dissociation of propeptide from the mature receptor and in the case of sortilin and SorLA, this conditions the receptors for ligand binding (Munck Petersen et al. 1999; Jacobsen et al. 2001). Accordingly, mutation of the furin cleavage sites results in receptor variants that are unable to bind Vps10p domain ligands (Munck Petersen et al. 1999; Jacobsen et al. 2001). Also, recombinant soluble propeptides are potent antagonists of sortilin and SorLA ligand binding, e.g., proneurotrophin binding by sortilin (Munck Petersen et al. 1999; Jacobsen et al. 2001; Nykjaer et al. 2004; Teng et al. 2005; Tauris et al. 2011). Neurotensin also completely inhibits this binding, implicating that the tunnel cavity is critically involved in the interaction with proneurotrophins (Nykjaer et al. 2004; Quistgaard et al. 2009). The above has important functional implications as it indicates that prosortilin cannot interact with Vps10p domain ligands prior to propeptide processing, whereas the SorCS3 propeptide appears to have no effect on its ability to bind pro-nerve growth factor (proNGF) (Westergaard et al. 2005). In the case of sortilin, SorLA, SorCS1, and SorCS3, it has been further demonstrated that the propeptide acts as a chaperone in the endoplasmic reticulum (ER) required for efficient folding of the newly synthesized receptors (Munck Petersen et al. 1999; Jacobsen et al. 2001; Westergaard et al. 2005; Hermey et al. 2003).

5 Cellular Trafficking of Sortilins

Sortilin and SorLA are mainly found intracellularly in perinuclear vesicles and in the TGN with less than 10 % of the receptor pool present on the cell surface (Petersen et al. 1997; Jacobsen et al. 1996; Nielsen et al. 2001). In neurons, sortilin and SorLA also show a vesicle-like staining in the soma, but receptors can also be found in axons and dendrites and at the nerve terminals (Sarret et al. 2003; Hermey et al. 2001). In contrast, SorCS3 is predominantly surface exposed (Westergaard et al. 2005). Notably, SorCS1 is unique among the sortilins as it exists in several distinct splice variants, denoted SorCS1-a, -b, and -c, that encode cytoplasmic domains differing in length and sequence. While the SorCS1-a variant is almost exclusively intracellular, the -b and -c isoforms mainly localize to the cell surface (Hermey et al. 2003; Nielsen et al. 2008).

As mentioned above, sortilins are matured in the TGN (Munck Petersen et al. 1999). This organelle is a sorting station important for distributing proteins between various cellular compartments. From here, proteins are directed to the constitutive or regulated secretory pathway, and to endosomes or lysosomes. Neuronal TGN is also involved in axonal transport and in the formation of signaling endosomes (Bonifacino and Rojas 2006). Trafficking between the TGN and the different cellular compartments is assisted by specific adaptor proteins that directly or indirectly connect the cytoplasmic tail of sortilins to the clathrin coat of transport vesicles, to lipid membranes, or to the cytoskeleton (Nielsen et al. 2001, 2008) (Fig. 3).

At the cell surface sortilin and SorLA are rapidly internalized into endosomes through clathrin coated pits. For SorLA, this is mediated by adaptor protein 2 (AP-2) by binding to an acidic cluster dileucine-like site in the cytoplasmic tail (Nielsen et al. 2007). In the case of sortilin, internalization is mediated by a tyrosine-based internalization motif (Nielsen et al. 2001). SorCS1-c, SorCS2, and SorCS3 also contain tyrosine-based internalization motifs, but their activity and adaptor proteins remain to be determined. Internalized receptors exit endosomes and are returned to the TGN through retrograde sorting pathways, thus escaping lysosomal degradation (Nielsen et al. 2001, 2007). Four distinct complexes of cargo adaptor proteins have been implicated in this transport pathway including the AP-1 complex that links cargo to the clathrin coat of endosomal and TGN vesicles, the clathrin adaptors Golgi-localizing, γ-adaptin ear homology domain, ARF-interacting proteins (GGA-1, -2, -3), the retromer complex, and the phosphofurin acidic cluster sorting protein (PACS1). The existing data suggest a model in which AP-1 and the GGAs transport sortilin and SorLA from the Golgi to endosomes whereas the retromer and possibly AP-1 mediate their return from the tubular endosomal network of early endosomes to the TGN (Nielsen et al. 2001, 2007). In the case of SorLA, PACS1 also appears to be implicated in TGN transport (Schmidt et al. 2007). At present, no data are available for SorCS1–3.

At the cell surface, sortilins are subject to events other than endocytosis as they can also be cleaved by ADAM10 or -17 and released from the cell surface (Hermey et al. 2006). Whether the soluble receptor product, which is capable of ligand

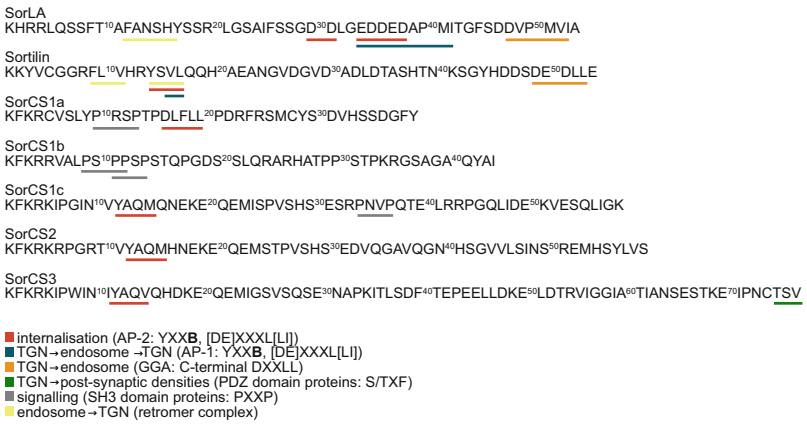

Fig. 3 Cytosolic adaptor sites in sortilins. Both putative and experimentally confirmed sites are indicated together with the type of trafficking they conduct. The responsible adaptor proteins and their consensus recognition motifs are listed in parenthesis (Nielsen et al. 2001, 2007, 2008; Schmidt *et al*. 2007; Hermey *et al*. 2003)

binding, serves as a decoy receptor is currently unclear. However, the remaining transmembrane fragment can undergo regulated intramembrane proteolysis (RIP) by the gamma-secretase complex. The fate of the resulting intracellular fragment is unclear, but in the case of the SorLA cytoplasmic tail, it is targeted to the nucleus where it possibly can induce gene expression as proposed by use of a reporter gene assay (Bohm et al. 2006). Whether RIP of sortilin is implicated signaling by neurotrophic factors remains to be determined, but the role of p75NTR RIP is well described in neuronal survival and death (see below).

6 Sortilin in Proneurotrophin-Induced Apoptosis

Contrasting their inherent name "neurotrophins," literally meaning "nerve nourishments," these molecules not only elicit neuronal survival, growth, and differentiation but are also capable of apoptotic signaling. The trophic signals are governed by binding of the neurotrophins (NT) to their respective Trk receptor. Nerve growth factor (NGF) binds to TrkA, brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) to TrkB, and neurotrophin-3 (NT-3) to TrkC, respectively. Co-expression with $p75^{NTR}$ fortifies the trophic activities as it strengthens both affinity and specificity of the NTs towards their cognate Trk receptor. Intriguingly, $p75^{NTR}$ is also required for NTs to stimulate apoptosis, suggesting that this receptor is able to switch between survival and death signaling depending on the cellular context. For some time this was a puzzling observation, but an indication of the underlying mechanism came with the recognition that while trophic activities are mediated by mature NTs, their proform, denoted proneurotrophins (proNT), can provoke apoptosis in a Trk-independent manner

(Lee et al. 2001). Earlier studies had reported that NT binding to $p75^{NTR}$ can induce apoptosis in cultured neurons and oligodendrocytes lacking the corresponding Trk receptor. However, the non-physiologically high ligand concentrations required suggests that proNTs most likely are accountable for the pro-apoptotic activity in vivo (Bamji et al. 1998; Casaccia-Bonnefil et al. 1996; Kenchappa et al. 2006; Yoon et al. 1998).

It has been intensively debated whether proNTs are merely intracellular precursors that require processing by pro-convertases in the TGN to mature before their secretion, or whether they can indeed be secreted in their unprocessed form. Recent reports have now settled this dispute by the demonstration of proNGF release from cultured sympathetic (Hasan et al. 2003) and cortical (Bruno and Cuello 2006; Hasan et al. 2003) neurons, microglia (Srinivasan et al. 2004), and astrocytes (Domeniconi et al. 2007). Likewise, release of proBDNF has been demonstrated in prenatal (Mowla et al. 1999; Teng et al. 2005) and postnatal (P0) (Yang et al. 2009) derived hippocampus cultures. Perhaps more convincing, in pathological conditions characterized by neurodegeneration such as Alzheimer's disease (AD), spongiform encephalomyelopathy, spinal cord injury, and seizures, proNT levels are increased (Fahnestock et al. 2001; Stoica et al. 2008; Harrington et al. 2004; Beattie et al. 2002; Volosin et al. 2006), and inhibitory antibodies against the proNTs significantly attenuate neuronal cell death associated with the latter two conditions (Harrington et al. 2004; Volosin et al. 2008).

Initially believed that $p75^{NTR}$ by itself was sufficient to bind and transmit the apoptotic signal, subsequent studies revealed a critical role of sortilin in this process. When analyzed separately, $p75^{NTR}$ and sortilin both bind proNGF with estimated affinities (Kd) of ~5–15 nM, but upon their co-expression in cells the affinity increases to ~160 pM (Nykjaer et al. 2004). This binding cooperativity is accomplished in part by a direct interaction between the two receptors, and in part by formation of a ternary receptor complex enabled by the simultaneous binding of the proNT pro-domain with sortilin and the mature part of the molecule with $p75^{NTR}$. In accordance with the dimeric confirmation of neurotrophins, a recent structural study revealed that proNGF shapes a 2:2 complex with $p75^{NTR}$ and that the binding of proNGF to sortilin is enhanced when proNGF is in a preformed complex with $p75^{NTR}$ (Feng et al. 2010). Although most ligands have been demonstrated to occupy sortilin in the tunnel (see above), $p75^{NTR}$ is unlikely to do so as its interaction with sortilin is not inhibited by the tunnel-specific inhibitor neurotensin. In contrast, the binding of proNGF is abolished by neurotensin, suggesting that sortilin potentially may bind proNT and $p75^{NTR}$ simultaneously by engaging different binding epitopes.

The important role of sortilin in neuronal cell death has been substantiated by in vivo studies demonstrating increased expression of sortilin as well as increased co-expression of sortilin with $p75^{NTR}$ following seizure (Volosin et al. 2006), facial nerve and spinal cord injury (Harrington et al. 2004; Provenzano et al. 2008; Jansen et al. 2007), retinal ischemia (Wei et al. 2007), spongiform encephalomyelopathy (Stoica et al. 2008), or aging (Al-Shawi et al. 2008), conditions where proNGF is also upregulated (see above). However, studies in mice deficient in sortilin

expression have provided the most compelling evidence. Cultured knockout neurons that lack sortilin (*Sort1*−/−) but still express $p75^{NTR}$ are resistant specifically to proNT-induced cell death. In vivo, *Sort1*−/− mice are characterized by reduced apoptosis in the developing retina that is indistinguishable from that observed in $p75^{NTR}$ knockout mice, and upon aging deficiency for sortilin protects sympathetic neurons against degeneration. Finally, in a neuronal injury protocol, lesioned corticospinal neurons were fully rescued from death in the *Sort1*−/− mice, a phenotype shared with mice treated with inhibitory antibodies to proNGF (Jansen et al. 2007; Harrington et al. 2004).

7 Sortilins and Proneurotrophin Signaling

What might be the molecular mechanisms by which sortilin affects proNT signaling in neurons? As described above, modulation of proNT affinity towards $p75^{NTR}$ would be a qualified suggestion as sortilin is required for high-affinity proNT binding to $p75^{NTR}$. Whereas this obviously appears to be the simplest mechanistic explanation, additional mechanisms could indeed be involved such as regulation of adaptors to $p75^{NTR}$ or modulation of $p75^{NTR}$ cleavage-dependent signaling. Finally, direct signaling by sortilin can also be envisioned.

While some $p75^{NTR}$ signaling pathways are initiated from the cell surface upon ligand binding (e.g., JNK activation (Reichardt 2006)), other signaling pathways require $p75^{NTR}$ internalization and subsequent receptor sorting from early to recycling endosomes, forming $p75^{NTR}$-signaling endosomes (Bronfman et al. 2003). The molecular mechanisms involved in $p75^{NTR}$ internalization and intracellular sorting are poorly characterized, and as previous studies have described the function of sortilin in internalization and intracellular sorting events, it is not unreasonable to speculate that sortilin could be involved. Thus, proNGF is rapidly internalized in cells expressing $p75^{NTR}$ and sortilin, whereas endocytosis of proNGF is absent in sortilin-deficient cells (Nykjaer et al. 2004). Interestingly, the expression of sortilin on the neuronal cell surface also appears to be positively regulated, thereby increasing responsiveness to proNT during specific developmental stages correlating with cell apoptosis (Nakamura et al. 2007). Thus, a mammalian $p75^{NTR}$ homologue NRH2 was reported to interact with sortilin and function as a trafficking switch, redistributing sortilin from the predominant TGN/perinuclear localization to the cell surface and promoting $p75^{NTR}$–sortilin receptor complex formation. Accordingly, knock down of NRH2 in sympathetic neurons significantly reduced proNT-induced apoptosis in these cells (Kim and Hempstead 2009).

The intracellular signaling cascades initiated by the sortilin–$p75^{NTR}$ complex upon proNT binding remain largely elusive. However, several signaling pathways activated following NT binding to $p75^{NTR}$ are mediated through the binding of adaptor proteins to the cytoplasmic domain of $p75^{NTR}$, including Traf6, neurotrophin receptor-interacting factor (NRIF), melanoma-associated antigen (MAGE), and neurotrophin receptor p75 interacting MAGE homologue (NRAGE) (Yamashita et al. 2005; Reichardt 2006). As the mechanisms that govern

ligand-induced adaptor docking to $p75^{NTR}$ are still largely unknown, it can be speculated whether the formation of a complex between $p75^{NTR}$ and sortilin is able to modulate/enhance adaptor binding to $p75^{NTR}$. Interestingly, Teng et al. (2005) investigated whether complexes of proBDNF and the soluble extracellular domain of sortilin was capable of initiating apoptosis of sympathetic neurons that express endogenous $p75^{NTR}$ and sortilin. Although a ternary complex likely forms between sortilin, $p75^{NTR}$, and proNT with only the extracellular domains of the receptors present (Feng et al. 2010), Teng and colleagues found no induction of apoptosis using a preformed, soluble sortilin–proBDNF complex (Teng et al. 2005). This argues that the transmembrane or intracellular part of sortilin is critical in $p75^{NTR}$- and proNT-mediated apoptosis, perhaps by contributing to the correct stoichiometry of the receptor–ligand complex to allow signaling. So far there has been no description of whether sortilin has any signaling property in its own right. However, it has been described how SorLA under certain circumstances can be processed by TNF-α converting enzyme (TACE) and subsequently by the γ-secretase, releasing both intra- and extracellular fragments. The cytoplasmic tail is subsequently translocated to the nucleus where it acts as a transcriptional activator and enhances proliferation of neuronal precursor cells (Bohm et al. 2006). Another indication that members of the Sortilins may directly signal came from the finding that SorLA interacts with the kinase Ste20-related proline–alanine-rich kinase (SPAK) in the distal nephron of the kidney. Intracellular trafficking of SPAK by SorLA is crucial in the regulation of Na^+-K^+-Cl^- cotransporter 2 (NKCC2) and hence in the maintenance of renal ion balance (Reiche et al. 2010).

Finally, a potential regulatory function of sortilin is upon $p75^{NTR}$ cleavage. On the cell surface and/or in the endosomal compartments, $p75^{NTR}$ is subject to cleavage by gamma-secretase (Bronfman 2007), and proNGF and proBDNF are reported to induce such cleavage in several neuronal systems, including sympathetic neurons, Schwann cells, and photoreceptors. The intracellular domain (ICD) of $p75^{NTR}$ is consequently released, and the $p75^{NTR}$ adaptor neurotrophin receptor interacting factor (NRIF) translocates to the nucleus to induce apoptosis (Kenchappa et al. 2006; Volosin et al. 2008; Srinivasan et al. 2007; Podlesniy et al. 2006). The binding of sortilin (and proNT) to $p75^{NTR}$ could potentially affect $p75^{NTR}$ cleavage as conformational changes upon complex formation might increase the affinity of $p75^{NTR}$ for the gamma-secretase (Fig. 4).

8 Potentiation of Neurotrophic Factor Signaling by Sortilins

As mentioned, the first evidence for a role of sortilin in proNT-mediated apoptosis was described in 2004 (Nykjaer et al. 2004), and this concept has subsequently been confirmed in numerous other studies. Surprisingly, recent data suggest that sortilin, like $p75^{NTR}$, may also engage in trophic signaling by mature neurotrophins. Thus, endogenous sortilin and Trks were found not only to be co-expressed in subgroups of sensory neurons as well as hippocampal and cortical neurons but also to be physically associated as determined by coimmunoprecipitation analysis and

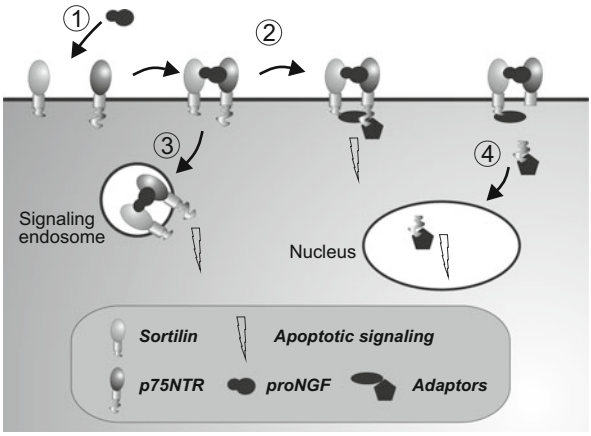

Fig. 4 Potential mechanisms of sortilin in $p75^{NTR}$-dependent proNT apoptotic signaling. (*1*) Sortilin and $p75^{NTR}$ constitute a high-affinity binding site for proNT, strongly increasing proNT binding to the cell surface. (*2*) Signaling of $p75^{NTR}$ depends on the binding of adaptors molecules to the cytoplasmic tail of $p75^{NTR}$, and the formation of a complex between $p75^{NTR}$ and sortilin might modulate/enhance adaptor binding to $p75^{NTR}$. (*3*) Some signaling pathways are reported to require $p75^{NTR}$ internalization with the formation of $p75^{NTR}$-signaling endosomes, perhaps assisted by sortilin. (*4*) $p75^{NTR}$ can be cleaved upon proNT binding, with the C-terminal fragment and adaptors translocating to the nucleus to induce apoptosis

fluorescence resonance energy transfer (FRET) microscopy. Studies in neuron cultures and in knockout mice revealed that sortilin facilitates efficient anterograde axonal transport and synaptic targeting of the Trks. However, the mechanism by which sortilin links to the microtubule motor is currently unknown. Somewhat surprisingly, the sortilin-deficient mice do not appear to be seriously affected by the reduction in peripheral Trk levels, at least when assessing sensory nerve morphology and functionality (Vaegter et al. 2011). These observations are, however, in accordance with previous work on Trk heterozygote mice which display reductions in Trk levels and activity of approximately 50 % (comparable to observations in the sortilin-deficient mouse) but are phenotypically normal (Ernfors et al. 1994; Klein et al. 1993; Minichiello et al. 1995) (Fig. 5).

The combined observations suggest the tripartite model for neurotrophin signaling illustrated in Fig. 6 ("the neurotrophin triangle"): Sortilin is essential to form a death complex with $p75^{NTR}$ activated by proNT. Signaling by Trk receptors, conversely, requires $p75^{NTR}$ on the plasma membrane to facilitate binding of NT and to strengthen trophic signals. To complete this triangular interaction, sortilin supports and fine-tunes trophic signaling by facilitating anterograde Trk transport along the axonal path.

Yet another function of sortilin in NT signaling was put forward by Chen and colleagues, who showed that sortilin is involved in sorting of BDNF from the TGN into the pathway for regulated secretion (Chen et al. 2005). In untreated primary hippocampal neurons and the neuroblastoma cell line PC12, (pro)BDNF

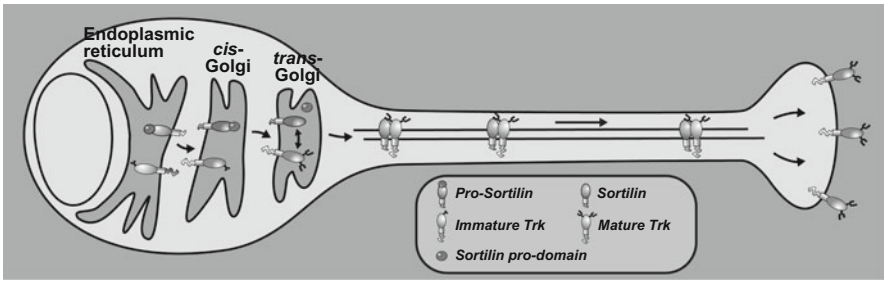

Fig. 5 Schematic model of sortilin involvement in Trk receptor trafficking. Prior to the trans-Golgi network (TGN), the pro-domain of sortilin inhibits binding of ligands to sortilin. However, following furin-mediated pro-convertase cleavage in the TGN, mature sortilin is now able to bind fully glycosylated Trk and facilitate anterograde transport of this receptor, assuring sufficient peripheral Trk levels to sustain efficient neurotrophin signaling by neurotrophins released from target tissues

colocalized with secretogranin II that labels vesicles destined for regulated secretion. Inhibiting sortilin activity by siRNA knockdown or overexpression of dominant-negative receptor mutants redistributed proBDNF from the regulated to the constitutive secretory pathway and reduced depolarization-induced (pro)BDNF secretion with a concomitant increase in constitutive release (Chen et al. 2005). Biochemical mapping subsequently identified a conserved binding motif in the pro-domain of BDNF that is capable of binding the luminal domain of sortilin. A recent study further supports the role of sortilin in vesicular transport and stabilization of proBDNF. Yang and colleagues found that proBDNF forms complex with sortilin and Huntingtin-associated protein-1 (HAP1) and that this complex is important for the transport of proBDNF/BDNF-containing vesicles to facilitate synaptic targeting of proBDNF in neurites of cortical neurons. Furthermore, the association of sortilin to the proBDNF/HAP1 complex prevents proBDNF degradation and facilitates the furin cleavage to release mature BDNF (Yang et al. 2011). How sortilin affects vesicular transport is unclear, but it is noteworthy that KIF1A, a subunit of kinesin-3 that transports synaptic vesicles, has been identified as a sortilin interaction partner (Vaegter et al. 2011) (Peder Madsen, personal communication).

Signaling by neurotrophic factors other than neurotrophins have also been reported to be positively regulated by sortilin. Ciliary neurotrophic factor (CNTF) belongs to the family of helical type 1 cytokines, which also includes interleukin-6 (IL-6), IL-11, leukemia inhibitory factor (LIF), and others. CNTF was initially identified (and named) for its ability to maintain survival of parasympathetic neurons of chicken ciliary ganglia (Adler et al. 1979). Since then it has been reported to support the survival of a variety of neuronal cell types, including sensory (Simon et al. 1995) and motor (Oppenheim et al. 1991) neurons. Furthermore, it is believed to act as a lesion factor released from tissues subjected to trauma as several studies have reported a marked change in the localization and expression of CNTF (Rudge et al. 1995; Sendtner et al. 1992; Friedman et al. 1992). CNTF signaling is elicited by the formation of a trimeric receptor complex composed of

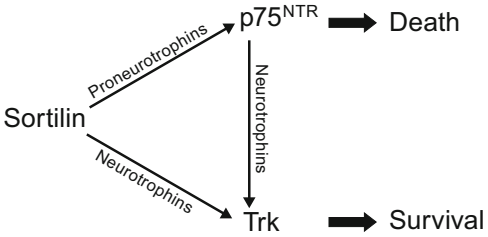

Fig. 6 Schematic illustration of "the neurotrophin triangle" concept, linking sortilin and key receptors in survival and death signaling by mature/pro-neutrophins

the GPI-anchored CNTF receptor α (CNTFRα), the signaling subunit 130-kDa glycoprotein (gp130), and the LIF receptor β (LIFRβ) (Davis et al. 1993). However, CNTFRα is not an absolute requirement for signaling because CNTF at relatively high concentrations is able to activate the gp130/LIFRβ heterodimer (Gearing et al. 1994). Interestingly, Larsen and colleagues demonstrated that sortilin interacts with LIFRβ, thereby facilitating CNTF signaling and mediating CNTF-dependent proliferation through the gp130/LIFRβ heterodimeric complex (Larsen et al. 2010). It will be interesting for future studies to investigate the effect of sortilin upon CNTF signaling in relation to, e.g., motor neuron regeneration following nerve injury in vivo.

9 Sortilins and Neuronal Disease

The first member of the family to be associated with a neurodegenerative disease was SorLA, with the finding of low levels of SorLA gene expression (*SORL1*) in patients with sporadic AD (Scherzer et al. 2004). Whereas several subsequent association studies confirmed this connection, some failed to do so. However, a recent comprehensive and unbiased meta-analysis of all published and unpublished data from studies on *SORL1* SNPs, including approximately 12,000 cases of AD and 17,000 controls, significantly substantiated the involvement of *SORL1* gene variants in AD and further suggested multiple causative gene variants in distinct regions of *SORL1* (Reitz et al. 2011a). Neuronal processing of amyloid-precurser protein (APP) by the β-secretase, with formation of the cleavage product Aβ and subsequently development of neurotoxic Aβ oligomers and senile plaques, are pathological hallmarks of AD. The involvement of SorLA in APP processing was described by Andersen and colleagues in 2005, demonstrating that the proteins colocalize in Golgi compartments and early endosomes. Further studies demonstrated that the neuronal production of Aβ inversely correlated with the level of SorLA, as APP is retained in the TGN by SorLA and thereby impairs transit to the plasma membrane or late endosomes for β-secretase cleavage (Andersen et al. 2005; Schmidt et al. 2007).

Intriguingly, another member of the sortilins has been associated with AD. Association of SorCS1 with AD was suggested approximately concurrently with the genetic association between SorLA and AD (Rogaeva et al. 2007; Grupe et al. 2006). A later study substantiated the association and found significantly lower SorCS1 expression in AD brains, suggesting an inverse correlation between SorCS1 levels and Aβ production, and this correlation was further supported by biochemical studies in cell lines (Reitz et al. 2011b). However, while genetic and biochemical data support a relationship between SorCS1 and AD, the mechanisms by which SorCS1 modulates Aβ is currently not clear.

Although AD is characterized by Aβ plaque formation, neurotrophins are likely involved in the subsequent process with loss of neurons. The cortex of AD brain is characterized by an increase in proNGF levels during disease progression and stable levels of $p75^{NTR}$ and sortilin but reduced levels of TrkA (Mufson et al. 2010; Counts et al. 2004; Al-Shawi et al. 2008; Peng et al. 2004; Fahnestock et al. 2001). As the balance of pro-survival versus pro-apoptotic signaling may depend on the stoichiometry of these proteins (Masoudi et al. 2009; Capsoni et al. 2010), the shift in ratio may very well change the functional outcome of proNGF on neurons in the brain. Because TrkA is necessary for NGF pro-survival signaling, this shift in NGF receptor stoichiometry paralleled with increased proNGF may favor the trimeric interactions of proNGF with $p75^{NTR}$ and sortilin, activating pro-apoptotic pathways during the early stages of AD. Further, although some studies indicate that proNGF can bind to TrkA (albeit with less with less affinity than NGF) to induce neurotrophic response (Fahnestock et al. 2004), the lower levels of TrkA may not be sufficient to initiate proNGF-induced cell survival signaling in the AD brain.

Sortilin has recently been functionally linked to frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U), a form of frontotemporal dementia (FTD) characterized by neuronal loss within, and atrophy of, the frontal and temporal lobes of the brain. FTLD-U cases are caused by haplo-insufficiency due to mutations in the GRN gene encoding progranulin (PGRN), a common feature in FTD with about 50 identified mutations in GRN linked to these disorders (Mackenzie et al. 2010). Despite intense investigation, the normal and pathological roles of PGRN within the CNS are still largely unknown. Apparently, PGRN can function as a nerve growth, protective, or survival factor (Bateman and Bennett 2009), and the reduction of PGRN levels observed in, e.g., FTLD-U would indeed be consistent with the observed neurodegeneration. Hu and colleagues identified sortilin as a major neuronal receptor for the PGRN, providing an important mechanistic link to understand normal CNS functions of PGRN and how partial loss of PGRN function may lead to neurodegenerative disease. Importantly, they showed that sortilin regulates PGRN levels as mice lacking sortilin had elevated brain and serum PGRN levels and further that PGRN binds sortilin and colocalizes with sortilin in endocytic vesicles and eventually with Lamp1, a marker for lysosomes (Hu et al. 2010). Together with the finding that mice lacking PGRN develop lysosomal dysfunction, this might implicate a normal role of PGRN in the lysosome (Ahmed et al. 2010).

A further hallmark of FTLD-U is the loss of nuclear localization of TAR DNA binding protein (TDP-43) but the presence of cytosolic accumulation of ubiquitinated inclusions of TDP-43. Two studies show that TDP-43 binds many target RNAs, approximately 30 % of the mouse transcriptome and preferably within the intron, suggesting a function in splicing regulation. Intriguingly, knock-down of TDP-43 affected in particular splicing of sortilin, suggesting another possible regulatory link between sortilin and key molecules in FTLD-U (Tollervey et al. 2011; Baum et al. 2008).

A single nucleotide polymorphism in the *bdnf* gene resulting in a valine (Val) to methionine (Met) mutation at amino acid 66 in the BDNF prodomain has been linked to neuropsychiatric disorders including depression, bipolar disorders, and memory impairment (Sen et al. 2003; Neves-Pereira et al. 2002; Sklar et al. 2002; Egan et al. 2003; Hariri et al. 2003; Rybakowski et al. 2003). The molecular mechanisms underlying the altered-variant function is not understood, but the Met-variant has been reported to have reduced activity-dependent (or regulated) secretion (Egan et al. 2003; Chen et al. 2004). Interestingly, Chen and colleagues reported in 2005 that the binding site of sortilin within the prodomain of BDNF is overlapping the region containing the Val–Met substitution and that the Met-variant has decreased interaction with sortilin (Chen et al. 2005). Thus, identification of the sortilin–BDNF interaction in regulated secretion of BDNF provides a possible molecular model in the attempt to understand the effect of the BDNF polymorphism in the selective impairment of CNS function.

Lastly, it should be noted that recent genome-wide association studies (GWAS) implicated SorCS2 in the etiology of bipolar disorder. Generally, the diagnosis and lack of quantitative physiological parameters in this disorder makes genomic studies challenging. However, a number of independent studies have now described association of the same three SNPs in the *SORCS2* gene to the risk of bipolar disorder, and *SORCS2* is in fact one of the top candidate genes to emerge from these GWAS (Baum et al. 2008; Christoforou et al. 2011; Ollila et al. 2009).

10 The Role of Sortilins in Metabolic Disorders

Although expression of sortilin family members predominates in neuronal tissues, they are also present in specific cell types in tissues outside the nervous system (skeletal muscle, pancreas, thyroid, liver, lung, heart) (Petersen et al. 1997; Hermey 2009; Jansen et al. 2007; Vaegter et al. 2011). The functions of the receptors outside the nervous system are still only beginning to be unraveled but appear to embrace involvement in many apparently unrelated molecular pathways. In particular, sortilin and SorCS1 have recently attracted attention due to their proposed roles in metabolic disorders such as regulation of plasma cholesterol levels/coronary heart disease (sortilin) and insulin metabolism/type 2 diabetes (sortilin and SorCS1).

Genome-wide association studies of large human cohorts showed a strong correlation between single-nucleotide polymorphisms (SNPs) in the chromosome

1p13.3 locus (that harbors the sortilin gene) and hypercholesterolemia as well as coronary heart disease (Kathiresan et al. 2008; Willer et al. 2008; Sandhu et al. 2008; Dube et al. 2011; Willnow et al. 2011). Effort has subsequently been mobilized to identify the mechanistic basis of this association, and independent groups have recently described their findings after focusing on the sortilin gene (*SORT1*), located at this locus. However, these studies find opposite effects of how sortilin might affect plasma cholesterol level. A study by Kjolby and colleagues found that loss of sortilin in a transgenic mouse model results in a reduction of plasma cholesterol. Furthermore, sortilin bound apoB100 containing lipoproteins in the secretory pathway, suggesting a stimulatory involvement in very-low-density lipoproteins (VLDL) secretion (Kjolby et al. 2010). In opposition to these findings, Musunuru and colleagues used a very different mouse model and reported that sortilin levels inversely correlate with plasma cholesterol, as sortilin impaired VLDL secretion from hepatocytes (Musunuru et al. 2010). While these contradictory findings may appear incompatible, they perhaps rather demonstrate that the specific function of regulatory proteins might significantly differ depending on the genetic background and chow and hence molecular conditions in which they are studied. Therefore, sortilin may partake in a broader range of functions in lipoprotein sorting/secretion depending on the overall metabolic milieu in vivo.

Other studies have linked sortilin and SorCS1 to insulin/glucose metabolism and the risk of type 2 diabetes development. Thus, SorCS1 was identified as a diabetes susceptibility gene, affecting fasting insulin and glucose plasma levels in mice (Clee et al. 2006; Stoehr et al. 2000). Genetic variants of the SORCS1 gene were subsequently associated with diabetes risk and age of onset of diabetes in a human genetic association study (Goodarzi et al. 2007), reducing in vivo insulin secretion and hence interfering with compensatory mechanism when type 2 diabetic patients become severely insulin resistant. Insulin resistance in fat and skeletal muscle tissues may be caused not only by defective insulin signaling but also by abnormal glucose transporter Glut4 regulation. Under basal conditions, Glut4 is present in multiple subcellular compartments but majorly in a distinct population of vesicles named insulin-responsive vesicles (IRV) or alternatively Glut4 storage vesicles (GSV). Upon insulin stimulation, glucose uptake in fat and skeletal muscle tissues is achieved by translocating Glut4 from the intracellular storage pool to the plasma membrane. In this context it is therefore interesting that sortilin shows a high degree of colocalization with Glut4 and represents one of the major component proteins of Glut4 vesicles (Lin et al. 1997; Morris et al. 1998). Furthermore, sortilin has been demonstrated to be essential for biogenesis of IRVs and for the acquisition of insulin responsiveness in adipose cells (Shi and Kandror 2005).

References

Adler R, Landa KB, Manthorpe M, Varon S (1979) Cholinergic neuronotrophic factors: intraocular distribution of trophic activity for ciliary neurons. Science 204(4400):1434–1436

Ahmed Z, Sheng H, Xu YF, Lin WL, Innes AE, Gass J, Yu X, Wuertzer CA, Hou H, Chiba S, Yamanouchi K, Leissring M, Petrucelli L, Nishihara M, Hutton ML, McGowan E, Dickson DW, Lewis J (2010) Accelerated lipofuscinosis and ubiquitination in granulin knockout mice suggest a role for progranulin in successful aging. Am J Pathol 177(1):311–324

Al-Shawi R, Hafner A, Olsen J, Chun S, Raza S, Thrasivoulou C, Lovestone S, Killick R, Simons P, Cowen T (2008) Neurotoxic and neurotrophic roles of proNGF and the receptor sortilin in the adult and ageing nervous system. Eur J Neurosci 27(8):2103–2114

Andersen OM, Reiche J, Schmidt V, Gotthardt M, Spoelgen R, Behlke J, von Arnim CA, Breiderhoff T, Jansen P, Wu X, Bales KR, Cappai R, Masters CL, Gliemann J, Mufson EJ, Hyman BT, Paul SM, Nykjaer A, Willnow TE (2005) Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. Proc Natl Acad Sci U S A 102(38):13461–13466

Bamji SX, Majdan M, Pozniak CD, Belliveau DJ, Aloyz R, Kohn J, Causing CG, Miller FD (1998) The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. J Cell Biol 140(4):911–923

Bateman A, Bennett HP (2009) The granulin gene family: from cancer to dementia. Bioessays 31 (11):1245–1254

Baum AE, Akula N, Cabanero M, Cardona I, Corona W, Klemens B, Schulze TG, Cichon S, Rietschel M, Nothen MM, Georgi A, Schumacher J, Schwarz M, Abou Jamra R, Hofels S, Propping P, Satagopan J, Detera-Wadleigh SD, Hardy J, McMahon FJ (2008) A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. Mol Psychiatry 13(2):197–207

Beattie MS, Harrington AW, Lee R, Kim JY, Boyce SL, Longo FM, Bresnahan JC, Hempstead BL, Yoon SO (2002) ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. Neuron 36(3):375–386

Bohm C, Seibel NM, Henkel B, Steiner H, Haass C, Hampe W (2006) SorLA signaling by regulated intramembrane proteolysis. J Biol Chem 281(21):14547–14553

Bonifacino JS, Rojas R (2006) Retrograde transport from endosomes to the trans-Golgi network. Nat Rev Mol Cell Biol 7(8):568–579

Botta R, Lisi S, Pinchera A, Giorgi F, Marcocci C, Taddei AR, Fausto AM, Bernardini N, Ippolito C, Mattii L, Persani L, de Filippis T, Calebiro D, Madsen P, Petersen CM, Marino M (2009) Sortilin is a putative postendocytic receptor of thyroglobulin. Endocrinology 150(1): 509–518

Bronfman FC (2007) Metalloproteases and gamma-secretase: new membrane partners regulating p75 neurotrophin receptor signaling? J Neurochem 103(Suppl 1):91–100

Bronfman FC, Tcherpakov M, Jovin TM, Fainzilber M (2003) Ligand-induced internalization of the p75 neurotrophin receptor: a slow route to the signaling endosome. J Neurosci 23(8): 3209–3220

Bruno MA, Cuello AC (2006) Activity-dependent release of precursor nerve growth factor, conversion to mature nerve growth factor, and its degradation by a protease cascade. Proc Natl Acad Sci U S A 103(17):6735–6740

Capsoni S, Tiveron C, Vignone D, Amato G, Cattaneo A (2010) Dissecting the involvement of tropomyosin-related kinase A and p75 neurotrophin receptor signaling in NGF deficit-induced neurodegeneration. Proc Natl Acad Sci U S A 107(27):12299–12304

Casaccia-Bonnefil P, Carter BD, Dobrowsky RT, Chao MV (1996) Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. Nature 383(6602): 716–719

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J Neurosci 24(18):4401–4411

Chen ZY, Ieraci A, Teng H, Dall H, Meng CX, Herrera DG, Nykjaer A, Hempstead BL, Lee FS (2005) Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. J Neurosci 25(26):6156–6166

Christoforou A, McGhee KA, Morris SW, Thomson PA, Anderson S, McLean A, Torrance HS, Le Hellard S, Pickard BS, StClair D, Muir WJ, Blackwood DH, Porteous DJ, Evans KL (2011) Convergence of linkage, association and GWAS findings for a candidate region for bipolar disorder and schizophrenia on chromosome 4p. Mol Psychiatry 16(3):240–242

Clee SM, Yandell BS, Schueler KM, Rabaglia ME, Richards OC, Raines SM, Kabara EA, Klass DM, Mui ET, Stapleton DS, Gray-Keller MP, Young MB, Stoehr JP, Lan H, Boronenkov I, Raess PW, Flowers MT, Attie AD (2006) Positional cloning of Sorcs1, a type 2 diabetes quantitative trait locus. Nat Genet 38(6):688–693

Counts SE, Nadeem M, Wuu J, Ginsberg SD, Saragovi HU, Mufson EJ (2004) Reduction of cortical TrkA but not p75(NTR) protein in early-stage Alzheimer's disease. Ann Neurol 56(4): 520–531

Davis S, Aldrich TH, Stahl N, Pan L, Taga T, Kishimoto T, Ip NY, Yancopoulos GD (1993) LIFR beta and gp130 as heterodimerizing signal transducers of the tripartite CNTF receptor. Science 260(5115):1805–1808

Domeniconi M, Hempstead BL, Chao MV (2007) Pro-NGF secreted by astrocytes promotes motor neuron cell death. Mol Cell Neurosci 34(2):271–279

Dube JB, Johansen CT, Hegele RA (2011) Sortilin: an unusual suspect in cholesterol metabolism: from GWAS identification to in vivo biochemical analyses, sortilin has been identified as a novel mediator of human lipoprotein metabolism. Bioessays 33(6):430–437

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112(2):257–269

Ernfors P, Lee KF, Kucera J, Jaenisch R (1994) Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. Cell 77(4):503–512

Fahnestock M, Michalski B, Xu B, Coughlin MD (2001) The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. Mol Cell Neurosci 18(2):210–220

Fahnestock M, Yu G, Michalski B, Mathew S, Colquhoun A, Ross GM, Coughlin MD (2004) The nerve growth factor precursor proNGF exhibits neurotrophic activity but is less active than mature nerve growth factor. J Neurochem 89(3):581–592

Fauchais AL, Lalloue F, Lise MC, Boumediene A, Preud'homme JL, Vidal E, Jauberteau MO (2008) Role of endogenous brain-derived neurotrophic factor and sortilin in B cell survival. J Immunol 181(5):3027–3038

Feng D, Kim T, Ozkan E, Light M, Torkin R, Teng KK, Hempstead BL, Garcia KC (2010) Molecular and structural insight into proNGF engagement of p75NTR and sortilin. J Mol Biol 396(4):967–984

Friedman B, Scherer SS, Rudge JS, Helgren M, Morrisey D, McClain J, Wang DY, Wiegand SJ, Furth ME, Lindsay RM et al (1992) Regulation of ciliary neurotrophic factor expression in myelin-related Schwann cells in vivo. Neuron 9(2):295–305

Gearing DP, Ziegler SF, Comeau MR, Friend D, Thoma B, Cosman D, Park L, Mosley B (1994) Proliferative responses and binding properties of hematopoietic cells transfected with low-affinity receptors for leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor. Proc Natl Acad Sci U S A 91(3):1119–1123

Goodarzi MO, Lehman DM, Taylor KD, Guo X, Cui J, Quinones MJ, Clee SM, Yandell BS, Blangero J, Hsueh WA, Attie AD, Stern MP, Rotter JI (2007) SORCS1: a novel human type 2 diabetes susceptibility gene suggested by the mouse. Diabetes 56(7):1922–1929

Grupe A, Li Y, Rowland C, Nowotny P, Hinrichs AL, Smemo S, Kauwe JS, Maxwell TJ, Cherny S, Doil L, Tacey K, van Luchene R, Myers A, Wavrant-De Vrieze F, Kaleem M, Hollingworth P, Jehu L, Foy C, Archer N, Hamilton G, Holmans P, Morris CM, Catanese J,

Sninsky J, White TJ, Powell J, Hardy J, O'Donovan M, Lovestone S, Jones L, Morris JC, Thal L, Owen M, Williams J, Goate A (2006) A scan of chromosome 10 identifies a novel locus showing strong association with late-onset Alzheimer disease. Am J Hum Genet 78(1):78–88

Hampe W, Riedel IB, Lintzel J, Bader CO, Franke I, Schaller HC (2000) Ectodomain shedding, translocation and synthesis of SorLA are stimulated by its ligand head activator. J Cell Sci 113 (Pt 24):4475–4485

Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR (2003) Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci 23(17):6690–6694

Harrington AW, Leiner B, Blechschmitt C, Arevalo JC, Lee R, Morl K, Meyer M, Hempstead BL, Yoon SO, Giehl KM (2004) Secreted proNGF is a pathophysiological death-inducing ligand after adult CNS injury. Proc Natl Acad Sci U S A 101(16):6226–6230

Hasan W, Pedchenko T, Krizsan-Agbas D, Baum L, Smith PG (2003) Sympathetic neurons synthesize and secrete pro-nerve growth factor protein. J Neurobiol 57(1):38–53

Hermans-Borgmeyer I, Hermey G, Nykjaer A, Schaller C (1999) Expression of the 100-kDa neurotensin receptor sortilin during mouse embryonal development. Brain Res Mol Brain Res 65(2):216–219

Hermey G (2009) The Vps10p-domain receptor family. Cell Mol Life Sci 66(16):2677–2689

Hermey G, Riedel IB, Rezgaoui M, Westergaard UB, Schaller C, Hermans-Borgmeyer I (2001) SorCS1, a member of the novel sorting receptor family, is localized in somata and dendrites of neurons throughout the murine brain. Neurosci Lett 313(1–2):83–87

Hermey G, Keat SJ, Madsen P, Jacobsen C, Petersen CM, Gliemann J (2003) Characterization of sorCS1, an alternatively spliced receptor with completely different cytoplasmic domains that mediate different trafficking in cells. J Biol Chem 278(9):7390–7396

Hermey G, Plath N, Hubner CA, Kuhl D, Schaller HC, Hermans-Borgmeyer I (2004) The three sorCS genes are differentially expressed and regulated by synaptic activity. J Neurochem 88 (6):1470–1476

Hermey G, Sjogaard SS, Petersen CM, Nykjaer A, Gliemann J (2006) Tumour necrosis factor alpha-converting enzyme mediates ectodomain shedding of Vps10p-domain receptor family members. Biochem J 395(2):285–293

Hu F, Padukkavidana T, Vaegter CB, Brady OA, Zheng Y, Mackenzie IR, Feldman HH, Nykjaer A, Strittmatter SM (2010) Sortilin-mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. Neuron 68(4):654–667

Jacobsen L, Madsen P, Moestrup SK, Lund AH, Tommerup N, Nykjaer A, Sottrup-Jensen L, Gliemann J, Petersen CM (1996) Molecular characterization of a novel human hybrid-type receptor that binds the alpha2-macroglobulin receptor-associated protein. J Biol Chem 271 (49):31379–31383

Jacobsen L, Madsen P, Jacobsen C, Nielsen MS, Gliemann J, Petersen CM (2001) Activation and functional characterization of the mosaic receptor SorLA/LR11. J Biol Chem 276(25): 22788–22796

Jansen P, Giehl K, Nyengaard JR, Teng K, Lioubinski O, Sjoegaard SS, Breiderhoff T, Gotthardt M, Lin F, Eilers A, Petersen CM, Lewin GR, Hempstead BL, Willnow TE, Nykjaer A (2007) Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. Nat Neurosci 10(11):1449–1457

Kathiresan S, Melander O, Guiducci C, Surti A, Burtt NP, Rieder MJ, Cooper GM, Roos C, Voight BF, Havulinna AS, Wahlstrand B, Hedner T, Corella D, Tai ES, Ordovas JM, Berglund G, Vartiainen E, Jousilahti P, Hedblad B, Taskinen MR, Newton-Cheh C, Salomaa V, Peltonen L, Groop L, Altshuler DM, Orho-Melander M (2008) Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 40(2):189–197

Kenchappa RS, Zampieri N, Chao MV, Barker PA, Teng HK, Hempstead BL, Carter BD (2006) Ligand-dependent cleavage of the P75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. Neuron 50(2):219–232

Kim T, Hempstead BL (2009) NRH2 is a trafficking switch to regulate sortilin localization and permit proneurotrophin-induced cell death. EMBO J 28(11):1612–1623

Kjolby M, Andersen OM, Breiderhoff T, Fjorback AW, Pedersen KM, Madsen P, Jansen P, Heeren J, Willnow TE, Nykjaer A (2010) Sort1, encoded by the cardiovascular risk locus 1p13.3, is a regulator of hepatic lipoprotein export. Cell Metab 12(3):213–223

Klein R, Smeyne RJ, Wurst W, Long LK, Auerbach BA, Joyner AL, Barbacid M (1993) Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. Cell 75(1):113–122

Kwon S, Christian JL (2011) Sortilin associates with transforming growth factor-{beta} family proteins to enhance lysosome-mediated degradation. J Biol Chem 286(24):21876–21885

Larsen JV, Hansen M, Moller B, Madsen P, Scheller J, Nielsen M, Petersen CM (2010) Sortilin facilitates signaling of ciliary neurotrophic factor and related helical type 1 cytokines targeting the gp130/leukemia inhibitory factor receptor beta heterodimer. Mol Cell Biol 30(17): 4175–4187

Lee R, Kermani P, Teng KK, Hempstead BL (2001) Regulation of cell survival by secreted proneurotrophins. Science 294(5548):1945–1948

Lefrancois S, Zeng J, Hassan AJ, Canuel M, Morales CR (2003) The lysosomal trafficking of sphingolipid activator proteins (SAPs) is mediated by sortilin. EMBO J 22(24):6430–6437

Lin BZ, Pilch PF, Kandror KV (1997) Sortilin is a major protein component of Glut4-containing vesicles. J Biol Chem 272(39):24145–24147

Mackenzie IR, Rademakers R, Neumann M (2010) TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. Lancet Neurol 9(10):995–1007

Marcusson EG, Horazdovsky BF, Cereghino JL, Gharakhanian E, Emr SD (1994) The sorting receptor for yeast vacuolar carboxypeptidase Y is encoded by the VPS10 gene. Cell 77(4): 579–586

Masoudi R, Ioannou MS, Coughlin MD, Pagadala P, Neet KE, Clewes O, Allen SJ, Dawbarn D, Fahnestock M (2009) Biological activity of nerve growth factor precursor is dependent upon relative levels of its receptors. J Biol Chem 284(27):18424–18433

Mazella J, Zsurger N, Navarro V, Chabry J, Kaghad M, Caput D, Ferrara P, Vita N, Gully D, Maffrand JP, Vincent JP (1998) The 100-kDa neurotensin receptor is gp95/sortilin, a non-G-protein-coupled receptor. J Biol Chem 273(41):26273–26276

Minichiello L, Piehl F, Vazquez E, Schimmang T, Hokfelt T, Represa J, Klein R (1995) Differential effects of combined trk receptor mutations on dorsal root ganglion and inner ear sensory neurons. Development 121(12):4067–4075

Morris NJ, Ross SA, Lane WS, Moestrup SK, Petersen CM, Keller SR, Lienhard GE (1998) Sortilin is the major 110-kDa protein in GLUT4 vesicles from adipocytes. J Biol Chem 273 (6):3582–3587

Mowla SJ, Pareek S, Farhadi HF, Petrecca K, Fawcett JP, Seidah NG, Morris SJ, Sossin WS, Murphy RA (1999) Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. J Neurosci 19(6):2069–2080

Mufson EJ, Wuu J, Counts SE, Nykjaer A (2010) Preservation of cortical sortilin protein levels in MCI and Alzheimer's disease. Neurosci Lett 471(3):129–133

Munck Petersen C, Nielsen MS, Jacobsen C, Tauris J, Jacobsen L, Gliemann J, Moestrup SK, Madsen P (1999) Propeptide cleavage conditions sortilin/neurotensin receptor-3 for ligand binding. EMBO J 18(3):595–604

Musunuru K, Strong A, Frank-Kamenetsky M, Lee NE, Ahfeldt T, Sachs KV, Li X, Li H, Kuperwasser N, Ruda VM, Pirruccello JP, Muchmore B, Prokunina-Olsson L, Hall JL, Schadt EE, Morales CR, Lund-Katz S, Phillips MC, Wong J, Cantley W, Racie T, Ejebe KG, Orho-Melander M, Melander O, Koteliansky V, Fitzgerald K, Krauss RM, Cowan CA, Kathiresan S, Rader DJ (2010) From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus. Nature 466(7307):714–719

Nakamura K, Namekata K, Harada C, Harada T (2007) Intracellular sortilin expression pattern regulates proNGF-induced naturally occurring cell death during development. Cell Death Differ 14(8):1552–1554

Neves-Pereira M, Mundo E, Muglia P, King N, Macciardi F, Kennedy JL (2002) The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. Am J Hum Genet 71(3):651–655

Nielsen MS, Jacobsen C, Olivecrona G, Gliemann J, Petersen CM (1999) Sortilin/neurotensin receptor-3 binds and mediates degradation of lipoprotein lipase. J Biol Chem 274(13): 8832–8836

Nielsen MS, Madsen P, Christensen EI, Nykjaer A, Gliemann J, Kasper D, Pohlmann R, Petersen CM (2001) The sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS domain of the GGA2 sorting protein. EMBO J 20(9):2180–2190

Nielsen MS, Gustafsen C, Madsen P, Nyengaard JR, Hermey G, Bakke O, Mari M, Schu P, Pohlmann R, Dennes A, Petersen CM (2007) Sorting by the cytoplasmic domain of the amyloid precursor protein binding receptor SorLA. Mol Cell Biol 27(19):6842–6851

Nielsen MS, Keat SJ, Hamati JW, Madsen P, Gutzmann JJ, Engelsberg A, Pedersen KM, Gustafsen C, Nykjaer A, Gliemann J, Hermans-Borgmeyer I, Kuhl D, Petersen CM, Hermey G (2008) Different motifs regulate trafficking of SorCS1 isoforms. Traffic 9(6):980–994

Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, Jacobsen C, Kliemannel M, Schwarz E, Willnow TE, Hempstead BL, Petersen CM (2004) Sortilin is essential for proNGF-induced neuronal cell death. Nature 427(6977):843–848

Ollila HM, Soronen P, Silander K, Palo OM, Kieseppa T, Kaunisto MA, Lonnqvist J, Peltonen L, Partonen T, Paunio T (2009) Findings from bipolar disorder genome-wide association studies replicate in a Finnish bipolar family-cohort. Mol Psychiatry 14(4):351–353

Oppenheim RW, Prevette D, Yin QW, Collins F, MacDonald J (1991) Control of embryonic motoneuron survival in vivo by ciliary neurotrophic factor. Science 251(5001):1616–1618

Peng S, Wuu J, Mufson EJ, Fahnestock M (2004) Increased proNGF levels in subjects with mild cognitive impairment and mild Alzheimer disease. J Neuropathol Exp Neurol 63(6):641–649

Petersen CM, Nielsen MS, Nykjaer A, Jacobsen L, Tommerup N, Rasmussen HH, Roigaard H, Gliemann J, Madsen P, Moestrup SK (1997) Molecular identification of a novel candidate sorting receptor purified from human brain by receptor-associated protein affinity chromatography. J Biol Chem 272(6):3599–3605

Podlesniy P, Kichev A, Pedraza C, Saurat J, Encinas M, Perez B, Ferrer I, Espinet C (2006) Pro-NGF from Alzheimer's disease and normal human brain displays distinctive abilities to induce processing and nuclear translocation of intracellular domain of p75NTR and apoptosis. Am J Pathol 169(1):119–131

Provenzano MJ, Xu N, Ver Meer MR, Clark JJ, Hansen MR (2008) p75NTR and sortilin increase after facial nerve injury. Laryngoscope 118(1):87–93

Quistgaard EM, Madsen P, Groftehauge MK, Nissen P, Petersen CM, Thirup SS (2009) Ligands bind to Sortilin in the tunnel of a ten-bladed beta-propeller domain. Nat Struct Mol Biol 16(1): 96–98

Reichardt LF (2006) Neurotrophin-regulated signalling pathways. Philos Trans R Soc Lond B Biol Sci 361(1473):1545–1564

Reiche J, Theilig F, Rafiqi FH, Carlo AS, Militz D, Mutig K, Todiras M, Christensen EI, Ellison DH, Bader M, Nykjaer A, Bachmann S, Alessi D, Willnow TE (2010) SORLA/SORL1 functionally interacts with SPAK to control renal activation of Na(+)-K(+)-Cl(−) cotransporter 2. Mol Cell Biol 30(12):3027–3037

Reitz C, Cheng R, Rogaeva E, Lee JH, Tokuhiro S, Zou F, Bettens K, Sleegers K, Tan EK, Kimura R, Shibata N, Arai H, Kamboh MI, Prince JA, Maier W, Riemenschneider M, Owen M, Harold D, Hollingworth P, Cellini E, Sorbi S, Nacmias B, Takeda M, Pericak-Vance MA, Haines JL, Younkin S, Williams J, van Broeckhoven C, Farrer LA, St George-Hyslop PH, Mayeux R (2011a) Meta-analysis of the association between variants in SORL1 and Alzheimer disease. Arch Neurol 68(1):99–106

Reitz C, Tokuhiro S, Clark LN, Conrad C, Vonsattel JP, Hazrati LN, Palotas A, Lantigua R, Medrano M, Z Jiménez-Velázquez I, Vardarajan B, Simkin I, Haines JL, Pericak-Vance MA, Farrer LA, Lee JH, Rogaeva E, George-Hyslop PS, Mayeux R (2011b) SORCS1 alters amyloid precursor protein processing and variants may increase Alzheimer's disease risk. Ann Neurol 69(1):47–64

Rezgaoui M, Hermey G, Riedel IB, Hampe W, Schaller HC, Hermans-Borgmeyer I (2001) Identification of SorCS2, a novel member of the VPS10 domain containing receptor family, prominently expressed in the developing mouse brain. Mech Dev 100(2):335–338

Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, Katayama T, Baldwin CT, Cheng R, Hasegawa H, Chen F, Shibata N, Lunetta KL, Pardossi-Piquard R, Bohm C, Wakutani Y, Cupples LA, Cuenco KT, Green RC, Pinessi L, Rainero I, Sorbi S, Bruni A, Duara R, Friedland RP, Inzelberg R, Hampe W, Bujo H, Song YQ, Andersen OM, Willnow TE, Graff-Radford N, Petersen RC, Dickson D, Der SD, Fraser PE, Schmitt-Ulms G, Younkin S, Mayeux R, Farrer LA, St George-Hyslop P (2007) The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet 39(2):168–177

Rudge JS, Pasnikowski EM, Holst P, Lindsay RM (1995) Changes in neurotrophic factor expression and receptor activation following exposure of hippocampal neuron/astrocyte cocultures to kainic acid. J Neurosci 15(10):6856–6867

Rybakowski JK, Borkowska A, Czerski PM, Skibinska M, Hauser J (2003) Polymorphism of the brain-derived neurotrophic factor gene and performance on a cognitive prefrontal test in bipolar patients. Bipolar Disord 5(6):468–472

Sandhu MS, Waterworth DM, Debenham SL, Wheeler E, Papadakis K, Zhao JH, Song K, Yuan X, Johnson T, Ashford S, Inouye M, Luben R, Sims M, Hadley D, McArdle W, Barter P, Kesaniemi YA, Mahley RW, McPherson R, Grundy SM, Bingham SA, Khaw KT, Loos RJ, Waeber G, Barroso I, Strachan DP, Deloukas P, Vollenweider P, Wareham NJ, Mooser V (2008) LDL-cholesterol concentrations: a genome-wide association study. Lancet 371(9611): 483–491

Sarret P, Krzywkowski P, Segal L, Nielsen MS, Petersen CM, Mazella J, Stroh T, Beaudet A (2003) Distribution of NTS3 receptor/sortilin mRNA and protein in the rat central nervous system. J Comp Neurol 461(4):483–505

Scherzer CR, Offe K, Gearing M, Rees HD, Fang G, Heilman CJ, Schaller C, Bujo H, Levey AI, Lah JJ (2004) Loss of apolipoprotein E receptor LR11 in Alzheimer disease. Arch Neurol 61 (8):1200–1205

Schmidt V, Sporbert A, Rohe M, Reimer T, Rehm A, Andersen OM, Willnow TE (2007) SorLA/LR11 regulates processing of amyloid precursor protein via interaction with adaptors GGA and PACS-1. J Biol Chem 282(45):32956–32964

Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB, Burmeister M (2003) A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology 28(2):397–401

Sendtner M, Stockli KA, Thoenen H (1992) Synthesis and localization of ciliary neurotrophic factor in the sciatic nerve of the adult rat after lesion and during regeneration. J Cell Biol 118 (1):139–148

Shi J, Kandror KV (2005) Sortilin is essential and sufficient for the formation of Glut4 storage vesicles in 3T3-L1 adipocytes. Dev Cell 9(1):99–108

Simon R, Thier M, Kruttgen A, Rose-John S, Weiergraber O, Heinrich PC, Schroder JM, Weis J (1995) Human CNTF and related cytokines: effects on DRG neurone survival. Neuroreport 7 (1):153–157

Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM, Tsan G, Schaffner S, Kirov G, Jones I, Owen M, Craddock N, DePaulo JR, Lander ES (2002) Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. Mol Psychiatry 7(6):579–593

Srinivasan B, Roque CH, Hempstead BL, Al-Ubaidi MR, Roque RS (2004) Microglia-derived pronerve growth factor promotes photoreceptor cell death via p75 neurotrophin receptor. J Biol Chem 279(40):41839–41845

Srinivasan B, Wang Z, Brun-Zinkernagel AM, Collier RJ, Black RA, Frank SJ, Barker PA, Roque RS (2007) Photic injury promotes cleavage of p75NTR by TACE and nuclear trafficking of the p75 intracellular domain. Mol Cell Neurosci 36(4):449–461

Stoehr JP, Nadler ST, Schueler KL, Rabaglia ME, Yandell BS, Metz SA, Attie AD (2000) Genetic obesity unmasks nonlinear interactions between murine type 2 diabetes susceptibility loci. Diabetes 49(11):1946–1954

Stoica G, Lungu G, Kim HT, Wong PK (2008) Up-regulation of pro-nerve growth factor, neurotrophin receptor p75, and sortilin is associated with retrovirus-induced spongiform encephalomyelopathy. Brain Res 1208:204–216

Tauris J, Gustafsen C, Christensen EI, Jansen P, Nykjaer A, Nyengaard JR, Teng KK, Schwarz E, Ovesen T, Madsen P, Petersen CM (2011) Proneurotrophin-3 may induce Sortilin-dependent death in inner ear neurons. Eur J Neurosci 33(4):622–631

Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD, Kermani P, Torkin R, Chen ZY, Lee FS, Kraemer RT, Nykjaer A, Hempstead BL (2005) ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. J Neurosci 25(22):5455–5463

Tollervey JR, Curk T, Rogelj B, Briese M, Cereda M, Kayikci M, Konig J, Hortobagyi T, Nishimura AL, Zupunski V, Patani R, Chandran S, Rot G, Zupan B, Shaw CE, Ule J (2011) Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. Nat Neurosci 14(4):452–458

Vaegter CB, Jansen P, Fjorback AW, Glerup S, Skeldal S, Kjolby M, Richner M, Erdmann B, Nyengaard JR, Tessarollo L, Lewin GR, Willnow TE, Chao MV, Nykjaer A (2011) Sortilin associates with Trk receptors to enhance anterograde transport and neurotrophin signaling. Nat Neurosci 14(1):54–61

Volosin M, Song W, Almeida RD, Kaplan DR, Hempstead BL, Friedman WJ (2006) Interaction of survival and death signaling in basal forebrain neurons: roles of neurotrophins and proneurotrophins. J Neurosci 26(29):7756–7766

Volosin M, Trotter C, Cragnolini A, Kenchappa RS, Light M, Hempstead BL, Carter BD, Friedman WJ (2008) Induction of proneurotrophins and activation of p75NTR-mediated apoptosis via neurotrophin receptor-interacting factor in hippocampal neurons after seizures. J Neurosci 28(39):9870–9879

Wahe A, Kasmapour B, Schmaderer C, Liebl D, Sandhoff K, Nykjaer A, Griffiths G, Gutierrez MG (2010) Golgi-to-phagosome transport of acid sphingomyelinase and prosaposin is mediated by sortilin. J Cell Sci 123(Pt 14):2502–2511

Wei Y, Wang N, Lu Q, Zhang N, Zheng D, Li J (2007) Enhanced protein expressions of sortilin and p75NTR in retina of rat following elevated intraocular pressure-induced retinal ischemia. Neurosci Lett 429(2–3):169–174

Westergaard UB, Kirkegaard K, Sorensen ES, Jacobsen C, Nielsen MS, Petersen CM, Madsen P (2005) SorCS3 does not require propeptide cleavage to bind nerve growth factor. FEBS Lett 579(5):1172–1176

Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, Heath SC, Timpson NJ, Najjar SS, Stringham HM, Strait J, Duren WL, Maschio A, Busonero F, Mulas A, Albai G, Swift AJ, Morken MA, Narisu N, Bennett D, Parish S, Shen H, Galan P, Meneton P, Hercberg S, Zelenika D, Chen WM, Li Y, Scott LJ, Scheet PA, Sundvall J, Watanabe RM, Nagaraja R, Ebrahim S, Lawlor DA, Ben-Shlomo Y, Davey-Smith G, Shuldiner AR, Collins R, Bergman RN, Uda M, Tuomilehto J, Cao A, Collins FS, Lakatta E, Lathrop GM, Boehnke M, Schlessinger D, Mohlke KL, Abecasis GR (2008) Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet 40(2):161–169

Willnow TE, Petersen CM, Nykjaer A (2008) VPS10P-domain receptors – regulators of neuronal viability and function. Nat Rev Neurosci 9(12):899–909

Willnow TE, Kjolby M, Nykjaer A (2011) Sortilins: new players in lipoprotein metabolism. Curr Opin Lipidol 22(2):79–85

Yamashita T, Fujitani M, Hata K, Mimura F, Yamagishi S (2005) Diverse functions of the p75 neurotrophin receptor. Anat Sci Int 80(1):37–41

Yang J, Siao CJ, Nagappan G, Marinic T, Jing D, McGrath K, Chen ZY, Mark W, Tessarollo L, Lee FS, Lu B, Hempstead BL (2009) Neuronal release of proBDNF. Nat Neurosci 12(2): 113–115

Yang M, Lim Y, Li X, Zhong JH, Zhou XF (2011) Precursor of brain-derived neurotrophic factor (proBDNF) forms a complex with Huntingtin associated protein-1 (HAP1) and sortilin that modulates proBDNF trafficking, degradation and processing. J Biol Chem 286(18): 16272–16284

Yano H, Torkin R, Martin LA, Chao MV, Teng KK (2009) Proneurotrophin-3 is a neuronal apoptotic ligand: evidence for retrograde-directed cell killing. J Neurosci 29(47):14790–14802

Yoon SO, Casaccia-Bonnefil P, Carter B, Chao MV (1998) Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival. J Neurosci 18(9):3273–3281

Part III

The Biology of Neurotrophins

Neurotrophins in the Regulation of Cellular Survival and Death

Claire Ceni, Nicolas Unsain, Michele P. Zeinieh, and Philip A. Barker

Abstract

The neurotrophins play crucial roles regulating survival and apoptosis in the developing and injured nervous system. The four neurotrophins exert profound and crucial survival effects on developing peripheral neurons, and their expression and action is intimately tied to successful innervation of peripheral targets. In the central nervous system, they are dispensable for neuronal survival during development but support neuronal survival after lesion or other forms of injury. Neurotrophins also regulate apoptosis of both peripheral and central neurons, and we now recognize that there are regulatory advantages to having the same molecules regulate life and death decisions. This chapter examines the biological contexts in which these events take place and highlights the specific ligands, receptors, and signaling mechanisms that allow them to occur.

Keywords

Proneurotrophins • p75NTR • Sortilin • Retrograde signaling • Tumor cells • Apoptosis • Death domains • NF-kB • Interleukin-1 receptor associated kinase (IRAK) • RIP2 • Receptor-interacting serine/Threonine-protein kinase 2 (RIP2) • Neurotrophin receptor interacting factor (NRIF) • Neurotrophin receptor-interacting MAGE homolog (NRAGE)

1 Introduction

The regulation of cell survival and death is a key aspect of the establishment of functional neuronal circuits. A remarkable feature of the developing vertebrate nervous system is that, for most populations, an excess of neurons is produced and

C. Ceni • N. Unsain • M.P. Zeinieh • P.A. Barker (✉)
Centre for Neuronal Survival, Montreal Neurological Institute, McGill University, 3801 University Street, Montreal, QC, Canada H3A 2B4
e-mail: phil.barker@mcgill.ca

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_8,

only those that successfully contact their target and form appropriate connections survive, while the remaining are removed by apoptosis. Several decades ago, analysis of limb ablation and target addition in developing vertebrate embryos demonstrated that naturally occurring cell death was regulated by target tissues [reviewed in Levi-Montalcini (1987) and Oppenheim (1991)]. This led to the hypothesis that target tissues produce limiting amounts of neurotrophic factors and that only neurons which successfully competed for this limited supply went on to survive the period of naturally occurring cell death. The pioneering work of Rita Levi-Montalcini and her colleagues (Levi-Montalcini and Angeletti 1968) led to the identification of nerve growth factor (NGF) as the first target-derived survival factor. This discovery initiated a new field of research in neurobiology devoted to characterizing the action of NGF, other neurotrophins, and non-neurotrophin survival factors.

The neurotrophins play important roles in neuronal life and death decisions. Two decades after the identification of NGF, Barde et al. (1982) succeeded in isolating a neuron survival factor from pig brain, termed brain-derived neurotrophic factor [BDNF—(Barde et al. 1982)]. BDNF was subsequently shown to be highly homologous to NGF (Leibrock et al. 1989) and this quickly led to the identification of neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), neurotrophin-6 (NT-6), and neurotrophin-7 (NT-7) (Hallbook et al. 1991; Hohn et al. 1990; Ip et al. 1992; Maisonpierre et al. 1990b; Gotz et al. 1994; Lai et al. 1998). NT-6 and NT-7 are expressed only in fish and will not be further discussed in this chapter. Given their central role as survival promoting factors, it was initially surprising to learn that the neurotrophins are also capable of initiating death pathways during development and after injury. In retrospect, we now recognize that the use of the same molecules and receptors in regulation of survival and death provides the tight regulatory control required to appropriately sculpt a functional nervous system.

2 Neurotrophins Promote the Survival of Neurons Through Trk Receptors

2.1 Neurotrophins and the Trk Receptors Support Survival of Developing Peripheral Neurons

About 50 % of the peripheral neurons that are generated go on to die during development through a process of programmed cell death. Initial in vivo gain and loss of function experiments that used exogenous NGF or neutralizing NGF antibodies showed that NGF is a survival factor for sympathetic neurons and a subpopulation of sensory dorsal root ganglia (DRG) neurons. The subsequent generation of mouse strains carrying null mutations in genes encoding each of the neurotrophins and their receptors unequivocally demonstrated that NGF, BDNF, NT-3, and NT-4/5, as well as their cognate Trk receptors, play an important role in regulating the survival of peripheral neurons [reviewed in Huang and Reichardt (2001)].

DRG neurons detect three distinct sensory modalities: nociception, elicited by noxious or thermal stimuli; mechanoreception, provoked by mechanical pressure in the skin; and proprioception, elicited by mechanical displacement of the muscles and joints. The subpopulation of neurons that detect these modalities show remarkable specificity in their neurotrophin requirements for survival during development [reviewed in Ernfors (2001) and Farinas (1999)] which is matched with the spatial distribution of neurotrophin expression. For example, NGF is expressed in the skin, a major target of pain sensory neurons, whereas NT-3 is expressed in muscle spindles, Golgi tendon organs, and Merkel cells, the targets of the NT-3-dependent neurons (Buchman and Davies 1993; Copray and Brouwer 1994; Ernfors 2001).

Nociceptive neurons invariably express TrkA at some time during their development, and essentially all of these neurons are lost in mice rendered null for TrkA or NGF (Crowley et al. 1994; Smeyne et al. 1994). Loss of a single allele of the NGF gene reduces survival of TrkA-expressing DRG neurons (Crowley et al. 1994) whereas transgenic mice that overexpress NGF skin display increased survival of TrkA-expressing neurons, both in wild-type neurons (Albers et al. 1994) and in NGF null mice (Harrison et al. 2004). Thus, it would appear that limiting quantities of skin-derived NGF normally support the survival of TrkA-expressing DRG neurons during development. Interestingly, recent evidence shows that after target innervation, nociceptive neurons also become dependent on locally produced BDNF for their survival (Valdes-Sanchez et al. 2010).

Most DRG neurons that express TrkC upon neurogenesis differentiate into proprioceptive neurons that convey information from muscle spindles and Golgi tendon organs. These neurons, and their corresponding end organs, are lost in NT-3 and TrkC mutants (Ernfors et al. 1994b; Klein et al. 1994). Proprioceptive neurons are lost almost immediately after neurogenesis in these null strains, which suggests that they depend on NT-3 before target innervation occurs, probably provided through intermediate targets (Farinas et al. 1996). NT-3 also supports the survival of primary sensory neurons that mediate slowly adapting mechanoreception. NT-3 null mice show a gradual loss of afferents and their corresponding end organs, the Merkel cells, shortly after birth. By 2 weeks of age, both are largely absent in the NT-3 null animals (Airaksinen et al. 1996; Fundin et al. 1997).

Some sensory neuron subpopulations switch their neurotrophin dependence during development (Farinas et al. 1998; White et al. 1996). Mechanoreceptive neurons termed D-hair receptors rely on NT-3 for survival during prenatal and early postnatal development but in mature animals, NT-4/5 is required to support their survival (Stucky et al. 2002). In fact, many DRG neurons (about 60 %) and virtually all sympathetic neurons rely on NT-3 before target innervation occurs (Ernfors et al. 1994b; Kuruvilla et al. 2004; Lefcort et al. 1996); the subsequent switch in neurotrophin dependence occurs once target is contacted, and this helps ensure that subsequent neuronal survival remains dependent on the target.

2.2 Neurotrophins and Central Neuron Survival

In contrast to their dramatic effects on the developmental neuronal survival of peripheral neurons, neurotrophins have a modest effect on the developmental survival of neurons in the central nervous system [reviewed in Huang and Reichardt (2001) and Rauskolb et al. (2010)]. This is somewhat surprising given that neurotrophins can support the survival of several types of primary CNS neurons in vitro and since successful target innervation and synaptic contact are key elements required for central neuron survival. With the exception of NGF, each of the neurotrophins promotes survival of purified motor neurons in vitro (Sendtner et al. 1996). Despite this, the vast majority of motor neurons are spared in mice lacking any single one of these factors (BDNF, NT-3, or NT-4/5) in vivo (Conover et al. 1995; Ernfors et al. 1994a, b; Farinas et al. 1994; Jones et al. 1994) and only slightly affected (20 % deficit in facial and spinal motor neurons) in triple null mice lacking BDNF, NT-3, and NT-4/5 (Agerman et al. 2000). Interestingly, TrkA and TrkC null mice do not show significant reduction in motor neurons, yet mice lacking TrkB show a dramatic decline in motor neurons in the facial nucleus and lumbar spinal cord. Nonetheless, mice lacking both BDNF and NT-4/5 do not show a corresponding deficit (Conover et al. 1995); the mechanisms that account for the specific TrkB-dependent motor neuron loss remain unresolved.

Other CNS neurons that are responsive to neurotrophins in vitro include basal forebrain and striatal cholinergic neurons. Although differentiation of these neurons is altered in NGF null mice (Smeyne et al. 1994), their perinatal survival is not affected in TrkA or NGF knockout animals. Postnatal atrophy of NGF-dependent populations of cholinergic forebrain neurons has been observed in adult NGF mutant heterozygotes indicating that these neurons appear to retain dependence on this neurotrophin (Chen et al. 1997). Cerebellar granule cells, mesencephalic dopaminergic neurons, and retinal ganglion cells are BDNF and NT-4/5–responsive neurons, and a modest increase in postnatal apoptosis of hippocampal and cerebellar granule cells is observed in TrkB and TrkB/TrkC mutants (Alcantara et al. 1997; Minichiello and Klein 1996). However, the effects on these cells are slight when compared to the dramatic losses observed in the peripheral nervous system of these same animals.

Because BDNF is the most abundant neurotrophin found in the brain, it is a strong candidate as a critical survival and growth factor for CNS neurons. The role of BDNF in the brain cannot be explored with conventional *bdnf* null mutant mice since this strain dies before the postnatal increase in BDNF expression occurs (Castren et al. 1992; Maisonpierre et al. 1990a; Zafra et al. 1992). Several studies have reported the effects of Cre-mediated excision of floxed *bdnf* alleles. The α-calcium/calmodulin-dependent protein kinase II (CamKII) promoter drives Cre expression in post-mitotic neurons in the forebrain (Chan et al. 2006a, 2008; Monteggia et al. 2007; Rios et al. 2001), and the depletion of BDNF in the hippocampus within CamKII-Cre animals causes a deficit in granule neuron differentiation in adult animals (Chan et al. 2008). BDNF depletion in the forebrain was achieved earlier in development by using the Emx1 promoter to drive Cre

expression in neuronal progenitors (at around E10.5). The postnatal striatum was reduced in these animals, and medium spiny neurons (MSNs) displayed abnormally small cell somas, thin dendrites, and few dendritic spines (Baquet et al. 2004). Significant striatal neuron losses were not detected at P180 but by 1 year of age, striatal neuron number had decreased by about 35 % in these mice (Baquet et al. 2004). Defects in these mice were not limited to the striatum; cortical thinning was observed in the visual cortex and was attributable, at least in part, to neuronal shrinkage and dendritic retraction (Gorski et al. 2003). By expressing Cre recombinase from the Tau locus, Rauskolb and colleagues (2010) were recently able to excise BDNF alleles from almost all differentiated neurons in the brain and spinal cord (Rauskolb et al. 2010). This study confirmed that BDNF is not a significant survival factor for most CNS neurons and, consistent with earlier studies, showed that BDNF is required for the postnatal growth of the striatal neurons; single-cell analyses revealed a marked decrease in dendritic complexity and spine density in these Tau-Cre:BDNF mice.

Studies analyzing conditional deletion of TrkB within the CNS complement those targeting BDNF. When CaMKII-Cre was used to drive TrkB deletion, pyramidal neurons within cortical layers II/III and V showed reduced dendritic arborization and layer thinning at early postnatal stages (Xu et al. 2000). At later developmental stages, loss of TrkB also results in progressive elimination of neurons in the somatosensory and visual cortices (Xu et al. 2000). Thus, the BDNF-TrkB axis functions to support striatal and cortical neuron size and dendrite structure rather than the initial development of these features. Thus, the BDNF-TrkB axis plays a role in stabilizing the "survival of circuitry" during activity-dependent reorganization of cortical connectivity (Gorski et al. 2003).

2.3 BDNF May Act as a Survival Factor After Injury

In contrast to its limited role in normal CNS development, neuronal survival after CNS axotomy does appear to require an intact TrkB-BDNF signaling axis. Early evidence for this emerged from studies showing that provision of exogenous BDNF reduced death of cortico-spinal neurons after axotomy (Giehl et al. 1998; Giehl and Tetzlaff 1996). TrkB seems to be capable of mediating an endogenous survival response in these circumstances as post-axotomy survival of hippocampal and facial motor neurons is sharply reduced in animals lacking TrkB receptors (Alcantara et al. 1997). Interestingly, survival of newly born neurons produced in the dentate gyrus following traumatic brain injury was sharply reduced in mice lacking BDNF expression in the hippocampus (Balthasar et al. 2004; Gao and Chen 2009). Thus, endogenous BDNF may have an important role maintaining neuronal survival after injury to the central nervous system.

2.4 Trk Signaling Promotes Survival

During development, survival of peripheral neurons requires neurotrophin signaling via Trk receptors. By binding neurotrophin, the receptor kinase domains are brought into proximity to facilitate trans-phosphorylation of residues within the kinase domain and elsewhere in the receptor's intracellular domain. Although most of the details of Trk survival signaling have emerged from studies in mammalian systems, Trks are ancient receptors that existed at the time of the protostome/deuterostome split, and its signaling mechanisms have likely been employed for several hundred million years (Wilson 2009).

Upon ligand binding, the initial activating event in Trk receptors is the phosphorylation of tyrosines Y670, Y674, Y675 (numbering scheme based on human TrkA) which are present within the activation loop of the tyrosine kinase domain. Structures of the kinase domains of the Trk receptor family have not been determined directly but in similar receptor tyrosine kinases (RTKs), the kinase is normally maintained in a catalytically repressed state through interactions of the activation loop with the membrane proximal domain. This "closed" confirmation blocks access of ATP and substrate residues; activation-loop phosphorylation relieves these inhibitory interactions and thus activates kinase activity (Hubbard and Miller 2007).

In addition to the three activation loop tyrosines, seven additional tyrosine residues are evolutionarily conserved among the Trks. Of these, Y490 and Y785 have been well characterized as adaptor protein docking sites that play crucial roles in Trk signaling. Phosphorylation of Y490 creates a binding site that can be occupied by several different cytosolic proteins. The first of these identified was Shc, an adaptor protein containing a central phosphotyrosine interaction domain (PID) and a C-terminal SH2 domain. The interaction of the PID domain with Y490 in TrkA results in Shc phosphorylation which in turn allows recruitment of the Grb2 adaptor protein and SOS, the RAS exchange factor. Once recruited to this complex, SOS-induced RAS activation elevates c-Raf and Erk activity and induces phosphatidylinositol-3-kinase (PI3K) activation.

A key feature that distinguishes Trk signaling from that of most other RTKs is sustained activation of the MEK/Erk pathway. This is accomplished through a secondary cascade initiated by the interaction of a Trk adaptor protein termed FRS2 which, like Shc, binds to Y490 (Meakin et al. 1999). Binding and phosphorylation of FRS2 allows Grb2 and the Crk adaptor protein to join the FRS2 complex and thereby engage C3G, an exchange factor that induces activation of Rap1, a Ras-related small GTPase. This in turn activates the b-Raf kinase which drives sustained Erk activity. Interestingly, NGF-induced Rap1 activation is reliant on PI3K activation and on TrkA internalization whereas Ras activation requires neither of these events (York et al. 2000). A number of additional adaptor proteins, including ARMS, PDZ-GEF1, RGS12, GIPC, and FRS3 (Dixon et al. 2006; Hisata et al. 2007; Varsano et al. 2006; Willard et al. 2007) have been shown to associate with the internalized Trk receptor signaling complex and facilitate sustained Erk activation. Although details of the developmental, cellular, and neurotrophin

specificity of each of these remain uncertain, the fact that the Trk signaling system invokes several complementary and overlapping systems to mediate prolonged Mek/Erk signaling emphasizes the crucial importance of this signaling cascade in neurotrophin function.

Activation of PI3K plays a central role regulating neurotrophin-dependent cell survival, and this pathway also relies on adaptors that bind to Y490; Ras directly binds to PI3K, and activation of this signaling pathway relies primarily on Ras activation. However, in some cases, PI3K activation can also occur in a Ras-independent manner through a pathway that involves the GAB1 adaptor protein. In this cascade, the PI3K regulatory subunit has been reported to directly bind TrkA and acts as an adaptor that brings GAB1 to the receptor complex, leading to its phosphorylation and enhancing its ability to act as a scaffold for downstream signaling cascades (Korhonen et al. 1999; Onishi-Haraikawa et al. 2001). Generation of phosphatidylinositides by PI3K leads to the activation of the protein kinase Akt, a central regulator of cell survival in neurons. Akt mediates its pro-survival effects by phosphorylating and inhibiting the action of targets such as BAD, a pro-apoptotic Bcl-2 family member (Datta et al. 1997), and FKHRL1, a forkhead transcription factor that drives expression of pro-apoptotic genes (Brunet et al. 1999).

Y785 on TrkA functions as a docking site that mediates binding and phosphorylation of phospholipase C-g1 (PLCg1); once activated, PLCg1 hydrolyzes phosphatidyl inositol to generate IP3 and DAG which in turn induces release of Ca^{2+} from internal stores and activates several forms of PKC. Activation of the PLCg1 cascade activates several pathways important for neurotrophin function [reviewed in Skaper (2008)] but does not appear to be required for neurotrophin-dependent survival or death signaling.

2.5 Retrograde Survival Signaling

Neurons that rely on target-derived neurotrophic support have the unique challenge of responding to survival factors that are produced at distances far from the cell body and nucleus. The retrograde transport of neurotrophin-Trk complexes play crucial roles in mediating neurotrophin-dependent survival responses (Ginty and Segal 2002). Activated Trk receptors that are complexed with ligand can be internalized via clathrin- or pincher-mediated endocytosis (Bhattacharyya et al. 2002; Grimes et al. 1996, 1997; Hendry et al. 1974; Howe et al. 2001); bead-bound NGF that activates cell surface Trk but cannot be internalized does not support retrograde signaling (Riccio et al. 1997), and dominant negative forms of dynamin that block internalization of NGF from distal axons reduce survival signaling in cell bodies (Watson et al. 2001). Thus, endocytosis of the NGF–TrkA complex is required for appropriate survival responses.

The intracellular vesicle that contains the activated ligand–receptor complex and is retrogradely transported to the cell body is termed the signaling endosome. This vesicle functions as an active signaling platform that contains TrkA and is bedecked

with activated components of the PI3K, MAPK, and PLCg pathways (Grimes et al. 1997; Howe et al. 2001; Yano et al. 2001).

As activated Trk receptors move from cell surface to initial endosome and then approach neuronal cell bodies, the signaling events generated change substantially. For example, PI3K activation is induced at the cell surface where it plays an important role initiating formation of the signaling endosome. In contrast, limited Mek/Erk signaling is initiated by cell surface Trk receptors and robust activation of this pathway requires receptor endocytosis and formation of signaling endosome (Grimes et al. 1997; Howe et al. 2001; Yano et al. 2001).

After internalization, the properties of the signaling endosome change during its retrograde journey. The Segal group has shown that Erk1/2 are preferentially activated in neuronal processes whereas Erk5, a related family member, is preferentially activated as the neurotrophin signal approaches the cell soma. This switch from Erk1/2 to Erk5 plays a crucial role in retrograde survival signaling since Erk5 activity is required for phosphorylation of CREB and MEF2, transcription factors that mediate production of anti-apoptotic gene products (Pazyra-Murphy et al. 2009; Watson et al. 2001).

Distinct neurotrophins differ in their ability to induce formation of signaling endosomes, and this has important consequences for development of the peripheral nervous system. Ginty and colleagues have shown that NGF and NT-3 both bind and activate TrkA present on sympathetic neurons, but only NGF drives TrkA into endosomes to mediate long-range survival effects (Kuruvilla et al. 2004). This difference emerges because the NGF–TrkA complex is capable of inducing a Rac1-cofilin signaling module that results in actin depolymerization that is essential for initiation of NGF/TrkA endosome trafficking. The NT-3–TrkA complex is incapable of mediating this effect, possibly because NT-3–TrkA complexes disassemble within the acidic environment of early endosomes (Harrington et al. 2011). Since NT-3 is produced at high levels in vasculature and NGF is only produced in target tissues and in vivo, local NT-3 allows TrkA-dependent neurite growth to occur on blood vessels during development whereas only NGF, produced by the ultimate target of the innervating neurons, is capable of eliciting long-range survival signals (Kuruvilla et al. 2004); this system uses local NT-3-TrkA effects to get sympathetic axons to their destination and then uses long-range NGF–TrkA signaling to support neuronal survival.

3 Promotion of Survival by the P75NTR

3.1 Genetic Evidence

When the p75exonIII−/− mouse was generated in 1992 (Lee et al. 1992), one of the most striking phenotypes identified was a defect in sensory innervation, most notably in developing paws (Lee et al. 1992), together with a striking decrease in the volume of dorsal root ganglia (DRG). Subsequent analyses using unbiased stereological counting methods revealed that the p75exonIII−/− animal showed

massive decrease in the numbers of DRG neurons surviving to P7, with 75 % fewer neurons in DRGs from the cervico-thoracic region and a 50 % fewer in lumbar DRGs (Murray et al. 1999).

DRG neurons are a heterogeneous population, and a number of studies have addressed whether p75NTR deletion selectively affects specific DRG subpopulations. Bergmann et al. (1997) reported that the increased neuronal loss observed in the p75NTR null occurred in DRG neurons of all sizes (Bergmann et al. 1997), and similar results were reported when neurons were classified as A- or B-cells by ultrastructural and cytochemical criteria (Gjerstad et al. 2002; Jiang et al. 2004). Other studies have shown that nociceptive neurons expressing CGRP and Substance P, nociceptive expressing isolectin IB4, and non-nociceptive RT97-positive neurons all show equivalent losses in the p75NTR null (Jiang et al. 2004; Vaegter et al. 2011). Furthermore, TrkA-, TrkB-, and TrkC-positive neurons are all lost to the same extent (40–60 %) in p75NTR null mice (Vaegter et al. 2011). Interestingly, genetic deletion of sortilin, a putative p75NTR co-receptor, had no effect on DRG survival but compound p75NTR:sortilin nulls had more severe DRG neuronal loss than that observed with p75NTR deletion alone.

In vitro data show that p75NTR enhances NGF-induced survival of primary trigeminal, DRG, and sympathetic neurons (Barrett and Bartlett 1994; Davies et al. 1993; Lee et al. 1994), contributes to Schwann cell survival (Gentry et al. 2000; von Schack et al. 2001), and normal myelination (Chan et al. 2006b; Cosgaya et al. 2002) and that it plays a role in neurogenesis in the adult rat subventricular zone (Young et al. 2007). However, the mechanisms that allow p75NTR to support survival and differentiation remain unknown, and many basic and fundamental questions remain unresolved. For example, we do not know when these defects appear during development or whether these phenotypes reflect a cell autonomous or a non-cell autonomous effect of p75NTR.

3.2 Prosurvival Signaling Pathways Activated by p75NTR

A number of studies suggest that p75NTR can activate survival through activation and/or positive modulation of the NF-kB and PI3K pathways. The NF-kB transcriptional complex regulates the expression of a number of genes involved in cell survival and is important for sustaining the survival of mature neurons, oligodendrocytes, DRG sensory neurons, and PC12 cells [reviewed in O'Neill and Kaltschmidt (1997)]. Since p75NTR is the founding member of the TNF receptor (TNFR) superfamily and many of the TNFR family members are potent NF-kB activators, p75NTR has been examined for its ability to activate NF-kB signaling. Activation of NF-kB by p75NTR was initially reported in primary cultures of Schwann cells, where NGF binding to p75NTR increased NF-kB DNA binding activity and p65 nuclear translocation (Carter et al. 1996). Subsequent studies have shown p75NTR-dependent NF-kB activation in oligodendrocytes, RN22 Schwannoma cells, sensory neurons, and PC12 cells (Bhakar et al. 1999; Gentry et al. 2000; Hamanoue et al. 1999; Hughes et al. 2001; Ladiwala et al. 1998).

Typically, p75NTR-dependent NF-kB activation is modest but is sharply enhanced in cells exposed to a variety of stressful stimuli (Carter et al. 1996; Bhakar et al. 1999; Hughes et al. 2001). In fibroblasts, p75NTR does not directly activate NF-kB, but instead indirectly enhances TNF-mediated NF-kB activation (Bhakar et al. 1999).

In the canonical NF-kB signaling system, the inhibitory IkB subunit binds and sequesters NF-kB dimers in the cytosol. Activation of the IkB kinases (IKK1 and IKK2) results in phosphorylation, ubiquitination, and proteosomal degradation of IkB which releases NF-kB dimers and allows them to translocate to the nucleus where they bind promoter elements to induce gene activation. In Schwann cells, neurotrophin treatment has been reported to induce NF-kB activation through a cascade involving the TRAF6 and RIP2 adaptor proteins. In this cascade, RIP2 and TRAF6 bind directly to the p75NTR intracellular domain (p75NTR-ICD) in a ligand-dependent manner, and this in turn leads to enhanced NF-kB activity that blocks Schwann cells apoptosis (Khursigara et al. 1999, 2001; Yeiser et al. 2004). A different scheme has been reported for PC12 cells, where the interleukin-1 receptor associated kinase (IRAK) is recruited to the p75NTR receptor and forms a complex that also contains the atypical protein kinase C interacting protein and TRAF6. Kinase activity of IRAK induced by NGF was found to be required for NF-kB activation, recruitment of p62 to p75NTR, and cell survival (Mamidipudi et al. 2002, 2004).

The PI3K/Akt pathway plays a major role in neuronal survival [reviewed in Brunet et al. (2001)], and p75NTR activates this pathway (Massa et al. 2006; Roux et al. 2001). An early study showed that moderate overexpression of p75NTR or a myristoylated form of the p75NTR-ICD results in enhanced Akt phosphorylation which is ligand-independent, blocked by inhibitors of PI3K, and associated with increased tyrosine phosphorylation of the p85 regulatory subunit of PI3K and the Shc adaptor protein (Roux et al. 2001). Another study reported that NGF modestly induces Akt phosphorylation in PC12nnr5 cells that express p75NTR but lack Trk receptor expression (Bui et al. 2002). An interesting series of non-peptidic NGF mimetics have been reported to activate Akt and NF-kB signaling in primary hippocampal neurons. Interestingly, these compounds activate these survival pathways in wild-type mice but not in neurons derived from p75NTR null mice, indicating that they achieve their pro-survival effect by functioning as p75NTR agonists. It is noteworthy in this regard that the phosphatase and tensin homolog (PTEN), a dual specific phosphatase that negatively regulates Akt activity by reducing PIP3 levels, undergoes NGF- and p75NTR-dependent phosphorylation on serine 380 in hippocampal neurons exposed to NGF (Arevalo and Rodriguez-Tebar 2006). Multiple targets of Akt could mediate p75NTR-dependent survival but since NGF-induced NF-kB activation is inhibited by the PI3K inhibitor LY294002 in PC12 cells (Bui et al. 2002), one likely possibility is that Akt induces phosphorylation of IkB kinase 1 (IKK1) to facilitate NF-kB induction (Kane et al. 1999; Ozes et al. 1999; Romashkova and Makarov 1999).

An important body of evidence indicates that p75NTR can promote survival through potentiation of Trk signaling. Primary sensory and sympathetic neurons

that lack p75NTR have reduced survival responses when maintained in low doses of neurotrophin (Barrett and Bartlett 1994; Davies et al. 1993; Lee et al. 1994). In cell lines and primary neurons, p75NTR clearly potentiates Trk signaling responses (Barker and Shooter 1994; Bibel et al. 1999; Ceni et al. 2010; Hantzopoulos et al. 1994; Brann et al. 2002; Bhakar et al. 2003; Twiss et al. 1998; Verdi et al. 1994; Yan et al. 1991). The ability of p75NTR to confer enhanced responses to low neurotrophin concentrations is an important property for neurons that compete for the low quantities of neurotrophins present in target tissues [reviewed in Barde (1989)]. The precise molecular mechanisms that allow p75NTR to enhance Trk responsiveness to neurotrophin and signaling remain uncertain, but two main hypotheses have been put forward: p75NTR may enhance Trk activation and/or p75NTR may activate signaling events that converge and/or synergize with Trk-dependent pathways.

p75NTR and Trk receptors interact independently with the neurotrophins with similar Kd, about 10^{-9} M (Kaplan et al. 1991; Klein et al. 1991; Lee et al. 1994; Rodriguez-Tebar et al. 1990, 1992; Squinto et al. 1991) yet high-affinity NGF binding sites (Kd $\sim 10^{-11}$ M) are present on PC12 cells and sensory neurons (Green and Greene 1986; Greene and Tischler 1976; Rodriguez-Tebar et al. 1990, 1992; Sutter et al. 1979). Some studies performed in the early 1990s indicated that co-expression of p75NTR with TrkA receptors in transformed cells produced high-affinity NGF binding sites (Hempstead et al. 1991; Rodriguez-Tebar et al. 1992) but others found no evidence for an effect of p75NTR on NGF binding to TrkA (Bothwell 1995; Jing et al. 1992; Klein et al. 1991; Wehrman et al. 2007). In an important study from the Hempstead group, it was found that TrkA expressed in the absence of p75NTR shows very slow association and dissociation kinetics. However, when the two receptors are co-expressed, the rate at which NGF can associate with TrkA increases by about 25-fold and this change in Trk association rate results in the generation of high-affinity binding sites (Mahadeo et al. 1994). Interestingly, neurotrophin binding to p75NTR does not seem to be required for creation of high affinity binding sites: high-affinity NGF binding sites can be generated when Trk is co-expressed with a p75NTR mutant deficient in neurotrophin binding but not with p75NTR constructs with disrupted transmembrane or ICD domains (Esposito et al. 2001).

Although NGF binding to p75NTR may be dispensable for the creation of high affinity sites, it is required for efficient NGF-induced TrkA activation (Barker and Shooter 1994; Clary and Reichardt 1994; Lachance et al. 1997; Ryden et al. 1997; Verdi et al. 1994). TrkA activation is increased by wild-type p75NTR, but not by a mutant form of p75NTR deficient in neurotrophin binding and not by a mutant form of NGF that cannot bind p75NTR (Hantzopoulos et al. 1994; Ryden et al. 1997), indicating that for this effect to be manifest, NGF must bind directly to p75NTR. Thus, while it seems certain that generation of kinetically distinguishable high-affinity sites is important for the enhanced activation of TrkA that is observed in the presence of p75NTR, it appears unlikely that the profound effects of p75NTR on Trk activity can be explained through this mechanism alone.

These observations suggest that NGF binding to p75NTR is necessary to facilitate TrkA activation in response to low levels of NGF. However, a recent crystallographic study argues against the existence of a ternary complex (where NGF can bind both p75NTR and TrkA at the same time) or even of a stable complex between p75NTR and Trk receptors (Wehrman et al. 2007). An alternative model is that p75NTR may act as a co-receptor that concentrates NGF locally or presents it to TrkA in a favorable binding conformation (Barker 2007; Barker and Shooter 1994). In this ligand-passing model, p75NTR functions to lower the energy barrier required for NGF binding to TrkA and thereby increases the NGF association rate. A prediction of this model is that intermediate affinity sites would be nonexistent or very transient and thus impossible to resolve kinetically using standard binding protocols.

A growing body of evidence indicates that p75NTR can potentiate Trk-induced Akt signaling, perhaps by acting on downstream signaling pathways. In PC12 cells, a p75NTR function blocking antibody significantly reduces NGF-induced Akt phosphorylation (Bui et al. 2002). In a PC12 subline that is deficient in p75NTR expression, termed PC84, NGF induces differentiation but does not induce Akt phosphorylation (Ito et al. 2003). Furthermore, acute siRNA-mediated knockdown of the p75NTR reduces NGF-induced or BDNF-induced Akt phosphorylation in PC12 cells and cerebellar granule neurons, respectively (Ceni et al. 2010). Given that p75NTR can induce Akt phosphorylation in the absence of Trk activation (Arevalo and Rodriguez-Tebar 2006; Bui et al. 2002; Massa et al. 2006; Roux et al. 2001) and that p75NTR rescues neuroblastoma cells from apoptosis via the PI3K pathway (Lachyankar et al. 2003), it seems likely that a p75NTR-derived pathway collaborates with Trk signaling to facilitate optimal Akt activation.

We have recently shown that Akt activation by p75NTR requires processing of the receptor and release of the p75NTR-ICD into the cytoplasm. p75NTR cleavage and generation of the p75NTR-ICD is induced through a Trk-dependent pathway involving activation of MEK and induction of ADAM17, a cell surface transmembrane metalloprotease (Ceni et al. 2010). This indicates that neurotrophin-dependent Trk activation propels a feed forward mechanism to generate the p75NTR-ICD and thus enhance Akt phosphorylation. Interestingly, p75NTR has also been shown to alter Shc phosphorylation. Antisense oligonucleotides targeting p75NTR were shown to decrease NGF-induced Shc phosphorylation (Epa et al. 2004), and p75NTR overexpression enhances Shc phosphorylation (Roux et al. 2001). Precisely how the p75NTR-ICD may alter Akt activation or Shc phosphorylation remains uncertain, but it is noteworthy that the ICD has been shown to reduce cytosolic protein tyrosine phosphatase activity (Roux et al. 2001) and that activity of the PTEN phosphatase can be reduced by p75NTR (Arevalo and Rodriguez-Tebar 2006). Therefore, it is possible that p75NTR enhances Akt activity by reducing activity of phosphatases that include PTEN.

A p75NTR-related protein called Neurotrophin Receptor Homologue 2 (NRH2) is co-expressed with p75NTR in many tissues (Murray et al. 2004), and recent studies indicate that NRH2 can collaborate with TrkA to create high-affinity NGF

binding sites and potentiate Trk signaling, at least in overexpression paradigms (Wong et al. 2008). NRH2 was recently shown to bind sortilin (Kim and Hempstead 2009), and it therefore seems likely that p75NTR and NRH2 may fulfill similar functions. Mice null for NRH2 have not yet been reported, but it will be very interesting to test whether NRH2 and p75NTR exhibit overlapping and/or compensatory activities.

4 Promotion of Cell Death by the p75NTR

As discussed previously, neurotrophins were first identified as promoters of neuronal survival, particularly in the PNS, and for decades researchers focused largely on the pro-survival effects of neurotrophins and their receptors on various neuronal populations. However, in the early 1990s, several studies showed that neurotrophins could also induce apoptosis, acting through the p75NTR, in several cell populations.

4.1 p75NTR and Cell Death: Evidence from Genetic Data

p75NTR is widely expressed in the peripheral and central nervous systems during development, and it can contribute to the elimination of neurons that are unable to obtain sufficient neurotrophic support [reviewed in Kaplan and Miller (2000)]. For instance, although p75NTR has a survival-promoting role for the DRG neurons, it was shown to promote death of other peripheral neurons during development. In the superior cervical ganglia (SCG), NGF mediates the survival of sympathetic neurons by activating TrkA. However, p75NTR induces apoptosis of these neurons in response to BDNF in vitro (Kenchappa et al. 2006). Consistent with this, p75NTR KO mice exhibit increased sympathetic neuron survival and delayed developmental apoptosis compared to wild-type mice (Bamji et al. 1998; Majdan et al. 2001). Eliminating p75NTR or NT-4/5 in mice leads to a marked attenuation of developmental apoptosis of trigeminal ganglion neurons, indicating that NT-4/5 can induce the death of these neurons through the p75NTR (Agerman et al. 2000).

The p75NTR has also been shown to mediate cell death in the CNS, both during development and after injury. NGF, BDNF, NT-3, and NT-4/5 can induce cell death of neurons maintained in vitro and this appears to be due to p75NTR, since this effect is lost in neurons derived from p75NTR null mice (Friedman 2000; Troy et al. 2002). p75NTR has been shown to facilitate seizure-induced death of hippocampal and entorhinal neurons in vivo, and seizure-induced death is reduced in p75NTR−/− mice (Roux et al. 1999; Troy et al. 2002).

p75NTR is highly expressed in the developing mouse retina at E15.5 and in the chick dorsal retina at E4, ages that correspond to the period of active developmental apoptosis in these species (Frade and Barde 1999; Frade et al. 1996). NGF binding to p75NTR induces apoptosis of retinal ganglion cells (RGC) and treatment of chick RGCs with NGF blocking antibodies significantly decreases cell death (Frade

et al. 1996). In addition, p75NTR and NGF null mice have less RGC death than wild-type mice (Frade et al. 1996), suggesting that NGF induces cell death via p75NTR during early retinal development. p75NTR has also been implicated in light-induced photoreceptor death in adult rodents in vivo (Harada et al. 2000). p75NTR may also play a role in the death of basal forebrain (BF) cholinergic neurons during development. p75NTR−/− mice show an increase in the number and the size of cholinergic neurons as well as in the cholinergic innervation and activity of the hippocampus (Van der Zee et al. 1996; Yeo et al. 1997).

p75NTR does not invariably induce cell death in the neurons that express it. p75NTR is highly expressed in spinal cord motoneurons between E13 and postnatal day 1, and inhibition of p75NTR with function blocking antibodies prevents NGF-induced death of motoneurons in culture (Sedel et al. 1999). Despite this, developmental loss of motoneurons proceeds normally in p75NTR null mice (Bertrand et al. 2008). Ferri et al. (1998) showed that p75NTR may contribute to post-axotomy motoneuron loss, but these early studies were performed by comparing p75NTR nulls with wild-type animals derived from different strain backgrounds (Ferri et al. 1998). When Gschwendtner et al. (2003) examined cell death in motoneurons after facial nerve transection, they found that injury-induced motoneuron death in the two distinct p75NTR null lines examined was identical to wild-type animals (Gschwendtner et al. 2003).

p75NTR also promotes cell death in oligodendrocytes, both in vitro (Casaccia-Bonnefil et al. 1996) and in vivo, after spinal cord injury (Beattie et al. 2002; Yune et al. 2007), and death of Schwann cells after sciatic nerve transfection is reduced in p75NTR null mice (Syroid et al. 2000). The ability of p75NTR to induce or facilitate survival of Schwann cells may depend on the expression of receptor-interacting serine/threonine-protein kinase 2 (RIP2), a p75NTR adaptor protein (Khursigara et al. 2001). Therefore, both in vivo and in vitro data brought evidence supporting the notion that p75NTR can induce death of neural cells during development and after injury.

4.2 p75NTR and Cell Death: Signaling

p75NTR belongs to the TNF receptor superfamily, a group of proteins characterized by 1–4 tandem arrays of a characteristic extracellular cysteine-rich motif (Grivennikov et al. 2006). p75NTR was the first member of this family identified but others, such as TNFR1, TNFR2, Fas, and CD40, were discovered soon after. In mammals, over 25 TNFR proteins have been identified, and TNFRs are also prevalent in nonmammalian vertebrates. In contrast, there are no TNFRs expressed in C. elegans and only one, termed Wengen, is expressed in Drosophila.

Several members of the TNFR superfamily, including p75NTR, function as death receptors that induce apoptosis in response to ligand binding. In TNFR1, Fas, and DR5, intracellular structures termed death domains play a crucial role in this process. Death domains consist of ~80 amino acids in a tight array of 6 alpha helices which, in response to ligand binding to the receptor, aggregate and produce

binding surfaces for downstream interactors that mediate the apoptotic response [reviewed in Park et al. (2007)]. This death-inducing signaling complex (DISC) contains adaptors such as FADD and TRADD that link the receptors to caspase 8, an initiator caspase. Oligomerization of caspase 8 within the DISC results in its activation and auto-cleavage (Wang et al. 2010). Activated caspase 8 can directly induce activation of downstream executioner caspases but also activates the mitochondrial apoptotic cascade, through cleavage and activation of BID, a BH3-domain-only protein [reviewed in Kantari and Walczak (2011)].

p75NTR contains a death domain but in contrast to the pro-apoptotic TNFR superfamily members discussed above, it does not form a DISC and does not activate caspase 8. Instead, p75NTR-dependent apoptosis seems to occur mainly through a pathway that involves activation of a Jun kinase cascade and activation of the BH3-domain-only family members, Bid and Bad. This leads to activation of Bax, permeabilization of the outer mitochondrial membrane, efflux of cytochrome C, and activation of initiator caspase 9 and executioner caspases 3, 6, and 7 (Bhakar et al. 2003; Friedman 2000; Salehi et al. 2002; Troy et al. 2002).

Substantial effort has gone into determining the events and players that are required for p75NTR activation and induction of p75NTR-dependent apoptosis. Since p75NTR has no intrinsic enzyme activity and must rely on adaptor proteins to transduce its signals, several studies have attempted to identify adaptor proteins that mediate the receptor's effect. Neurotrophin receptor interacting factor (NRIF) (Casademunt et al. 1999; Linggi et al. 2005) is a DNA binding protein that becomes ubiquitinated and then transported to the nucleus upon p75NTR activation (Kenchappa et al. 2006). The p75NTR-associated cell death executor (NADE) has been reported to bind to the p75NTR death domain and induce caspase activation and cell death within primary cortical neurons and in transfected non-neuronal cells (Mukai et al. 2000; Park et al. 2000). NRAGE interacts with the juxtamembrane domain of p75NTR and induces apoptosis by activating JNK and caspase 3 (Salehi et al. 2000, 2002). Genetic loss of function data indicates that NRIF and NRAGE play important roles regulating p75NTR-dependent death in vivo (Salehi et al. 2000, 2002), but the role of NADE in mediating p75NTR-dependent death in vivo remains unexplored.

p75NTR undergoes a cleavage process, termed regulated intramembrane proteolysis (RIP), that first releases the receptor's intracellular domain from its transmembrane tether and then processes the carboxy fragment to generate a soluble form of the p75NTR-ICD. Interestingly, cleavage of p75NTR and generation of the p75NTR-ICD have been shown to play a key role in apoptotic events. Early studies showed that transgenic mice expressing p75NTR -ICD showed increased neuronal apoptosis, both peripherally and centrally, and more recent work has shown that the p75NTR-ICD induces activation of JNK (Kenchappa et al. 2010). Interestingly, blockade of p75NTR cleavage prevents NRIF nuclear translocation and inhibits apoptosis (Kenchappa et al. 2006).

4.3 p75NTR and Cell Death: Role of pro-NGF

Neurotrophin precursors, the pro-neurotrophins, bind p75NTR and induce apoptosis downstream of this receptor [reviewed in Hempstead (2006)]. The effect of proneurotrophins on p75NTR requires the participation of a co-receptor termed sortilin. This Type I transmembrane protein contains a Vps10p domain and specifically recognizes the pro-domain of proneurotrophins. Sortilin is highly expressed in the vertebrate CNS and when co-expressed with p75NTR, forms a high-affinity co-receptor complex that mediates proneurotrophin-induced apoptosis in primary SCG neurons, cerebellar granule neurons, and basal forebrain cholinergic neurons [reviewed in Nykjaer and Willnow (2012)].

Proneurotrophin-induced death has been observed after injury in several settings. ProNGF levels rise after spinal cord injury and contribute to the elimination of injured oligodendrocytes through activation of caspase 3 (Beattie et al. 2002) and Schwann cell death that occurs after facial nerve axotomy similarly relies on the association of p75NTR, sortilin, and proNGF (Provenzano et al. 2008). Apoptosis of corticospinal neurons after internal capsule lesion is blocked by neutralizing antibodies to p75NTR (Giehl et al. 2001) and reduced in sortilin knockout mice (Jansen et al. 2007), indicating that both receptors may be involved in this form of lesion-induced death.

The proneurotrophin signaling pathways that activate cell death seem to overlap with those discussed above, with caspase activation being a prominent feature in proNGF-induced apoptosis. Interestingly, proneurotrophins may also act to suppress pro-survival signals as proNGF has been reported to activate the phosphatase PTEN which in turn acts to suppress the PI3K survival pathway in basal cholinergic neurons (Song et al. 2010; Volosin et al. 2006). However, the proximal signaling partners that allow the p75NTR-sortilin complex to transduce its signal remain unclear and a p75NTR death-inducing signaling complex, analogous to that described for TNFR1, has not yet emerged.

p75NTR has also been shown to contribute to neuronal cell degeneration in a non-cell-autonomous fashion. A recent study has shown that proNGF promotes the death of adult retinal ganglion cells via p75NTR signaling from Müller glia (Lebrun-Julien et al. 2009), a specialized type of glia present in the vertebrate eye and the only cell type in the retina that expresses p75NTR (Hu et al. 1998). ProNGF induced robust expression of tumor necrosis factor alpha (TNFα) in Müller cells which was required for proNGF-induced death of retinal neurons. Moreover, retinas from mice lacking p75NTR or sortilin were resistant to the effects of proNGF on TNFα expression and cell death. Similar observations were also made in retinal degeneration as a consequence of glaucoma (Bai et al. 2010). These results provided an explanation for the apparent lack of neuroprotective effects of NGF in retinal injury, despite expression of prosurvival TrkA receptors in retinal ganglion cells (Bai et al. 2010; Shi et al. 2007).

5 The Emergence of the Trk Receptors as Death Receptors

It is well established that the Trk family of RTK support survival of neurons during development yet paradoxically, TrkA and TrkC have more recently emerged as able to induce cell death of tumor cells [for review see Harel et al. (2010)] and developing neurons.

5.1 Trk Receptors Can Induce Death of Tumor Cells

The pro-death potential role of Trk receptors emerged from studies of neuroblastoma and medulloblastoma, pediatric tumors of neuronal origin. Neuroblastomas are solid tumors derived from the sympathoadrenal neural crest lineage whereas medulloblastomas are malignant CNS tumors, usually derived from the cerebellum. TrkA expression is a robust indicator of positive prognosis in neuroblastoma [reviewed in Brodeur et al. (2009)], and TrkC levels correlate with a positive prognosis in medulloblastoma [reviewed in Gulino et al. (2008)].

NGF treatment of medulloblastoma cells engineered to overexpress TrkA induces apoptotic cell death; this is blocked by the Trk kinase inhibitor K252a or by co-expression of a kinase-inactive form of TrkA, indicating a role for TrkA kinase activity pathway (Chou et al. 2000; Muragaki et al. 1997). Similarly, chronic overexpression of TrkA followed by exposure to NGF induces caspase 3-dependent apoptosis in two distinct neuroblastoma cell lines (Lavoie et al. 2005). Jung and Kim (2008) developed a Tet-On system to drive TrkA overexpression in neuroblastoma cells and found that, with TrkA expression, apoptosis occurred even in the absence of NGF but cell death increased further when NGF was present (Jung and Kim 2008).

The mechanism(s) that mediate TrkA-induced death remain unclear. In one study, TrkA-induced apoptosis of medulloblastoma cells was reported to require Ras and p53 activity and another has shown that TrkA-dependent death activates caspases via the mitochondrial apoptotic pathway and that Bcl-XL overexpression can block TrkA-induced death. Alternatively, experiments on TrkA-expressing glioblastoma and osteosarcoma cells have suggested that TrkA induces autophagy via a ERK- and JNK-dependent pathways (Hansen et al. 2007) or through a combination of apoptosis and autophagy (Dadakhujaev et al. 2009).

Recently, CCM2, the protein product of the cerebral cavernous malformation 2 gene, was identified as a novel TrkA interactor that functions as a key mediator of TrkA-induced cell death (Harel et al. 2009). Knockdown of CCM2 in TrkA-expressing medulloblastoma and neuroblastoma cells attenuated NGF-induced death, and neuroblastoma patients that co-expressed CCM2 and TrkA had improved outcomes over those that did not. This study rules out the possibility that p75NTR may play a role in TrkA-induced death and instead suggested that CCM2 acts as an adaptor that binds directly to TrkA and links it to as yet unknown downstream effectors.

The role of TrkC in tumor cell death has been less well studied, but TrkC expression in medulloblastomas has been correlated with a positive prognosis (Segal et al. 1994). NT-3-induced apoptosis of primary medulloblastoma cells is highly correlated with TrkC expression levels and in these cells, NT-3-induced death can be blocked by K252a, suggesting that RTK activity is required. However, there may be alternative means for TrkC to induce cell death since transfection of the receptor in HEK293 cells reportedly causes cell death which is independent of its endogenous tyrosine kinase activity (Tauszig-Delamasure et al. 2007).

5.2 Trk Receptors in the Death of Neurons

The Trk receptors play a crucial role in promoting neuronal survival in vivo, yet emerging data support the provocative concept that Trks may also promote neuronal cell death. An initial study on this topic showed that overexpression of TrkC in HEK293 cells induced apoptosis and found that this was rescued by exposure to exogenous NT-3 (Tauszig-Delamasure et al. 2007). Further support for this concept emerged from the work of Nikoletopoulou et al. (2010) who produced ES cells in which the locus was engineered to drive expression of cDNAs encoding TrkA, TrkB, or TrkC. When the ES cells were differentiated to glutamatergic neurons, overexpression of TrkA and TrkC was initiated and the cells quickly died (Nikoletopoulou et al. 2010). This cell death was not prevented by addition of K252A but was blocked by NGF (on TrkA-expressing cells) or NT-3 (on TrkC expressing cells). Further, the ES cell death was significantly reduced when p75NTR expression was suppressed by RNA interference.

The same study also examined the role of TrkA as a death receptor in primary sympathetic neurons. Interestingly, death induced by NGF withdrawal was reduced by about 50 % in sympathetic neurons derived from TrkA−/− embryos and by about 20 % in neurons derived from p75exonIII−/− embryos. One interpretation of this data is that NGF withdrawal and loss of ligand from TrkA activates a signaling pathway that converges on p75NTR and related receptors which act as the bona fide executioners in this pathway (Nikoletopoulou et al. 2010).

It is noteworthy that hippocampal neurons were also reported to undergo cell death when withdrawn from NGF (Matrone et al. 2009). Interestingly, in this study, TrkA and PLCg were phosphorylated 24 h after NGF withdrawal and pharmacologic inhibition of TrkA activity or partial silencing of TrkA or p75NTR receptors blocked neuronal apoptosis. In an interesting twist, antibodies against amyloid-beta were found to block NGF-withdrawal-induced TrkA phosphorylation, and authors proposed that NGF deprivation induces amyloid-beta production, which in turn activates TrkA and induces cell death in a p75NTR-dependent manner.

Although many details remain to be determined, these data indicate that TrkA and TrkC may have dual roles, both promoting survival and facilitating cell death in specific contexts. Determining the physiological relevance of these pathways and identifying the precise requirements for ligand and co-receptors, the signaling

cascades involved, and their cell-specific and temporal and spatial organization are likely to be an exciting area of exploration in the years to come.

References

Agerman K, Baudet C, Fundin B, Willson C, Ernfors P (2000) Attenuation of a caspase-3 dependent cell death in NT4- and p75-deficient embryonic sensory neurons. Mol Cell Neurosci 16(3):258–268

Airaksinen MS, Koltzenburg M, Lewin GR, Masu Y, Helbig C, Wolf E, Brem G, Toyka KV, Thoenen H, Meyer M (1996) Specific subtypes of cutaneous mechanoreceptors require neurotrophin-3 following peripheral target innervation. Neuron 16(2):287–295

Albers KM, Wright DE, Davis BM (1994) Overexpression of nerve growth factor in epidermis of transgenic mice causes hypertrophy of the peripheral nervous system. J Neurosci 14(3 Pt 2):1422–1432

Alcantara S, Frisen J, del Rio JA, Soriano E, Barbacid M, Silos-Santiago I (1997) TrkB signaling is required for postnatal survival of CNS neurons and protects hippocampal and motor neurons from axotomy-induced cell death. J Neurosci 17(10):3623–3633

Arevalo MA, Rodriguez-Tebar A (2006) Activation of casein kinase II and inhibition of phosphatase and tensin homologue deleted on chromosome 10 phosphatase by nerve growth factor/p75NTR inhibit glycogen synthase kinase-3beta and stimulate axonal growth. Mol Biol Cell 17 (8):3369–3377

Bai Y, Dergham P, Nedev H, Xu J, Galan A, Rivera JC, ZhiHua S, Mehta HM, Woo SB, Sarunic MV, Neet KE, Saragovi HU (2010) Chronic and acute models of retinal neurodegeneration TrkA activity are neuroprotective whereas p75NTR activity is neurotoxic through a paracrine mechanism. J Biol Chem 285(50):39392–39400

Balthasar N, Coppari R, McMinn J, Liu SM, Lee CE, Tang V, Kenny CD, McGovern RA, Chua SC Jr, Elmquist JK, Lowell BB (2004) Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. Neuron 42(6):983–991

Bamji SX, Majdan M, Pozniak CD, Belliveau DJ, Aloyz R, Kohn J, Causing CG, Miller FD (1998) The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. J Cell Biol 140(4):911–923

Baquet ZC, Gorski JA, Jones KR (2004) Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. J Neurosci 24(17):4250–4258

Barde YA (1989) Trophic factors and neuronal survival. Neuron 2(6):1525–1534

Barde YA, Edgar D, Thoenen H (1982) Purification of a new neurotrophic factor from mammalian brain. EMBO J 1(5):549–553

Barker PA (2007) High affinity not in the vicinity? Neuron 53(1):1–4

Barker PA, Shooter EM (1994) Disruption of NGF binding to the low affinity neurotrophin receptor p75LNTR reduces NGF binding to TrkA on PC12 cells. Neuron 13(1):203–215

Barrett GL, Bartlett PF (1994) The p75 nerve growth factor receptor mediates survival or death depending on the stage of sensory neuron development. Proc Natl Acad Sci USA 91 (14):6501–6505

Beattie MS, Harrington AW, Lee R, Kim JY, Boyce SL, Longo FM, Bresnahan JC, Hempstead BL, Yoon SO (2002) ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. Neuron 36(3):375–386

Bergmann I, Priestley JV, McMahon SB, Brocker EB, Toyka KV, Koltzenburg M (1997) Analysis of cutaneous sensory neurons in transgenic mice lacking the low affinity neurotrophin receptor p75. Eur J Neurosci 9(1):18–28

Bertrand MJ, Kenchappa RS, Andrieu D, Leclercq-Smekens M, Nguyen HN, Carter BD, Muscatelli F, Barker PA, De Backer O (2008) NRAGE, a p75NTR adaptor protein, is required for developmental apoptosis in vivo. Cell Death Differ 15(12):1921–1929

Bhakar AL, Roux PP, Lachance C, Kryl D, Zeindler C, Barker PA (1999) The p75 neurotrophin receptor (p75NTR) alters tumor necrosis factor-mediated NF-kappaB activity under physiological conditions, but direct p75NTR-mediated NF-kappaB activation requires cell stress. J Biol Chem 274(30):21443–21449

Bhakar AL, Howell JL, Paul CE, Salehi AH, Becker EB, Said F, Bonni A, Barker PA (2003) Apoptosis induced by p75NTR overexpression requires Jun kinase-dependent phosphorylation of Bad. J Neurosci 23(36):11373–11381

Bhattacharyya A, Watson FL, Pomeroy SL, Zhang YZ, Stiles CD, Segal RA (2002) High-resolution imaging demonstrates dynein-based vesicular transport of activated Trk receptors. J Neurobiol 51(4):302–312

Bibel M, Hoppe E, Barde YA (1999) Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR. EMBO J 18(3):616–622

Bothwell M (1995) Functional interactions of neurotrophins and neurotrophin receptors. Annu Rev Neurosci 18:223–253

Brann AB, Tcherpakov M, Williams IM, Futerman AH, Fainzilber M (2002) Nerve growth factor-induced p75-mediated death of cultured hippocampal neurons is age-dependent and transduced through ceramide generated by neutral sphingomyelinase. J Biol Chem 277(12):9812–9818

Brodeur GM, Minturn JE, Ho R, Simpson AM, Iyer R, Varela CR, Light JE, Kolla V, Evans AE (2009) Trk receptor expression and inhibition in neuroblastomas. Clin Cancer Res 15 (10):3244–3250

Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96(6):857–868

Brunet A, Datta SR, Greenberg ME (2001) Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. Curr Opin Neurobiol 11(3):297–305

Buchman VL, Davies AM (1993) Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. Development 118 (3):989–1001

Bui NT, Konig HG, Culmsee C, Bauerbach E, Poppe M, Krieglstein J, Prehn JH (2002) p75 neurotrophin receptor is required for constitutive and NGF-induced survival signalling in PC12 cells and rat hippocampal neurones. J Neurochem 81(3):594–605

Carter BD, Kaltschmidt C, Kaltschmidt B, Offenhauser N, Bohm-Matthaei R, Baeuerle PA, Barde YA (1996) Selective activation of NF-kappa B by nerve growth factor through the neurotrophin receptor p75. Science 272(5261):542–545

Casaccia-Bonnefil P, Carter BD, Dobrowsky RT, Chao MV (1996) Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. Nature 383 (6602):716–719

Casademunt E, Carter BD, Benzel I, Frade JM, Dechant G, Barde YA (1999) The zinc finger protein NRIF interacts with the neurotrophin receptor p75(NTR) and participates in programmed cell death. EMBO J 18(21):6050–6061

Castren E, Zafra F, Thoenen H, Lindholm D (1992) Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. Proc Natl Acad Sci USA 89(20):9444–9448

Ceni C, Kommaddi RP, Thomas R, Vereker E, Liu X, McPherson PS, Ritter B, Barker PA (2010) The p75NTR intracellular domain generated by neurotrophin-induced receptor cleavage potentiates Trk signaling. J Cell Sci 123(Pt 13):2299–2307

Chan JP, Unger TJ, Byrnes J, Rios M (2006a) Examination of behavioral deficits triggered by targeting Bdnf in fetal or postnatal brains of mice. Neuroscience 142(1):49–58

Chan JR, Jolicoeur C, Yamauchi J, Elliott J, Fawcett JP, Ng BK, Cayouette M (2006b) The polarity protein Par-3 directly interacts with p75NTR to regulate myelination. Science 314 (5800):832–836

Chan JP, Cordeira J, Calderon GA, Iyer LK, Rios M (2008) Depletion of central BDNF in mice impedes terminal differentiation of new granule neurons in the adult hippocampus. Mol Cell Neurosci 39(3):372–383

Chen KS, Nishimura MC, Armanini MP, Crowley C, Spencer SD, Phillips HS (1997) Disruption of a single allele of the nerve growth factor gene results in atrophy of basal forebrain cholinergic neurons and memory deficits. J Neurosci 17(19):7288–7296

Chou TT, Trojanowski JQ, Lee VM (2000) A novel apoptotic pathway induced by nerve growth factor-mediated TrkA activation in medulloblastoma. J Biol Chem 275(1):565–570

Clary DO, Reichardt LF (1994) An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. Proc Natl Acad Sci USA 91 (23):11133–11137

Conover JC, Erickson JT, Katz DM, Bianchi LM, Poueymirou WT, McClain J, Pan L, Helgren M, Ip NY, Boland P et al (1995) Neuronal deficits, not involving motor neurons, in mice lacking BDNF and/or NT4. Nature 375(6528):235–238

Copray JC, Brouwer N (1994) Selective expression of neurotrophin-3 messenger RNA in muscle spindles of the rat. Neuroscience 63(4):1125–1135

Cosgaya JM, Chan JR, Shooter EM (2002) The neurotrophin receptor p75NTR as a positive modulator of myelination. Science 298(5596):1245–1248

Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S, Armanini MP, Ling LH, McMahon SB, Shelton DL, Levinson AD et al (1994) Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 76(6):1001–1011

Dadakhujaev S, Jung EJ, Noh HS, Hah YS, Kim CJ, Kim DR (2009) Interplay between autophagy and apoptosis in TrkA-induced cell death. Autophagy 5(1):103–105

Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91(2):231–241

Davies AM, Lee KF, Jaenisch R (1993) p75-deficient trigeminal sensory neurons have an altered response to NGF but not to other neurotrophins. Neuron 11(4):565–574

Dixon SJ, MacDonald JI, Robinson KN, Kubu CJ, Meakin SO (2006) Trk receptor binding and neurotrophin/fibroblast growth factor (FGF)-dependent activation of the FGF receptor substrate (FRS)-3. Biochim Biophys Acta 1763(4):366–380

Epa WR, Markovska K, Barrett GL (2004) The p75 neurotrophin receptor enhances TrkA signalling by binding to Shc and augmenting its phosphorylation. J Neurochem 89(2):344–353

Ernfors P (2001) Local and target-derived actions of neurotrophins during peripheral nervous system development. Cell Mol Life Sci 58(8):1036–1044

Ernfors P, Lee KF, Jaenisch R (1994a) Mice lacking brain-derived neurotrophic factor develop with sensory deficits. Nature 368(6467):147–150

Ernfors P, Lee KF, Kucera J, Jaenisch R (1994b) Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. Cell 77(4):503–512

Esposito D, Patel P, Stephens RM, Perez P, Chao MV, Kaplan DR, Hempstead BL (2001) The cytoplasmic and transmembrane domains of the p75 and Trk A receptors regulate high affinity binding to nerve growth factor. J Biol Chem 276(35):32687–32695

Farinas I (1999) Neurotrophin actions during the development of the peripheral nervous system. Microsc Res Tech 45(4–5):233–242

Farinas I, Jones KR, Backus C, Wang XY, Reichardt LF (1994) Severe sensory and sympathetic deficits in mice lacking neurotrophin-3. Nature 369(6482):658–661

Farinas I, Yoshida CK, Backus C, Reichardt LF (1996) Lack of neurotrophin-3 results in death of spinal sensory neurons and premature differentiation of their precursors. Neuron 17 (6):1065–1078

Farinas I, Wilkinson GA, Backus C, Reichardt LF, Patapoutian A (1998) Characterization of neurotrophin and Trk receptor functions in developing sensory ganglia: direct NT-3 activation of TrkB neurons in vivo. Neuron 21(2):325–334

Ferri CC, Moore FA, Bisby MA (1998) Effects of facial nerve injury on mouse motoneurons lacking the p75 low-affinity neurotrophin receptor. J Neurobiol 34(1):1–9

Frade JM, Barde YA (1999) Genetic evidence for cell death mediated by nerve growth factor and the neurotrophin receptor p75 in the developing mouse retina and spinal cord. Development 126(4):683–690

Frade JM, Rodriguez-Tebar A, Barde YA (1996) Induction of cell death by endogenous nerve growth factor through its p75 receptor. Nature 383(6596):166–168

Friedman WJ (2000) Neurotrophins induce death of hippocampal neurons via the p75 receptor. J Neurosci 20(17):6340–6346

Fundin BT, Silos-Santiago I, Ernfors P, Fagan AM, Aldskogius H, DeChiara TM, Phillips HS, Barbacid M, Yancopoulos GD, Rice FL (1997) Differential dependency of cutaneous mechanoreceptors on neurotrophins, trk receptors, and P75 LNGFR. Dev Biol 190(1):94–116

Gao X, Chen J (2009) Conditional knockout of brain-derived neurotrophic factor in the hippocampus increases death of adult-born immature neurons following traumatic brain injury. J Neurotrauma 26(8):1325–1335

Gentry JJ, Casaccia-Bonnefil P, Carter BD (2000) Nerve growth factor activation of nuclear factor kappaB through its p75 receptor is an anti-apoptotic signal in RN22 schwannoma cells. J Biol Chem 275(11):7558–7565

Giehl KM, Tetzlaff W (1996) BDNF and NT-3, but not NGF, prevent axotomy-induced death of rat corticospinal neurons in vivo. Eur J Neurosci 8(6):1167–1175

Giehl KM, Schutte A, Mestres P, Yan Q (1998) The survival-promoting effect of glial cell line-derived neurotrophic factor on axotomized corticospinal neurons in vivo is mediated by an endogenous brain-derived neurotrophic factor mechanism. J Neurosci 18(18):7351–7360

Giehl KM, Rohrig S, Bonatz H, Gutjahr M, Leiner B, Bartke I, Yan Q, Reichardt LF, Backus C, Welcher AA, Dethleffsen K, Mestres P, Meyer M (2001) Endogenous brain-derived neurotrophic factor and neurotrophin-3 antagonistically regulate survival of axotomized corticospinal neurons in vivo. J Neurosci 21(10):3492–3502

Ginty DD, Segal RA (2002) Retrograde neurotrophin signaling: Trk-ing along the axon. Curr Opin Neurobiol 12(3):268–274

Gjerstad MD, Tandrup T, Koltzenburg M, Jakobsen J (2002) Predominant neuronal B-cell loss in L5 DRG of p75 receptor-deficient mice. J Anat 200(Pt 1):81–87

Gorski JA, Zeiler SR, Tamowski S, Jones KR (2003) Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites. J Neurosci 23(17):6856–6865

Gotz R, Koster R, Winkler C, Raulf F, Lottspeich F, Schartl M, Thoenen H (1994) Neurotrophin-6 is a new member of the nerve growth factor family. Nature 372(6503):266–269

Green SH, Greene LA (1986) A single Mr approximately 103,000 125I-beta-nerve growth factor-affinity-labeled species represents both the low and high affinity forms of the nerve growth factor receptor. J Biol Chem 261(32):15316–15326

Greene LA, Tischler AS (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc Natl Acad Sci USA 73 (7):2424–2428

Grimes ML, Zhou J, Beattie EC, Yuen EC, Hall DE, Valletta JS, Topp KS, LaVail JH, Bunnett NW, Mobley WC (1996) Endocytosis of activated TrkA: evidence that nerve growth factor induces formation of signaling endosomes. J Neurosci 16(24):7950–7964

Grimes ML, Beattie E, Mobley WC (1997) A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. Proc Natl Acad Sci USA 94(18):9909–9914

Grivennikov SI, Kuprash DV, Liu ZG, Nedospasov SA (2006) Intracellular signals and events activated by cytokines of the tumor necrosis factor superfamily: from simple paradigms to complex mechanisms. Int Rev Cytol 252:129–161

Gschwendtner A, Liu Z, Hucho T, Bohatschek M, Kalla R, Dechant G, Raivich G (2003) Regulation, cellular localization, and function of the p75 neurotrophin receptor (p75NTR) during the regeneration of facial motoneurons. Mol Cell Neurosci 24(2):307–322

Gulino A, Arcella A, Giangaspero F (2008) Pathological and molecular heterogeneity of medulloblastoma. Curr Opin Oncol 20(6):668–675

Hallbook F, Ibanez CF, Persson H (1991) Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. Neuron 6(5):845–858

Hamanoue M, Middleton G, Wyatt S, Jaffray E, Hay RT, Davies AM (1999) p75-mediated NF-kappaB activation enhances the survival response of developing sensory neurons to nerve growth factor. Mol Cell Neurosci 14(1):28–40

Hansen K, Wagner B, Hamel W, Schweizer M, Haag F, Westphal M, Lamszus K (2007) Autophagic cell death induced by TrkA receptor activation in human glioblastoma cells. J Neurochem 103(1):259–275

Hantzopoulos PA, Suri C, Glass DJ, Goldfarb MP, Yancopoulos GD (1994) The low affinity NGF receptor, p75, can collaborate with each of the Trks to potentiate functional responses to the neurotrophins. Neuron 13(1):187–201

Harada T, Harada C, Nakayama N, Okuyama S, Yoshida K, Kohsaka S, Matsuda H, Wada K (2000) Modification of glial-neuronal cell interactions prevents photoreceptor apoptosis during light-induced retinal degeneration. Neuron 26(2):533–541

Harel L, Costa B, Tcherpakov M, Zapatka M, Oberthuer A, Hansford LM, Vojvodic M, Levy Z, Chen ZY, Lee FS, Avigad S, Yaniv I, Shi L, Eils R, Fischer M, Brors B, Kaplan DR, Fainzilber M (2009) CCM2 mediates death signaling by the TrkA receptor tyrosine kinase. Neuron 63 (5):585–591

Harel L, Costa B, Fainzilber M (2010) On the death Trk. Dev Neurobiol 70(5):298–303

Harrington AW, St Hillaire C, Zweifel LS, Glebova NO, Philippidou P, Halegoua S, Ginty DD (2011) Recruitment of actin modifiers to TrkA endosomes governs retrograde NGF signaling and survival. Cell 146(3):421–434

Harrison SM, Davis BM, Nishimura M, Albers KM, Jones ME, Phillips HS (2004) Rescue of NGF-deficient mice I: transgenic expression of NGF in skin rescues mice lacking endogenous NGF. Brain Res Mol Brain Res 122(2):116–125

Hempstead BL (2006) Dissecting the diverse actions of pro- and mature neurotrophins. Curr Alzheimer Res 3(1):19–24

Hempstead BL, Martin-Zanca D, Kaplan DR, Parada LF, Chao MV (1991) High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. Nature 350(6320):678–683

Hendry IA, Stockel K, Thoenen H, Iversen LL (1974) The retrograde axonal transport of nerve growth factor. Brain Res 68(1):103–121

Hisata S, Sakisaka T, Baba T, Yamada T, Aoki K, Matsuda M, Takai Y (2007) Rap1-PDZ-GEF1 interacts with a neurotrophin receptor at late endosomes, leading to sustained activation of Rap1 and ERK and neurite outgrowth. J Cell Biol 178(5):843–860

Hohn A, Leibrock J, Bailey K, Barde YA (1990) Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. Nature 344 (6264):339–341

Howe CL, Valletta JS, Rusnak AS, Mobley WC (2001) NGF signaling from clathrin-coated vesicles: evidence that signaling endosomes serve as a platform for the Ras-MAPK pathway. Neuron 32(5):801–814

Hu B, Yip HK, So KF (1998) Localization of p75 neurotrophin receptor in the retina of the adult SD rat: an immunocytochemical study at light and electron microscopic levels. Glia 24 (2):187–197

Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24:677–736

Hubbard SR, Miller WT (2007) Receptor tyrosine kinases: mechanisms of activation and signaling. Curr Opin Cell Biol 19(2):117–123

Hughes AL, Messineo-Jones D, Lad SP, Neet KE (2001) Distinction between differentiation, cell cycle, and apoptosis signals in PC12 cells by the nerve growth factor mutant delta9/13, which is selective for the p75 neurotrophin receptor. J Neurosci Res 63(1):10–19

Ip NY, Ibanez CF, Nye SH, McClain J, Jones PF, Gies DR, Belluscio L, Le Beau MM, Espinosa R 3rd, Squinto SP et al (1992) Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity. Proc Natl Acad Sci USA 89(7):3060–3064

Ito H, Nomoto H, Furukawa S (2003) Growth arrest of PC12 cells by nerve growth factor is dependent on the phosphatidylinositol 3-kinase/Akt pathway via p75 neurotrophin receptor. J Neurosci Res 72(2):211–217

Jansen P, Giehl K, Nyengaard JR, Teng K, Lioubinski O, Sjoegaard SS, Breiderhoff T, Gotthardt M, Lin F, Eilers A, Petersen CM, Lewin GR, Hempstead BL, Willnow TE, Nykjaer A (2007) Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. Nat Neurosci 10(11):1449–1457

Jiang Y, Nyengaard JR, Zhang JS, Jakobsen J (2004) Selective loss of calcitonin gene-related Peptide-expressing primary sensory neurons of the a-cell phenotype in early experimental diabetes. Diabetes 53(10):2669–2675

Jing S, Tapley P, Barbacid M (1992) Nerve growth factor mediates signal transduction through trk homodimer receptors. Neuron 9(6):1067–1079

Jones KR, Farinas I, Backus C, Reichardt LF (1994) Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. Cell 76 (6):989–999

Jung EJ, Kim DR (2008) Apoptotic cell death in TrkA-overexpressing cells: kinetic regulation of ERK phosphorylation and caspase-7 activation. Mol Cells 26(1):12–17

Kane LP, Shapiro VS, Stokoe D, Weiss A (1999) Induction of NF-kappaB by the Akt/PKB kinase. Curr Biol 9(11):601–604

Kantari C, Walczak H (2011) Caspase-8 and bid: caught in the act between death receptors and mitochondria. Biochim Biophys Acta 1813(4):558–563

Kaplan DR, Miller FD (2000) Neurotrophin signal transduction in the nervous system. Curr Opin Neurobiol 10(3):381–391

Kaplan DR, Hempstead BL, Martin-Zanca D, Chao MV, Parada LF (1991) The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. Science 252 (5005):554–558

Kenchappa RS, Zampieri N, Chao MV, Barker PA, Teng HK, Hempstead BL, Carter BD (2006) Ligand-dependent cleavage of the P75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. Neuron 50(2):219–232

Kenchappa RS, Tep C, Korade Z, Urra S, Bronfman FC, Yoon SO, Carter BD (2010) p75 neurotrophin receptor-mediated apoptosis in sympathetic neurons involves a biphasic activation of JNK and up-regulation of tumor necrosis factor-alpha-converting enzyme/ADAM17. J Biol Chem 285(26):20358–20368

Khursigara G, Orlinick JR, Chao MV (1999) Association of the p75 neurotrophin receptor with TRAF6. J Biol Chem 274(5):2597–2600

Khursigara G, Bertin J, Yano H, Moffett H, DiStefano PS, Chao MV (2001) A prosurvival function for the p75 receptor death domain mediated via the caspase recruitment domain receptor-interacting protein 2. J Neurosci 21(16):5854–5863

Kim T, Hempstead BL (2009) NRH2 is a trafficking switch to regulate sortilin localization and permit proneurotrophin-induced cell death. EMBO J 28(11):1612–1623

Klein R, Jing SQ, Nanduri V, O'Rourke E, Barbacid M (1991) The trk proto-oncogene encodes a receptor for nerve growth factor. Cell 65(1):189–197

Klein R, Silos-Santiago I, Smeyne RJ, Lira SA, Brambilla R, Bryant S, Zhang L, Snider WD, Barbacid M (1994) Disruption of the neurotrophin-3 receptor gene trkC eliminates la muscle afferents and results in abnormal movements. Nature 368(6468):249–251

Korhonen JM, Said FA, Wong AJ, Kaplan DR (1999) Gab1 mediates neurite outgrowth, DNA synthesis, and survival in PC12 cells. J Biol Chem 274(52):37307–37314

Kuruvilla R, Zweifel LS, Glebova NO, Lonze BE, Valdez G, Ye H, Ginty DD (2004) A neurotrophin signaling cascade coordinates sympathetic neuron development through differential control of TrkA trafficking and retrograde signaling. Cell 118(2):243–255

Lachance C, Belliveau DJ, Barker PA (1997) Blocking nerve growth factor binding to the p75 neurotrophin receptor on sympathetic neurons transiently reduces trkA activation but does not affect neuronal survival. Neuroscience 81(3):861–871

Lachyankar MB, Condon PJ, Daou MC, De AK, Levine JB, Obermeier A, Ross AH (2003) Novel functional interactions between Trk kinase and p75 neurotrophin receptor in neuroblastoma cells. J Neurosci Res 71(2):157–172

Ladiwala U, Lachance C, Simoneau SJ, Bhakar A, Barker PA, Antel JP (1998) p75 neurotrophin receptor expression on adult human oligodendrocytes: signaling without cell death in response to NGF. J Neurosci 18(4):1297–1304

Lai KO, Fu WY, Ip FC, Ip NY (1998) Cloning and expression of a novel neurotrophin, NT-7, from carp. Mol Cell Neurosci 11(1–2):64–76

Lavoie JF, Lesauteur L, Kohn J, Wong J, Furtoss O, Thiele CJ, Miller FD, Kaplan DR (2005) TrkA induces apoptosis of neuroblastoma cells and does so via a p53-dependent mechanism. J Biol Chem 280(32):29199–29207

Lebrun-Julien F, Duplan L, Pernet V, Osswald I, Sapieha P, Bourgeois P, Dickson K, Bowie D, Barker PA, Di Polo A (2009) Excitotoxic death of retinal neurons in vivo occurs via a non-cell-autonomous mechanism. J Neurosci 29(17):5536–5545

Lee KF, Li E, Huber LJ, Landis SC, Sharpe AH, Chao MV, Jaenisch R (1992) Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. Cell 69(5):737–749

Lee KF, Davies AM, Jaenisch R (1994) p75-deficient embryonic dorsal root sensory and neonatal sympathetic neurons display a decreased sensitivity to NGF. Development 120(4):1027–1033

Lefcort F, Clary DO, Rusoff AC, Reichardt LF (1996) Inhibition of the NT-3 receptor TrkC, early in chick embryogenesis, results in severe reductions in multiple neuronal subpopulations in the dorsal root ganglia. J Neurosci 16(11):3704–3713

Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengerer B, Masiakowski P, Thoenen H, Barde YA (1989) Molecular cloning and expression of brain-derived neurotrophic factor. Nature 341 (6238):149–152

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

Levi-Montalcini R, Angeletti PU (1968) Nerve growth factor. Physiol Rev 48(3):534–569

Linggi MS, Burke TL, Williams BB, Harrington A, Kraemer R, Hempstead BL, Yoon SO, Carter BD (2005) Neurotrophin receptor interacting factor (NRIF) is an essential mediator of apoptotic signaling by the p75 neurotrophin receptor. J Biol Chem 280(14):13801–13808

Mahadeo D, Kaplan L, Chao MV, Hempstead BL (1994) High affinity nerve growth factor binding displays a faster rate of association than p140trk binding. Implications for multi-subunit polypeptide receptors. J Biol Chem 269(9):6884–6891

Maisonpierre PC, Belluscio L, Friedman B, Alderson RF, Wiegand SJ, Furth ME, Lindsay RM, Yancopoulos GD (1990a) NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. Neuron 5(4):501–509

Maisonpierre PC, Belluscio L, Squinto S, Ip NY, Furth ME, Lindsay RM, Yancopoulos GD (1990b) Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. Science 247(4949 Pt 1):1446–1451

Majdan M, Walsh GS, Aloyz R, Miller FD (2001) TrkA mediates developmental sympathetic neuron survival in vivo by silencing an ongoing p75NTR-mediated death signal. J Cell Biol 155(7):1275–1285

Mamidipudi V, Li X, Wooten MW (2002) Identification of interleukin 1 receptor-associated kinase as a conserved component in the p75-neurotrophin receptor activation of nuclear factor-kappa B. J Biol Chem 277(31):28010–28018

Mamidipudi V, Lin C, Seibenhener ML, Wooten MW (2004) Regulation of interleukin receptor-associated kinase (IRAK) phosphorylation and signaling by iota protein kinase C. J Biol Chem 279(6):4161–4165

Massa SM, Xie Y, Yang T, Harrington AW, Kim ML, Yoon SO, Kraemer R, Moore LA, Hempstead BL, Longo FM (2006) Small, nonpeptide p75NTR ligands induce survival signaling and inhibit proNGF-induced death. J Neurosci 26(20):5288–5300

Matrone C, Marolda R, Ciafre S, Ciotti MT, Mercanti D, Calissano P (2009) Tyrosine kinase nerve growth factor receptor switches from prosurvival to proapoptotic activity via Abeta-mediated phosphorylation. Proc Natl Acad Sci USA 106(27):11358–11363

Meakin SO, MacDonald JI, Gryz EA, Kubu CJ, Verdi JM (1999) The signaling adapter FRS-2 competes with Shc for binding to the nerve growth factor receptor TrkA. A model for discriminating proliferation and differentiation. J Biol Chem 274(14):9861–9870

Minichiello L, Klein R (1996) TrkB and TrkC neurotrophin receptors cooperate in promoting survival of hippocampal and cerebellar granule neurons. Genes Dev 10(22):2849–2858

Monteggia LM, Luikart B, Barrot M, Theobold D, Malkovska I, Nef S, Parada LF, Nestler EJ (2007) Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biol Psychiatry 61(2):187–197

Mukai J, Hachiya T, Shoji-Hoshino S, Kimura MT, Nadano D, Suvanto P, Hanaoka T, Li Y, Irie S, Greene LA, Sato TA (2000) NADE, a p75NTR-associated cell death executor, is involved in signal transduction mediated by the common neurotrophin receptor p75NTR. J Biol Chem 275 (23):17566–17570

Muragaki Y, Chou TT, Kaplan DR, Trojanowski JQ, Lee VM (1997) Nerve growth factor induces apoptosis in human medulloblastoma cell lines that express TrkA receptors. J Neurosci 17 (2):530–542

Murray SS, Bartlett PF, Cheema SS (1999) Differential loss of spinal sensory but not motor neurons in the p75NTR knockout mouse. Neurosci Lett 267(1):45–48

Murray SS, Perez P, Lee R, Hempstead BL, Chao MV (2004) A novel p75 neurotrophin receptor-related protein, NRH2, regulates nerve growth factor binding to the TrkA receptor. J Neurosci 24(11):2742–2749

Nikoletopoulou V, Lickert H, Frade JM, Rencurel C, Giallonardo P, Zhang L, Bibel M, Barde YA (2010) Neurotrophin receptors TrkA and TrkC cause neuronal death whereas TrkB does not. Nature 467(7311):59–63

Nykjaer A, Willnow TE (2012) Sortilin: a receptor to regulate neuronal viability and function. Trends Neurosci 35(4):261–270

O'Neill LA, Kaltschmidt C (1997) NF-kappa B: a crucial transcription factor for glial and neuronal cell function. Trends Neurosci 20(6):252–258

Onishi-Haraikawa Y, Funaki M, Gotoh N, Shibuya M, Inukai K, Katagiri H, Fukushima Y, Anai M, Ogihara T, Sakoda H, Ono H, Kikuchi M, Oka Y, Asano T (2001) Unique phosphorylation mechanism of Gab1 using PI 3-kinase as an adaptor protein. Biochem Biophys Res Commun 288(2):476–482

Oppenheim RW (1991) Cell death during development of the nervous system. Annu Rev Neurosci 14:453–501

Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB (1999) NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. Nature 401(6748):82–85

Park JA, Lee JY, Sato TA, Koh JY (2000) Co-induction of p75NTR and p75NTR-associated death executor in neurons after zinc exposure in cortical culture or transient ischemia in the rat. J Neurosci 20(24):9096–9103

Park HH, Lo YC, Lin SC, Wang L, Yang JK, Wu H (2007) The death domain superfamily in intracellular signaling of apoptosis and inflammation. Annu Rev Immunol 25:561–586

Pazyra-Murphy MF, Hans A, Courchesne SL, Karch C, Cosker KE, Heerssen HM, Watson FL, Kim T, Greenberg ME, Segal RA (2009) A retrograde neuronal survival response: target-derived neurotrophins regulate MEF2D and bcl-w. J Neurosci 29(20):6700–6709

Provenzano MJ, Xu N, Ver Meer MR, Clark JJ, Hansen MR (2008) p75NTR and sortilin increase after facial nerve injury. Laryngoscope 118(1):87–93

Rauskolb S, Zagrebelsky M, Dreznjak A, Deogracias R, Matsumoto T, Wiese S, Erne B, Sendtner M, Schaeren-Wiemers N, Korte M, Barde YA (2010) Global deprivation of brain-

derived neurotrophic factor in the CNS reveals an area-specific requirement for dendritic growth. J Neurosci 30(5):1739–1749
Riccio A, Pierchala BA, Ciarallo CL, Ginty DD (1997) An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons. Science 277(5329):1097–1100
Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, Lechan RM, Jaenisch R (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol Endocrinol 15(10):1748–1757
Rodriguez-Tebar A, Dechant G, Barde YA (1990) Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. Neuron 4(4):487–492
Rodriguez-Tebar A, Dechant G, Gotz R, Barde YA (1992) Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. EMBO J 11(3):917–922
Romashkova JA, Makarov SS (1999) NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. Nature 401(6748):86–90
Roux PP, Colicos MA, Barker PA, Kennedy TE (1999) p75 neurotrophin receptor expression is induced in apoptotic neurons after seizure. J Neurosci 19(16):6887–6896
Roux PP, Bhakar AL, Kennedy TE, Barker PA (2001) The p75 neurotrophin receptor activates Akt (protein kinase B) through a phosphatidylinositol 3-kinase-dependent pathway. J Biol Chem 276(25):23097–23104
Ryden M, Hempstead B, Ibanez CF (1997) Differential modulation of neuron survival during development by nerve growth factor binding to the p75 neurotrophin receptor. J Biol Chem 272 (26):16322–16328
Salehi AH, Roux PP, Kubu CJ, Zeindler C, Bhakar A, Tannis LL, Verdi JM, Barker PA (2000) NRAGE, a novel MAGE protein, interacts with the p75 neurotrophin receptor and facilitates nerve growth factor-dependent apoptosis. Neuron 27(2):279–288
Salehi AH, Xanthoudakis S, Barker PA (2002) NRAGE, a p75 neurotrophin receptor-interacting protein, induces caspase activation and cell death through a JNK-dependent mitochondrial pathway. J Biol Chem 277(50):48043–48050
Sedel F, Bechade C, Triller A (1999) Nerve growth factor (NGF) induces motoneuron apoptosis in rat embryonic spinal cord in vitro. Eur J Neurosci 11(11):3904–3912
Segal RA, Goumnerova LC, Kwon YK, Stiles CD, Pomeroy SL (1994) Expression of the neurotrophin receptor TrkC is linked to a favorable outcome in medulloblastoma. Proc Natl Acad Sci USA 91(26):12867–12871
Sendtner M, Holtmann B, Hughes RA (1996) The response of motoneurons to neurotrophins. Neurochem Res 21(7):831–841
Shi Z, Birman E, Saragovi HU (2007) Neurotrophic rationale in glaucoma: a TrkA agonist, but not NGF or a p75 antagonist, protects retinal ganglion cells in vivo. Dev Neurobiol 67(7):884–894
Skaper SD (2008) The biology of neurotrophins, signalling pathways, and functional peptide mimetics of neurotrophins and their receptors. CNS Neurol Disord Drug Targets 7(1):46–62
Smeyne RJ, Klein R, Schnapp A, Long LK, Bryant S, Lewin A, Lira SA, Barbacid M (1994) Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature 368(6468):246–249
Song W, Volosin M, Cragnolini AB, Hempstead BL, Friedman WJ (2010) ProNGF induces PTEN via p75NTR to suppress Trk-mediated survival signaling in brain neurons. J Neurosci 30 (46):15608–15615
Squinto SP, Stitt TN, Aldrich TH, Davis S, Bianco SM, Radziejewski C, Glass DJ, Masiakowski P, Furth ME, Valenzuela DM et al (1991) trkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. Cell 65(5):885–893
Stucky CL, Shin JB, Lewin GR (2002) Neurotrophin-4: a survival factor for adult sensory neurons. Curr Biol 12(16):1401–1404
Sutter A, Riopelle RJ, Harris-Warrick RM, Shooter EM (1979) Nerve growth factor receptors. Characterization of two distinct classes of binding sites on chick embryo sensory ganglia cells. J Biol Chem 254(13):5972–5982

Syroid DE, Maycox PJ, Soilu-Hanninen M, Petratos S, Bucci T, Burrola P, Murray S, Cheema S, Lee KF, Lemke G, Kilpatrick TJ (2000) Induction of postnatal schwann cell death by the low-affinity neurotrophin receptor in vitro and after axotomy. J Neurosci 20(15):5741–5747

Tauszig-Delamasure S, Yu LY, Cabrera JR, Bouzas-Rodriguez J, Mermet-Bouvier C, Guix C, Bordeaux MC, Arumae U, Mehlen P (2007) The TrkC receptor induces apoptosis when the dependence receptor notion meets the neurotrophin paradigm. Proc Natl Acad Sci USA 104 (33):13361–13366

Troy CM, Friedman JE, Friedman WJ (2002) Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. J Biol Chem 277(37):34295–34302

Twiss JL, Wada HG, Fok KS, Chan SD, Verity AN, Baxter GT, Shooter EM, Sussman HH (1998) Duration and magnitude of nerve growth factor signaling depend on the ratio of p75LNTR to TrkA. J Neurosci Res 51(4):442–453

Vaegter CB, Jansen P, Fjorback AW, Glerup S, Skeldal S, Kjolby M, Richner M, Erdmann B, Nyengaard JR, Tessarollo L, Lewin GR, Willnow TE, Chao MV, Nykjaer A (2011) Sortilin associates with Trk receptors to enhance anterograde transport and neurotrophin signaling. Nat Neurosci 14(1):54–61

Valdes-Sanchez T, Kirstein M, Perez-Villalba A, Vega JA, Farinas I (2010) BDNF is essentially required for the early postnatal survival of nociceptors. Dev Biol 339(2):465–476

Van der Zee CE, Ross GM, Riopelle RJ, Hagg T (1996) Survival of cholinergic forebrain neurons in developing p75NGFR-deficient mice. Science 274(5293):1729–1732

Varsano T, Dong MQ, Niesman I, Gacula H, Lou X, Ma T, Testa JR, Yates JR 3rd, Farquhar MG (2006) GIPC is recruited by APPL to peripheral TrkA endosomes and regulates TrkA trafficking and signaling. Mol Cell Biol 26(23):8942–8952

Verdi JM, Birren SJ, Ibanez CF, Persson H, Kaplan DR, Benedetti M, Chao MV, Anderson DJ (1994) p75LNGFR regulates Trk signal transduction and NGF-induced neuronal differentiation in MAH cells. Neuron 12(4):733–745

Volosin M, Song W, Almeida RD, Kaplan DR, Hempstead BL, Friedman WJ (2006) Interaction of survival and death signaling in basal forebrain neurons: roles of neurotrophins and proneurotrophins. J Neurosci 26(29):7756–7766

von Schack D, Casademunt E, Schweigreiter R, Meyer M, Bibel M, Dechant G (2001) Complete ablation of the neurotrophin receptor p75NTR causes defects both in the nervous and the vascular system. Nat Neurosci 4(10):977–978

Wang L, Yang JK, Kabaleeswaran V, Rice AJ, Cruz AC, Park AY, Yin Q, Damko E, Jang SB, Raunser S, Robinson CV, Siegel RM, Walz T, Wu H (2010) The Fas-FADD death domain complex structure reveals the basis of DISC assembly and disease mutations. Nat Struct Mol Biol 17(11):1324–1329

Watson FL, Heerssen HM, Bhattacharyya A, Klesse L, Lin MZ, Segal RA (2001) Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. Nat Neurosci 4(10):981–988

Wehrman T, He X, Raab B, Dukipatti A, Blau H, Garcia KC (2007) Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors. Neuron 53 (1):25–38

White FA, Silos-Santiago I, Molliver DC, Nishimura M, Phillips H, Barbacid M, Snider WD (1996) Synchronous onset of NGF and TrkA survival dependence in developing dorsal root ganglia. J Neurosci 16(15):4662–4672

Willard MD, Willard FS, Li X, Cappell SD, Snider WD, Siderovski DP (2007) Selective role for RGS12 as a Ras/Raf/MEK scaffold in nerve growth factor-mediated differentiation. EMBO J 26(8):2029–2040

Wilson KH (2009) The genome sequence of the protostome Daphnia pulex encodes respective orthologues of a neurotrophin, a Trk and a p75NTR: evolution of neurotrophin signaling components and related proteins in the bilateria. BMC Evol Biol 9:243

Wong AW, Willingham M, Xiao J, Kilpatrick TJ, Murray SS (2008) Neurotrophin receptor homolog-2 regulates nerve growth factor signaling. J Neurochem 106(4):1964–1976

Xu B, Zang K, Ruff NL, Zhang YA, McConnell SK, Stryker MP, Reichardt LF (2000) Cortical degeneration in the absence of neurotrophin signaling: dendritic retraction and neuronal loss after removal of the receptor TrkB. Neuron 26(1):233–245

Yan H, Schlessinger J, Chao MV (1991) Chimeric NGF-EGF receptors define domains responsible for neuronal differentiation. Science 252(5005):561–563

Yano H, Lee FS, Kong H, Chuang J, Arevalo J, Perez P, Sung C, Chao MV (2001) Association of Trk neurotrophin receptors with components of the cytoplasmic dynein motor. J Neurosci 21 (3):RC125

Yeiser EC, Rutkoski NJ, Naito A, Inoue J, Carter BD (2004) Neurotrophin signaling through the p75 receptor is deficient in traf6−/− mice. J Neurosci 24(46):10521–10529

Yeo TT, Chua-Couzens J, Butcher LL, Bredesen DE, Cooper JD, Valletta JS, Mobley WC, Longo FM (1997) Absence of p75NTR causes increased basal forebrain cholinergic neuron size, choline acetyltransferase activity, and target innervation. J Neurosci 17(20):7594–7605

York RD, Molliver DC, Grewal SS, Stenberg PE, McCleskey EW, Stork PJ (2000) Role of phosphoinositide 3-kinase and endocytosis in nerve growth factor-induced extracellular signal-regulated kinase activation via Ras and Rap1. Mol Cell Biol 20(21):8069–8083

Young KM, Merson TD, Sotthibundhu A, Coulson EJ, Bartlett PF (2007) p75 neurotrophin receptor expression defines a population of BDNF-responsive neurogenic precursor cells. J Neurosci 27(19):5146–5155

Yune TY, Lee JY, Jung GY, Kim SJ, Jiang MH, Kim YC, Oh YJ, Markelonis GJ, Oh TH (2007) Minocycline alleviates death of oligodendrocytes by inhibiting pro-nerve growth factor production in microglia after spinal cord injury. J Neurosci 27(29):7751–7761

Zafra F, Lindholm D, Castren E, Hartikka J, Thoenen H (1992) Regulation of brain-derived neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes. J Neurosci 12(12):4793–4799

BDNF and Synaptic Plasticity, Cognitive Function, and Dysfunction

B. Lu, G. Nagappan, and Y. Lu

Abstract

Among all neurotrophins, brain-derived neurotrophic factor (BDNF) stands out for its high level of expression in the brain and its potent effects on synapses. It is now widely accepted that the main function of BDNF in the adult brain is to regulate synapses, with structural and functional effects ranging from short-term to long-lasting, on excitatory or inhibitory synapses, in many brain regions. The diverse effects of BDNF on brain synapses stem from its complex downstream signaling cascades, as well as the diametrically opposing effects of the pro- and mature form through distinct receptors, TrkB and $p75^{NTR}$. Many aspects of BDNF cell biology are regulated by neuronal activity. The synergistic interactions between neuronal activity and synaptic plasticity by BDNF make it an ideal and essential regulator of cellular processes that underlie cognition and other complex behaviors. Indeed, numerous studies firmly established that BDNF plays a critical role in hippocampal long-term potentiation (LTP), a long-term enhancement of synaptic efficacy thought to underlie learning and memory. Converging evidence now strongly suggest that deficits in BDNF signaling contribute to the pathogenesis of several major diseases and disorders such as Huntington's disease, Alzheimer's disease, and depression. Thus, manipulating BDNF pathways represents a viable treatment approach to a variety of neurological and psychiatric disorders.

Keywords

Brain-derived neurotrophic factor • Synaptic plasticity • mRNA trafficking

B. Lu (✉) • G. Nagappan
GlaxoSmithKline, R&D China, Building 3, 898 Halei Road, Zhangjiang Hi-tech Park, Pudong, Shanghai 201203, China
e-mail: bai.b.lu@gsk.com

Y. Lu
Department of Psychiatry, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA, USA

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_9,

Abbreviations

BDNF	Brain-derived neurotrophic factor
BDNF-KIV	BDNF GFP knockin in exon IV
CA1/CA3	Cornu ammonis areas 1 and 3
E-LTP	Early phase long-term potentiation
GAD	Glutamate decarboxylase
HA	Hemagglutinin
HFS	High frequency stimulation
IgG	Immunoglobulin
KO	Knockout
L-LTP	Late phase long-term potentiation
LTD	Long-term depression
mBDNF	Mature BDNF
MMP	Matrix metalloprotease
MRI	Magnetic resonance imaging
NMDAR	*N*-methyl-D-aspartate receptor
$p75^{NTR}$	p75 neurotrophin receptor
proBDNF	Precursor BDNF
PRP	Plasticity-related protein
PV	Parvalbumin
SNP	Single nucleotide polymorphism
STDP	Spike time-dependent plasticity
TBS	Theta burst stimulation
tDCS	Transcranial direct current stimulation
tPA	Tissue plasminogen activator
TrkB	Tropomyosin-related kinase B
UTR	Untranslated region
$Val^{66}Met$	Valine 66 to methionine

1 BDNF Regulation of Early Phase-LTP

1.1 Initial Discovery

The hint that BDNF might be involved in synaptic plasticity came from the observation that the expression of BDNF in the hippocampus can be induced by high frequency stimulation (HFS) that is often used to induce LTP (Castren et al. 1993; Patterson et al. 1992). The first paper on pharmacological regulation of LTP by BDNF was the report by Figurov et al. (1996) demonstrating that treatment of hippocampal slices with BDNF facilitates early phase LTP (E-LTP) induced by theta burst stimulation (TBS). Neonatal hippocampus generally expresses a low level of BDNF, and TBS induces only short-term synaptic

potentiation (STP). Application of exogenous BDNF enhances the synaptic response to TBS, leading to LTP. In adult hippocampus, where the endogenous BDNF levels are high, inhibition of BDNF activity by the BDNF scavenger TrkB-IgG reduces the magnitude of LTP. In parallel, genetic experiments using two independent lines of BDNF knockout mice demonstrate that a reduction in BDNF expression is associated with a significant impairment in hippocampal LTP (Korte et al. 1995; Patterson et al. 1996). Moreover, heterozygous (+/−) and homozygous (−/−) BDNF-KO mice exhibit similar degrees of impairment in LTP, suggesting that a certain level of BDNF in the hippocampus is required for LTP induction and/or maintenance. Incubation with recombinant BDNF for a few hours (Patterson et al. 1996) rescues the LTP deficits seen in BDNF-KO mice, suggesting that the genetic impairment is amenable for pharmacological manipulations. Subsequent experiments using more sophisticated genetic (TrkB conditional knockout, regional or inducible BDNF knockout, chemical genetic model) and pharmacological (BDNF (Chen et al. 1999) or TrkB (Kang et al. 1997) antibody) approaches have ascertained unequivocally the obligatory role of BDNF-TrkB pathway in hippocampal LTP. BDNF regulation of LTP has also been demonstrated in other brain regions such as visual cortex (Akaneya et al. 1997; Huber et al. 1998; Jiang et al. 2001).

1.2 Acute Versus Chronic Synaptic Modulation by BDNF

In addition to its role in LTP, bath application of BDNF has also been shown to induce long-lasting increase in basal synaptic transmission at hippocampal CA1 synapses (Kang and Schuman 1995). However, similar experiments by a number of laboratories, where BDNF was slowly perfused (as opposed to bath application) acutely showed no such enhancement (Figurov et al. 1996; Patterson et al. 1996; Tanaka et al. 1997). Why would different methods of BDNF application (bath or acute application versus slow or chronic perfusion) elicit such distinct effects? Would different modes of BDNF delivery (or secretion under the physiological conditions) lead to different functional outcomes? To address this question, Ji et al. (2010) applied the same amount of BDNF (final concentration: 1 nM) either acutely as a single bath application or gradually by increasing BDNF concentration from 0.0001 to 1 nM with increments of tenfold every 30 min. Remarkably, the kinetics of TrkB activation and its downstream signaling molecules (Erk, PLCγ1, GSK-3β activation) differed dramatically depending on the mode of BDNF delivery. When BDNF was applied acutely, the activation was robust but transient and declined to baseline within 2 h of application. However, when BDNF concentration increased gradually, the kinetics of TrkB activation was slow, reached the maximal in 1 h, and persisted for up to 8 h without decline. The difference in TrkB signaling kinetics is not due to differential degradation or synthesis of TrkB. Rather, the gradual but not acute delivery of BDNF appears to allow more TrkB receptor to recycle back to the cell surface. Moreover, TrkB activation by acute BDNF application elicited transient activation of both Ras- and Rap-dependent activation

of Erk, whereas gradual BDNF increase resulted in a sustained, Rap-dependent activation of Erk. These differences in downstream signaling pathways suggest that TrkB in different compartments (plasma membrane, endocytic vesicles/signaling endosomes) activate different signaling molecules as reported earlier (Arimura et al. 2009; Heerssen and Segal 2002; Huang and Reichardt 2003; Watson et al. 1999, 2001; Zhou et al. 2007; Zweifel et al. 2005). For instance, stimulation of both Erk1/2 and PI3K/Akt signaling at the plasma membrane is important for axonal elongation. However, preventing endocytosis using genetic or pharmacological inhibitors reduce Erk1/2 phosphorylation but not PI3K/Akt activation suggesting PI3K/Akt activation precedes Trk internalization, while Erk1/2 activation follows receptor endocytosis (York et al. 2000; Zhang et al. 2000). The acute and gradual modes of BDNF signaling also lead to differential expression of TrkB-responsive genes such as Homer1 and Arc. The steady state levels of these proteins increased and lasted longer when BDNF was applied gradually as opposed to acute application, where the levels only increased transiently.

In addition to the differences in the kinetics of TrkB activation and its downstream signaling, different modes of BDNF application also induced differential morphological changes. For instance, acute BDNF application promoted neurite elongation and spine head enlargement, whereas gradual application increased dendritic branching and filopodia-like spines. This is in parallel to changes in different downstream signaling pathways causing relevant morphological changes to establish homeostasis. Mimicking the gradual and acute increases in BDNF concentrations in neonatal rat hippocampal slices showed that slow perfusion of BDNF (slow and chronic) facilitated LTP induced by weak TBS without changing baseline synaptic strength. In contrast, fast perfusion of BDNF (acute) to adult hippocampal slices induced a rapid increase in activation of BDNF signaling that promotes synaptic growth required for establishing neuronal networks during development. It may also be beneficial for long-term, activity-induced structural and functional changes in synapses. In contrast, transient activation of TrkB as a consequence of acute BDNF secretion may rapidly potentiate synaptic transmission in the adult brain (Ji et al. 2010).

1.3 Activity-Dependent Secretion of BDNF and Its Role in Synapse Plasticity and Memory

Similar to all neurotrophins, BDNF is synthesized first as a precursor, proBDNF, which is proteolytically cleaved either inside the cells (Mowla et al. 2001) or after its secretion (Nagappan et al. 2009; Yang et al. 2009b) to form mature BDNF (mBDNF). Unlike other neurotrophins, BDNF is secreted through constitutive as well as regulated pathways. BDNF has been localized to both 200 and 400 nm diameter vesicles by electron microscopy, suggesting that BDNF is trafficked in vesicles that fuse with the plasma membrane either stochastically or in a regulated fashion. While the secretion of mBDNF has been shown to be induced by depolarization, high frequency electric stimulation (HFS), and some chemical inducers,

relatively little is known about the secretion of proBDNF until 2001. Teng et al. reported that proBDNF was detectable in neuronal culture medium, if collected in the presence of α2 anti-plasmin inhibitors and in the absence of glial cells (Lee et al. 2001; Yang et al. 2009b). In contrast, pulse-chase experiments by Matsumoto et al. (2008) detected only mBDNF but not proBDNF extracellularly in hippocampal cultures even after stimulation by the GABA antagonist bicuculline (Matsumoto et al. 2008). This finding questioned whether proBDNF is secreted by neurons at all. To resolve this discrepancy, Yang et al. (2009b) used the BDNF-HA, knockin mice, in which BDNF is tagged with HA fragment to help detection of secreted BDNF, as well as an antibody that specifically detected proBDNF but not mBDNF. Results showed that proBDNF is highly expressed, especially during postnatal development, and secreted in response to neuronal depolarization. The following key measures helped demonstrate activity-dependent secretion of proBDNF: (1) pure neuronal culture with minimum glial contamination; (2) a potent plasmin inhibitor to prevent secreted proBDNF from converting to mBDNF in the culture medium; (3) more sensitive antibodies to detect secreted proBDNF.

Nagappan et al. (2009) reported that hippocampal neurons secrete proBDNF both constitutively and also in a regulated fashion. Moreover, they showed that proBDNF isoform is the major species secreted in response to physiological stimuli such as the LTD-inducing low frequency stimulation (LFS). Interestingly, tissue plasminogen activator (tPA), the enzyme identified to be responsible for converting proBDNF to mBDNF isoform, was secreted only under LTP, but not in LTD stimulating conditions. These results further substantiate that proBDNF secreted from neurons is converted to mBDNF extracellularly in situ and is regulated by neuronal activity. Pharmacological inhibition of tPA in different phases of L-LTP suggests that extracellular conversion of proBDNF by a tPA/plasminogen mechanism may be necessary for the induction phase, whereas the intracellular production of mBDNF may be involved in the maintenance phase (Pang et al, SfN Abstract, 2007). In addition to the tPA/plasmin system, proBDNF can also be converted extracellularly by matrixmetalloprotease 2, 3, 7, and 9 and tolloid-like metalloproteinase (Hwang et al. 2005; Keifer et al. 2009; Lee et al. 2001; Yang et al. 2009a). Further studies are necessary to establish the specificities of proBDNF→mBDNF converting enzymes involved in different brain regions and their physiological functions.

An important question is how cells sort BDNF into different vesicular (constitutive and regulated) trafficking system. The discovery of the association between the single nucleotide polymorphism (SNP) in humans (Egan et al. 2003) and Val^{66}Met (dbSNP number rs6265, with nucleotide change G196A; occurrence: 20–30 % in Caucasian population) greatly facilitated the study of BDNF cell biology and functions. Remarkably, cell culture experiments demonstrate that depolarization-induced secretion of Met^{66}BDNF from hippocampal neurons is significantly reduced compared with Val^{66}BDNF (Chen et al. 2004). Subjects with this SNP exhibit lower levels of hippocampal *N*-acetyl aspartate (an indicator of cell health) as measured by MRI spectroscopy, abnormal hippocampal activation in fMRI,

poorer verbal episodic memory (Egan et al. 2003), as well as reduced hippocampal volume (Pezawas et al. 2004; Szeszko et al. 2005). Interestingly, the Val^{66}Met polymorphism resides in the pro-domain of BDNF and not in mBDNF. How does a SNP in the pro-domain affect activity-dependent BDNF secretion? In vitro experiments using the Val^{66} and Met^{66} forms of BDNF indicate that Met^{66}BDNF protein tends to be clustered in neuronal cell bodies and the proximal regions of the dendritic compartment, whereas the Val^{66} BDNF is distributed as punctates throughout neuronal cell bodies and can travel to the distal dendrites. It is important to note that the functional properties of Met^{66} derived mBDNF were not altered. However, Val^{66}BDNF, but not Met^{66}BDNF, is co-localized with *SecII*, a regulated secretory granule marker (Egan et al. 2003). Moreover, a large fraction of Val^{66}BDNF, but not Met^{66}BDNF, is co-localized with synaptic markers such as synapsin I and PSD95. Taken together, these results suggest that the majority of BDNF is normally sorted into regulated secretory vesicles from Golgi compartments. These vesicles are capable of being transported to distal dendrites or axons, localized to synapses, and released in an activity-dependent manner.

Identification of Val^{66}Met in BDNF trafficking and therefore its consequential function in human episodic memory opened a new area for research in BDNF biology. To further understand the impact of Val^{66}Met substitution, Chen et al. (2006) generated a genetic knockin line of mice in which the Val^{66}BDNF is replaced by Met^{66}BDNF. Similar to the human results reported by Egan et al., neurons derived from the transgenic mice also exhibited reduced BDNF secretion (~30 %), and Met^{66}BDNF mice showed reduced hippocampal volume, due to changes in dendritic complexity, as well as deficits in hippocampal-dependent contextual memory. Moreover, these mice exhibit anxiety-like behaviors, and treatment with antidepressants such as fluoxetine did not alleviate the anxiety phenotype, suggesting that this antidepressant may achieve its anxiolytic effects through activity-dependent BDNF secretion. Consistent with reduction in regulated secretion of BDNF, synaptic plasticity in Met^{66}BDNF mice was significantly altered (Ninan et al. 2010). While the basal glutamatergic transmission remained unaltered in the Met^{66}BDNF animals (no changes in input/output curve, paired pulse facilitation), both NMDAR-dependent LTP and LTD were significantly reduced. Interestingly, mGluR-dependent LTD remained intact. These results suggest that activity-dependent BDNF secretion is selectively involved in the NMDA-dependent forms of synaptic plasticity. Future detailed studies should investigate the specific mechanisms by which Val^{66}MetBDNF alters NMDA receptor function. Considering the rarity of the Met/Met allele frequency in humans (<0.3 %), the Met^{66}BDNF knockin line could serve as a good model to study synaptic dysfunction and effects of pharmacological interventions.

1.4 Effect of tDCS on LTP and Motor Learning

The genetics of BDNF polymorphism offers an opportunity to study the functional consequences of alteration of activity-dependent BDNF secretion in human. It is

conceivable that a reduction in BDNF secretion throughout development may lead to structural alterations in neuronal circuits. It is therefore important to determine whether some of the changes observed in Met^{66}BDNF carriers could be reversed through acute manipulations. Unfortunately, studies of synaptic plasticity have been limited to animal models. Among the few available approaches for use in man, transcranial direct current stimulation (tDCs) has emerged as a safe, simple, noninvasive, and effective manipulation of cortical activity in humans (Antal et al. 2004; Fregni et al. 2005; Gandiga et al. 2006; Iyer et al. 2005; Nitsche et al. 2003). It has been shown that when the anode electrode is placed over the target cortical area on a subject's head and a weak direct current (mA) is applied, stimulation can enhance cortical excitability and function (Webster et al. 2006). In a simple experimental design, Reis et al. (2009) demonstrated that anodal tDCS applied over the human motor cortex (M1) during training facilitates motor skill learning, resulting in substantial improvements in long-term retention of motor memories. In line with these findings, BDNF levels are reported to be elevated in rat motor cortex following motor learning (Klintsova et al. 2004). Moreover, training-induced potentiation of motor-evoked potentials is reduced in human Met^{66}BDNF carriers (Kleim et al. 2006). Thus, one could speculate that motor learning is facilitated by tDCS-induced BDNF secretion in M1 cortex.

To test this hypothesis, Fritsch et al. (2010) developed a method that allows direct application of DCS to mouse slices from M1 cortex, mimicking tDCS in humans (Fritsch et al. 2010). Using this approach, they have identified a novel, long-lasting synaptic potentiation induced by DCS (DCS-LTP), which is polarity (anodal)-specific, NMDA-receptor dependent, and requires coupling of DCS with simultaneous low frequency (0.1 Hz) synaptic activation (mimicking training). Several lines of evidence suggest that DCS-LTP is mediated by DCS-induced secretion of BDNF. First, DCS-LTP is completely blocked in M1 slices derived from BDNF or TrkB knockout mice. Second, combined DCS and low frequency stimulation results in TrkB phosphorylation suggesting BDNF secretion. Finally, scavenging secreted BDNF by TrkB-IgG eliminated DCS-LTP. Thus, activity-dependent BDNF secretion appears to mediate this novel DCS-induced synaptic plasticity in mouse M1 motor cortex.

How activity-dependent secretion of BDNF could alter motor learning in vivo (mouse and humans) was further examined using BDNF Val66Met allele careers (Reis et al. 2009) and $BDNF^{Met/Met}$ knockin mice (Fritsch et al. 2010). Interestingly, acquisition of a fine motor skill over multiple days was found to be significantly impaired in human Met allele careers as well as in $BDNF^{Met/Met}$ knockin mice. Furthermore, Met allele careers exhibited an attenuated response to combined anodal tDCS and training. Taken together, these findings suggest that BDNF is an important player in human motor learning, likely through its contribution to synaptic plasticity at M1, and therefore may have implications in the treatment of motor deficits in neurological and psychiatric conditions.

1.5 Role of TrkB Trafficking

As a diffusible factor, how does BDNF achieve synapse-specific modulation? In addition to local synthesis and/or secretion of BDNF at the active synapse, it is likely that active synapses may also respond better to BDNF compared to less active ones. Therefore, neuronal/synaptic activity may enhance TrkB signaling selectively at active synapses, without affecting the neighboring less active ones. Indeed, multiple studies have revealed several mechanisms conferring activity-dependent regulation of TrkB signaling. First, TrkB mRNA is localized at synapses, especially in the dendritic regions and in synaptosomal fractions, suggesting that similar to BDNF, TrkB mRNA may be locally translated (Righi et al. 2000; Simonato et al. 2002; Tongiorgi et al. 1997). Tongiorgi et al. (1997) have shown that neuronal activity induces translocation of TrkB mRNA into dendrites in vitro. BDNF also induce dendritic translocation of TrkB mRNA, suggesting that activity-dependent local secretion of BDNF may mobilize TrkB mRNA into the dendrites (Tongiorgi and Baj 2008; Tongiorgi et al. 1997). Second, contrary to TrkB mRNA transport into the dendrites, which occur in hours, dendritic TrkB protein levels increased within minutes (~10 min) following neuronal activity. TrkB mRNA local translation may serve as the first node of regulation by neuronal activity. Third, in addition to local translation, BDNF regulation of active synapses may also be mediated through selective insertion of TrkB receptors, providing a positive feed forward regulation (Meyer-Franke et al. 1998). Corroborating this notion are the results from Du et al., demonstrating that the physiologically relevant tetanic stimulation, but not the low frequency stimulation, increase the number of surface TrkB receptors (Du et al. 2000). Neuronal activity or BDNF stimulation led to rapid insertion of TrkB receptors (<30 min) and was dependent on intracellular increase in Ca^{2+} and activation of CaMKII. However, surface expression of TrkB is tightly regulated depending on how TrkB is exposed to BDNF. For instance, acute exposure to BDNF rapidly increases surface expression (Du et al. 2000), whereas chronic exposure results in decrease in surface TrkB levels (Frank et al. 1996; Haapasalo et al. 2002; Sommerfeld et al. 2000), possibly due to TrkB endocytosis and proteasome-mediated degradation. However, if neuronal activity significantly elevates the surface levels of TrkB rapidly in a random fashion, then how does BDNF-TrkB signaling provide synapse-specific regulation?

One mechanism that could potentially constrain BDNF regulation to highly active synapses is through the lateral movement of surface TrkB receptors that are inserted at extrasynaptic sites to move into active synapses (spines/active zones). Presence of lipid rafts (cholesterol and sphingolipid-rich microdomains) at the synapses does offer specialized signaling platform for TrkB regulation (Assaife-Lopes et al. 2010; Suzuki et al. 2004; Wu et al. 1997). Interestingly, translocation of TrkB into lipid rafts selectively activates the Ras/MAPK/Erk pathway, but not PI3K/Akt pathway, suggesting that lipid rafts could compartmentalize downstream signaling events of TrkB (Suzuki et al. 2004). Moreover, blocking TrkB translocation into lipid rafts abolished the potentiating effects of BDNF on evoked synaptic transmission in culture and blocked evoked synaptic

responses in hippocampal slices in response to tetanic stimulation (Suzuki et al. 2004). Finally, alternate mechanisms do exist that can specifically regulate the responsiveness of TrkB receptors at synapses. Along with BDNF secretion, neuronal activity also increases the intracellular concentration of cAMP ([cAMP]i) in situ (spines and active zones), which has been shown to be responsible for regulating BDNF-induced TrkB phosphorylation as well as facilitating the movement of TrkB into the postsynaptic density in dendritic spines (Ji et al. 2005). Together, multiple mechanisms have been discovered that can regulate BDNF actions in a synapse-specific manner by modulating its receptor, TrkB.

2 BDNF Regulation of Late Phase-LTP and Long-Term Memory

2.1 proBDNF Cleavage by tPA/Plasmin System Regulates Late Phase-LTP

In addition to its role in E-LTP, substantial evidence suggests that BDNF is also critical for late phase LTP (L-LTP). Reduction of BDNF levels either genetically by BDNF gene knockout (BDNF+/− mice) (Patterson et al. 1996) or pharmacologically by the application of a BDNF scavenger (Chen et al. 1999) (TrkB-IgG) results in impairment in L-LTP in rat hippocampal slices. Moreover, application of BDNF after hippocampal slices were stimulated with a weak TBS (three sets of four pulses at 100 Hz), which normally only induce E-LTP, resulted in sustained L-LTP. These results suggest that BDNF is necessary and sufficient for L-LTP. In addition, tPA has also been implicated in L-LTP (Frey et al. 1996; Huang et al. 1996). The biochemical function of tPA is to cleave and convert the inactive zymogen plasminogen into active protease plasmin. The finding by Lee et al. (2001) that plasmin can convert proBDNF into mBDNF in vitro (Lee et al. 2001) prompted Pang et al. (2004) to hypothesize that if proBDNF is produced and secreted in the brain, then conversion of proBDNF to mBDNF by the tPA/plasmin system may be involved in L-LTP. Using different transgenic knockout animals (tPA, plasmin, BDNF), this hypothesis was tested systematically to establish the functional relationship between tPA/plasmin and BDNF. First, L-LTP was severely impaired in both tPA and plasminogen knockout mice, and this impairment was completely rescued by perfusing cleaved mBDNF (Pang et al. 2004). Remarkably, perfusion of cleavage-resistant proBDNF (mutated at furin cleavage site) was unable to rescue the L-LTP deficit in tPA (−/−) and plasminogen (−/−) mice, suggesting that conversion of proBDNF to mBDNF is essential for expressing L-LTP. Second, in vitro biochemical experiments showed that tPA together with plasmin was necessary for the conversion of proBDNF to mBDNF, and proBDNF is not a direct substrate of tPA (Pang et al. 2004). Consistent with this finding, tPA knockout animals showed elevated levels of proBDNF. Third, perfusion of tPA failed to rescue the L-LTP deficit in plasminogen (−/−) or BDNF (+/−) mice, whereas perfusion of plasmin rescued the L-LTP deficit in tPA (−/−) mice but not in BDNF

(+/−) mice. These results, together with the finding that mBDNF rescued the L-LTP deficit in both tPA (−/−) and plasminogen (−/−) mice, suggest that tPA, by activating the extracellular protease plasmin, converts the precursor proBDNF to mBDNF in the hippocampus, and such conversion is required for L-LTP (Pang et al. 2004).

An even more remarkable finding is that application of mBDNF after tetanus is sufficient to allow L-LTP to occur even when all protein synthesis is blocked (Pang et al. 2004). It is well established that both long-term memory and L-LTP require new protein synthesis (Govindarajan et al. 2011; Klann and Sweatt 2008). An essential and yet unresolved question is what is (are) the specific product (s) mediating the long-term changes at synapses. The results by Pang et al. (2004) suggest that mBDNF is likely to be the key (or only) protein synthesis product that is essential to convert E-LTP to L-LTP. This is truly a provocative idea that surprised many in the field.

2.2 BDNF Regulation of Long-Term Memory

L-LTP is considered as a cellular basis for long-term memory (LTM). Substantial evidence supports a critical role of BDNF in LTM. An elevation in BDNF mRNA level in the hippocampus has been observed following acquisition of spatial tasks such as Morris water maze and radial arm maze (Kesslak et al. 1998; Mizuno et al. 2000); inhibitory avoidance (Alonso et al. 2002a; Ma et al. 1998); contextual fear conditioning (Hall et al. 2000); olfactory recognition (Broad et al. 2002); and conditioned taste aversion memory (Ma et al. 2011). In addition, the retrieval of spatial memories increases the level of BDNF mRNA in hippocampus following contextual fear conditioning and Morris water maze training (Hall et al. 2000; Kesslak et al. 1998). Moreover, significant increase of BDNF expression is observed to accompany a new form of learning, the extinction of previously acquired memories (e.g., conditioned fear) in the prefrontal cortex (Bredy et al. 2007) and amygdale (Chhatwal et al. 2006).

On the other hand, LTM is impaired by disrupting BDNF signaling. Morris water maze acquisition (Linnarsson et al. 1997) and contextual fear conditioning (Liu et al. 2004) are impaired in BDNF (+/−) mice. Intraventricular injection of anti-BDNF neutralizing antibody into rat brain prior to training also impaired LTM in the Morris water maze task (Mu et al. 1999). In addition, over-expression of truncated TrkB impaired long-term spatial memory (Saarelainen et al. 2000), while over-expression of TrkB resulted in improved learning and memory in the water maze, contextual fear conditioning, and conditioned taste aversion tests (Koponen et al. 2004). Surprisingly, over-expression of BDNF also resulted in modest learning deficits in spatial memory tasks, potentially due to precocious effects of BDNF on the development of multiple circuits, leading to abnormal wiring in the CNS (Cunha et al. 2009).

Region-specific genetic and pharmacological manipulations have helped delineate the role of BDNF signaling in specific brain regions. Inhibition of BDNF

mRNA expression via hippocampal infusion of BDNF antisense oligonucleotides or anti-BDNF antibody before training also blocks acquisition in inhibitory avoidance and radial arm maze tasks (Alonso et al. 2002a; Ma et al. 1998; Mizuno et al. 2000). Gorski et al. (2003) deleted BDNF gene from the forebrain using site-specific Cre recombinase and found that such mice failed to learn Morris water maze task. Prelimbic cortical-specific deletion of BDNF resulted in robust deficits in consolidation of cued fear (Choi et al. 2010). In addition, decreased BDNF mRNA expression in the hippocampus by targeted deletion of BDNF gene using lentiviral vector engineered to express Cre recombinase led to impairments in spatial learning in Morris water maze and the extinction of fear-potentiated startle (Heldt et al. 2007). A recent study using post-training CA1 intrahippocampal infusion of anti-BDNF antibody also revealed a critical role of BDNF in object recognition LTM retention (Furini et al. 2009). Moreover, deletion of TrkB gene in forebrain results in severe behavioral deficits in a spatial water maze task and moderate deficits in a radial arm maze task (Minichiello et al. 1999), while expression of a dominant-negative TrkB in amygdala specifically impaired consolidation of conditioned fear extinction (Chhatwal et al. 2006).

Unfortunately, due to the lack of temporally restricted and reversible manipulation of BDNF signaling, it is very difficult to discriminate the role of BDNF signaling in specific processes of LTM such as formation (acquisition or encoding), retention, retrieval, and extinction. However, using intra-hippocampal infusion of BDNF antibodies or antisense oligonucleotide, recent studies demonstrated the existence of two-time windows in LTM that requires BDNF: one at 1–4 h after encoding, which is critical for LTM lasting for 1–2 days (Alonso et al. 2002a, b) and the other at 12 h after memory formation that is essential for LTM 7 days later (Bekinschtein et al. 2007). It remains unclear whether the second wave of BDNF is induced by initial memory acquisition or it is the result of subsequent signaling cascades initiated post-acquisition.

2.3 BDNF-TrkB Signaling in Synaptic and Behavior Tagging

Like LTM, L-LTP requires gene transcription and de novo protein synthesis. Since gene expression occurs at the neuronal soma, how can the newly synthesized proteins (known as "plasticity-related proteins" or PRPs) specifically modify the stimulated or activated synapses but not the nearby, less active ones? The "synaptic tagging hypothesis," proposed by Frey and Morris (1997), states that local synaptic activity generates a tag, which "captures" the soma-derived PRPs. Several lines of evidence strongly suggest BDNF as a PRP.

First, BDNF mRNA levels are significantly increased 1–3 h after the induction of L-LTP in hippocampal CA1 neurons (Castren et al. 1993; Dragunow et al. 1993; Morimoto et al. 1998; Patterson et al. 1992). Such an increase is probably mediated by enhanced BDNF transcription through activity-dependent transcription. Second, application of mBDNF can rescue the impaired L-LTP in mice with reduced BDNF expression (Pang et al. 2004; Patterson et al. 2001). Third, in mice with elevated

levels of BDNF, a weak TBS, which can create a "synaptic tag" but not PRP, can induce L-LTP (Barco et al. 2005). Moreover, application of BDNF to wild-type mouse hippocampal slices also converts E-LTP induced by weak TBS to L-LTP. Finally, BDNF application completely rescued L-LTP blocked by protein synthesis inhibition.

In an insightful review, Tonegawa and colleagues proposed several criteria for molecules to function as a synaptic tags (Kelleher et al. 2004): (1) a tag can be generated by weak stimulation that induces only E-LTP, which is protein synthesis-independent; (2) the lifetime of a tag must be about 1–2 h; (3) the activation of a tag must not require protein synthesis; (4) a tag must be induced in an input-specific manner and should be spatially restricted; and (5) a tag must interact with (and therefore capture) PRP to facilitate L-LTP. If BDNF is a PRP, TrkB is an obvious candidate for a synaptic tag. Using combined biochemical, genetic, electrophysiological, and cell biological approaches, Lu and colleagues have recently demonstrated that TrkB satisfies four of the five criteria (Lu et al. 2011). For example, TrkB phosphorylation (and therefore activation) was induced in hippocampal slices by weak TBS that only induces E-LTP, and this TrkB activation is transient (about 1 h) and protein synthesis-independent. To demonstrate that TrkB activation is input-specific and spatially restricted, BDNF-conjugated beads were locally applied to cultured hippocampal neurons to mimic BDNF release at synapses upon local stimulation. Imaging studies demonstrated that TrkB activation is confined to stimulated synapses (Lu et al. 2011). A litmus test for TrkB to act as a synaptic tag is the two-pathway experiment in which induction of L-LTP by strong stimulation (12 sets of TBS) in one pathway converts E-LTP induced by weak stimulation (four sets of TBS) to L-LTP in a second, independent pathway. Taking advantages of the pharmacologically regulatable $TrkB^{F616A}$ transgenic mice (Chen et al. 2005), in which the ATP binding site of TrkB is genetically modified to be reversibly inhibited by the compound 1NMPP1, it was shown that application of 1NMPP1 at the time of stimulation with a weak stimulus in the second pathway diminished L-LTP in that pathway but had no effect on the first one. Since TrkB is the natural receptor for BDNF, there is no conceptual difficulty for TrkB to capture the potential PRP: BDNF (the fifth criterion).

Since L-LTP is considered as the cellular model for LTM, "synaptic tagging" may serve as a cellular mechanism underlying "behavioral tagging"—a conversion of short-term memory (STP) provided by weak training to LTM, if a PRP could be induced by strong training of completely different modality. Specifically, it was found in rats that are exposed to a strong stimulation such as a novel environment or a novel taste before or after a weak training could provide the PRPs necessary to convert STM to LTM (Ballarini et al. 2009; Moncada and Viola 2007). This behavioral paradigm was adapted to mice: weak inhibitory avoidance conditioning (IA) normally results in a STM detectable at 1 h but not 24 h after training. However, exposure to a novel environment at 1 h before the IA training results in LTM lasting for 24 h after training. Remarkably, inhibition of TrkB activation by 1NMPP1 in $TrkB^{F616A}$ mice prior to IA training blocked the conversion of STM to LTM by novelty (Lu et al. 2011). These findings demonstrate that BDNF/TrkB has

the strongest potential to serve as a PRP/tag for L-LTP and LTM both in vitro and in vivo, respectively.

2.4 Role of Untranslated Region (UTR) of BDNF mRNA

Various isoforms of BDNF mRNAs are detected in neuronal dendrites, and such dendritic localization of BDNF mRNAs has been shown to be regulated by neuronal activity (Chiaruttini et al. 2009; Tongiorgi et al. 1997). A remarkable feature of the BDNF transcripts is that they are processed at two alternative polyadenylation sites, giving rise to two pools of BDNF mRNAs that harbor either a short or a long 3′UTR of 0.35 kb and 2.85 kb in length, respectively (Liu et al. 2005, 2006). These two pools of BDNF mRNA isoforms encode the same BDNF protein. Recently, a study by An et al. (2008) showed that short 3′UTR BDNF mRNA is restricted to somata while the long 3′UTR BDNF mRNA can be localized to dendrites of cultured hippocampal neurons. A line of transgenic mice that express only the short 3′UTR but not the long 3′UTR BDNF mRNA ($BDNF^{klox/klox}$) was used to investigate the functional role of long 3′UTR in vivo. Truncation of the long 3′UTR disrupts dendritic localization of BDNF mRNA in the brain, leading to pruning and enlargement of dendritic spines, and selective impairment in LTP at apical dendrites but not in somata, of hippocampal neurons. In addition, lack of dendritic BDNF ($BDNF^{klox/klox}$) in layer 2/3 pyramidal neurons of the visual cortex also showed altered spine pruning, late phase spine maturation, and recovery of cortical responsiveness following monocular deprivation (Kaneko et al. 2012). These results reveal a critical role for local BDNF synthesis in the structural and functional plasticity in dendrites of hippocampal neurons. Furthermore, this study provides an example that mRNAs containing the same coding sequence but distinct 3′UTRs can have distinct physiological functions due to their selective subcellular localization and translation. Interestingly, dendritically localized BDNF mRNAs remain translationally silent and are made competent in response to neuronal activity (Lau et al. 2010). Pilocarpine, a muscarinic cholinergic receptor agonist known to exacerbate excitatory neuronal activity leading to seizures, specifically mobilized long 3′UTR BDNF transcripts into the polyribosomal fractions in neurites. Further investigations are required to reveal how the long 3′UTR silences BDNF mRNA translation, and how neuronal stimulation removes the silencing.

The above data suggest that activity-dependent regulation of BDNF expression could be achieved at the levels of trafficking and/or translation. These could be mediated by one or more *trans*-acting factors, including but not limited to RNA binding proteins and microRNAs that may be associated with short or long 3′UTR transcripts. Clues to the *cis*-elements in the BDNF transcripts and the *trans*-acting factors involved in this process are beginning to emerge. Chiaruttini et al. (2009) proposed G196A (rs6265) as a critical *cis* element in the 5′UTR for BDNF mRNA trafficking into dendrites. Evidence for additional *cis* elements in BDNF mRNA also came from genetic association studies of the human SNP C270T (rs56164415) in the 5′UTR in idiopathic temporal lobe epilepsy (Kanemoto et al. 2003).

The 5′UTRs encoded by human BDNF gene exons V and VIII are proposed to contain putative internal ribosome entry sequence (IRES), which may serve as alternate sites for ribosomal binding and translation. In addition to the *cis* elements, Chiaruttini et al. (2009) also proposed the role for the *trans*-acting complex translin/trax in transporting BDNF mRNA into the dendrites. However, recent studies using translin knockout mice showed that translin/trax complex-independent mechanisms may also be involved in dendritic trafficking of BDNF mRNA (Wu et al. 2011). Corroborating this idea, CArG box binding factor A or A2RE/RTS binding factor (CBF-A) was shown to be a *trans* factor (other than staufen-1, DDX3 translin) responsible for facilitating dendritic transport of different mRNAs including BDNF, Arc, CaMKIIα (Raju et al. 2011). Similarly, fragile X mental retardation protein (FMRP) has been suggested as a *trans* factor for dendritic BDNF mRNA transport (Louhivuori et al. 2010). More interestingly, the mutant protein huntingtin (htt), in which the change in the CAG repeat length is responsible for causing Huntington's disease, has also been shown to be associated with BDNF mRNA granules (Ma et al. 2010). Other non-proteinaceous *trans*-acting factors, like microRNAs 134, 381, and 495 that regulate BDNF mRNA translation, are beginning to emerge (Wu et al. 2010).

While these findings unveiled multiple mechanisms of BDNF regulation by the 3′UTRs, it is important to emphasize that BDNF transcripts also contain different 5′UTRs and may impart additional regulatory mechanisms. BDNF mRNA trafficking into different neuronal compartments, their local regulation of translation, and association with factors that play a causal role in different neurological diseases have opened up a new area in BDNF biology, which will be one of the key areas for research focus in near future.

3 BDNF Regulation of Long-Term Depression

3.1 proBDNF Effect on LTD

Compared with the vast literature supporting the role of mBDNF in LTP, relatively few studies have focused on BDNF regulation of other forms of plasticity such as long-term depression (LTD). A clue came from outside of the synaptic plasticity field. Hempstead and colleagues elegantly demonstrated that proNGF (also proBDNF) induced neuronal apoptosis through the pan-neurotrophin receptor, $p75^{NTR}$, along with the co-receptor, sortilin (Lee et al. 2001). This result suggested that proneurotrophins through a distinct receptor ($p75^{NTR}$) may elicit effects opposite to mature neurotrophins. However, although there was no obvious cellular phenotype, $p75^{NTR}$ homozygous (−/−) mice (Lee et al. 1992) did show impairments in several learning and memory tasks (in C57Bl/6 background) (Peterson et al. 1999; Wright et al. 2004). These results remain controversial, as a recent study demonstrated that spatial memory and hippocampal LTP are significantly enhanced in the $p75^{NTR}$-knockout mice (in 129/Sv background) (Barrett et al. 2009). These data strongly suggest that proBDNF-$p75^{NTR}$ interaction may

regulate synaptic function, rather than apoptosis, in adult mice. Given that a significant proportion of BDNF secreted in the brain is proBDNF (Mowla et al. 2001; Nagappan et al. 2009) and that cleavage of proBDNF facilitates L-LTP, it was hypothesized that uncleaved proBDNF might have an opposite role—regulation of long-term depression (LTD). Indeed, Korte and colleagues reported that LTD could not be induced in two lines (exon III and exon IV) of $p75^{NTR}$ transgenic mice (Rosch et al. 2005). A systematic analysis by Woo et al. (2005) showed that $p75^{NTR}$ (−/−) mice indeed exhibit selective impairment in the NMDA-dependent LTD (called NR-LTD), without affecting basal synaptic transmission or other forms of synaptic plasticity. LTD could be reliably induced either by application of a train of low frequency stimulation (LFS) or perfusion of NMDA to the hippocampal slices from wild-type juvenile mice but not the $p75^{NTR}$−/− mice of the same age. This effect is very specific since NMDA-dependent LTP and NMDA-independent LTD are completely normal in p75−/− mice (Woo et al. 2005).

More direct evidence for the role of proBDNF in LTD came from pharmacological studies (Woo et al. 2005). Uncleavable proBDNF facilitated NR-LTD, but not LTP, not only in young mice (3–4 weeks when LTD is normally measurable) but also in older mice (7–8 weeks old). Moreover, proBDNF promotes NR-LTD through $p75^{NTR}$, as deletion of the $p75^{NTR}$ gene or inhibition of $p75^{NTR}$ by functionally blocking $p75^{NTR}$ (REX) antibody completely inhibited the potentiating effect of proBDNF on NR-LTD. These results, together with the electron microscopic evidence that $p75^{NTR}$ is localized in the dendritic spines of CA1 pyramidal neurons, suggest that proBDNF is the endogenous ligand acting on postsynaptic $p75^{NTR}$ in the CA1 neurons to control NR-LTD. This conclusion was unexpected, since the traditional thinking was that $p75^{NTR}$ is only expressed at the cholinergic afferents projecting from the basal forebrain neurons into the hippocampus. Further experimentation revealed that NR2B, but not NR2A, is responsible for $p75^{NTR}$/NR-LTD. In hippocampal CA1 synapses from the $p75^{NTR}$ mutant mice, synaptic currents mediated by NR2B, but not those by NR2A, were selectively eliminated. Further, activation of $p75^{NTR}$ by proBDNF enhanced NR2B-mediated synaptic currents. A selective impairment in NR2B expression could therefore explain the specific failure of NR-LTD, but not LTP or NR-independent LTD, in $p75^{NTR}$−/− mice. Together, these findings revealed a novel role of proBDNF-$p75^{NTR}$ signaling in LTD in hippocampal slices and its potential mechanism of action (Woo et al. 2005).

In vivo studies in awake and behaving rats suggest a possible role for endogenous proBDNF in regulating memory. During recall, a fully consolidated memory can undergo either reconsolidation or be subject to extinction, depending on whether the memory is enforced or not. Extinction memory competes with consolidated memory to control behavior. Memories encoded in rats that are conditioned in two different contexts can be retrieved and manipulated without interference from each other. In one such experiment, Barnes et al. established an extinction protocol in rats that were fear conditioned by foot shock in two different contexts (Barnes and Thomas 2008). Interestingly, proBDNF levels in the

hippocampal CA1 region were found to increase by ~2.5-fold only during extinction but not in acquisition or recall. Moreover, when proBDNF levels increased by inhibiting the proBDNF processing enzymes tPA/plasmin using tPA-STOP (a small molecule inhibitor), the extinction of conditioned fear memory was potentiated. In parallel, tPA-STOP attenuated consolidation of memory during recall testing. Together these studies suggest that the extent of proBDNF cleavage may be precisely controlled by neuronal activity induced during memory recall: higher levels of proBDNF may promote extinction while suppressing consolidation. This study provides a mechanistic link from molecular events (proBDNF conversion by tPA/plasmin cascade) to circuits (LTD facilitated by proBDNF) and behavior (extinction memory).

3.2 Opposing Effects of proBDNF and Mature BDNF: Yin-Yang Hypothesis

The studies highlighted above not only established a bidirectional regulation of hippocampal plasticity by proBDNF and mBDNF but also helped formulate a "yin-yang hypothesis": the uncleaved proBDNF (pro-neurotrophins) leads to negative effects such as apoptosis and LTD through $p75^{NTR}$, while mBDNF (mature neurotrophins) elicit positive functions such as cell survival and LTP through TrkB. This hypothesis is based on several major findings that are now well validated. First, pro-neurotrophins are secreted, and they could serve as signaling molecules, rather than inactive precursors (Lee et al. 2001; Yang et al. 2009b). It is now clear that the pro and mature neurotrophins elicit distinct signal transduction pathways (Koshimizu et al. 2010; Koshimizu et al. 2009; Sun et al. 2012). Second, in contrast to mature neurotrophins which preferentially bind Trk receptors, pro-neurotrophins bind with high affinity to $p75^{NTR}$, which previously was considered a low affinity pan neurotrophin receptor (Nykjaer et al. 2004). Third, pro and mature neurotrophins often elicit opposite effects. Under this simple model, the binary actions of neurotrophins depend on *both* the forms of the neurotrophin (pro vs. mature) and the class of receptors activated ($p75^{NTR}$ vs. Trk's). In addition to cell survival and synaptic plasticity, recent studies have also shown that proBDNF elicits axonal retraction (Sun et al. 2012; Yang et al. 2009a), inhibits neuronal migration (Xu et al. 2011), and reduces dendritic growth and spines (Koshimizu et al. 2009), through $p75^{NTR}$. Finally, proNGF and proBDNF can be cleaved by extracellular proteases such as MMP7 and plasmin (Lee et al. 2001; Pang et al. 2004). An important concept emerged from the Yin-yang hypothesis is that cleavage of pro-neurotrophins (or not) by extracellular proteases becomes a critical control mechanism for bidirectional neurotrophin regulation. These results may have implications in neural development, synaptic plasticity, and even nervous system diseases. It remains to be established whether the yin and yang actions of neurotrophins are equally prevalent.

3.3 Role of p75 and LTD in Stress Coping and Anxiety

The unexpected discovery that proBDNF promotes NR-LTD in the juvenile hippocampus through $p75^{NTR}$ raised more questions. Since in adults $p75^{NTR}$ is primarily expressed in basal forebrain cholinergic neurons but rarely in other brain regions, what is the role of $p75^{NTR}$ in the adult brain? Given that NR-LTD is also restricted to the juvenile brain, one may also ask can LTD ever be induced in the adult, and if so, what is its physiological function? Further, what role does $p75^{NTR}$ play in pathological conditions such as during stress?

Martinowich and colleagues have performed a series of experiments to address these questions using the $p75^{NTR}$ (−/−) mice (Martinowich et al. 2011b). First, acute stress (placing the mice on a small elevated platform) could enhance NMDA-dependent LTD in hippocampus with weak low frequency stimulation (LFS), which by itself will not enhance LTD in adult wild-type mice. Remarkably, this "stress-enabled" NR-LTD was completely absent in the $p75^{NTR}$ (−/−) mice. The effect of $p75^{NTR}$ gene deletion on LTD is very specific: there was no change in LTP, basal synaptic transmission, or even LTD induced by a perfusion of NMDA or muscarinic receptor agonist carbachol in adult $p75^{NTR}$ (−/−) slices. These results identified a new form of LTD in the adult hippocampus that is dependent on NMDA receptor, $p75^{NTR}$, as well as cholinergic inputs to the hippocampus.

Second, upon stress, the $p75^{NTR}$ mutants exhibit a selective increase in anxiety-like, but not depressive-like, behaviors, as well as a decreased stress resiliency (Martinowich et al. 2011b). These mice mount a normal stress-induced glucocorticoid surge and hyperthermia (a transient increase in body temperature, which recovers upon removal of stress), but their ability to recover from this stress is impaired, suggesting their inability to cope with stressful conditions. The muscarinic receptor antagonist scopolamine also blocked stress-enabled LTD, leading to anxiety. In contrast, an increase in cholinergic transmission by the acetylcholinesterase inhibitor (−)-phenserine resulted in anxiolytic effects. Taken together, these results support a hypothetical pathway for stress coping (Martinowich et al. 2011b): $p75^{NTR}$ → cholinergic transmission → stress-enabled hippocampal LTD → control of stress-induced anxiety.

Finally, to test this hypothesis, a membrane permeable and brain-penetrating peptide, Tat-$GluA2_{3Y}$, was used to block GluR2 endocytosis. Remarkably, systemic administration of the peptide attenuated the recovery of wild-type animals from stress-induced hyperthermia and exacerbated anxiety-like behavior after exposure to an acute stressor. Thus, LTD is a coping mechanism for stress-induced anxiety, which is regulated by $p75^{NTR}$-mediated cholinergic transmission in the hippocampus. Piecing together these results suggest that acute stress leads to acetylcholine release, which can be modulated by $p75^{NTR}$ in the basal forebrain cholinergic afferents, and these cholinergic inputs facilitate hippocampal LTD, which in turn suppresses the development of anxiety-like behaviors in response to stress.

4 BDNF Regulation of GABAergic Network

4.1 Activity-Dependent Transcription and GABAergic Interneurons

While a majority of the studies have focused on BDNF regulation of excitatory synapses, evidence for the role of BDNF in GABAergic inhibitory synapses is in fact quite substantial (Holm et al. 2009; Huang et al. 2011; Olofsdotter et al. 2000). For example, pharmacological treatment of brain slices with mature BDNF, but not proBDNF, has been shown to decrease inhibitory synaptic transmission (Frerking et al. 1998; Holm et al. 2009; Tanaka et al. 1997). A series of recent studies have now pointed to a major role of activity-dependent BDNF transcription in the development and function of GABAergic synapses.

BDNF gene is transcribed through multiple discrete promoters (I–VIII); each drives a unique 5′exon (exons I–VIII) that is spliced on to the common 3′ coding exon (exon IX). Thus, a total of nine BDNF transcripts are synthesized in rodents (Aid et al. 2007; Timmusk et al. 1993) and ~17 transcripts in humans (Pruunsild et al. 2007). Why are there so many different BDNF mRNAs that code for exactly the same BDNF protein? Different transcripts are expressed in different brain regions, cell types, and even different subcellular loci. They are also expressed during different developmental stages and regulated by different environmental factors. An emerging concept is that some promoters control the basal levels of *bdnf* expression necessary for neuronal survival and differentiation, whereas others drive activity-dependent *bdnf* expression, which may be involved in experience-dependent circuit maturation and plasticity in vivo (Hong et al. 2008; Sakata et al. 2009). Two groups have used sophisticated mouse genetics to address the role of activity-dependent *bdnf* expression, which is mediated largely by promoter IV. In one study, Hong et al. generated a mouse line in which the CaRE3/CRE (CREm) in endogenous promoter IV was mutated. CREm mice exhibit reduced spontaneous inhibitory postsynaptic currents (sIPSCs) in cortical culture and fewer GABAergic synapses in the cortex (Hong et al. 2008). In another study, Sakata and colleagues disrupted the promoter IV-mediated *bdnf* gene expression completely by a GFP-STOP cassette after *bdnf* exon IV (the BDNF-KIV line) (Sakata et al. 2009). These mice exhibit fewer parvalbumin (PV)-expressing, fast-spiking GABAergic interneurons in the prefrontal cortex (PFC), reduced frequency and amplitude of sIPSCs in cortical culture, as well as an altered spike-time dependent synaptic potentiation (STDP) in PFC slices. Interestingly, the structure and function of cortical glutamatergic synapses appear to be normal in both lines. These studies demonstrate specific requirements for activity-dependent *bdnf* expression in the development of inhibitory circuits in cortex.

To determine how activity-driven *bdnf* gene expression shapes the GABAergic network in specific cortical circuits in vivo, Jiao et al. (2011) crossed the BDNF-KIV line with the GAD67–GFP mouse line, in which all GABAergic neurons are genetically labeled with GFP. Two interesting observations were made. First, BDNF immunoreactivity in the barrel cortex was found to be distributed in an

orderly barrel shape in the control, wild-type mice, but this barrel pattern of BDNF distribution was completely abolished in the BDNF-KIV. This implies that it is the activity-driven, not the constitutive, *bdnf* transcription that is responsible for the barrel-shaped BDNF distribution in somatosensory cortex. Whisker trimming markedly reduced BDNF expression in the barrel cortex of control mice, but not in BDNF-KIV, suggesting that whisker sensory activities drive activity-dependent BDNF expression at local barrel cortical circuits in an input-specific manner. Second, whisker trimming deprived sensory inputs to the barrel cortex, leading to fewer perisomatic GABAergic boutons on the pyramidal neurons, as well as barrel-specific attenuation of GABAergic transmission. All these occur only in wild-type mice, but not in BDNF-KIV. It is remarkable that a relatively mild manipulation on activity-dependent but not basal BDNF expression machinery could completely abolish whisker-trimming-induced plasticity of GABAergic circuit in the barrel cortex in vivo.

While the BDNF-KIV was initially generated with the intent to block the promoter IV driven *bdnf* transcription, detailed characterization indicates that the activities of promoters I and III, which also contribute to activity-dependent *bdnf* transcription, were also reduced in this line. Further analyses revealed that activity-driven increase in BDNF protein is completely blocked while baseline BDNF level has only a mild reduction in the BDNF-KIV brain (Jiao et al. 2011; Martinowich et al. 2011a; Sakata et al. 2009). Thus, the BDNF-KIV line should serve as a tool to study the function of activity-dependent BDNF expression, rather than that of promoter-IV. To begin addressing the functional role of activity-dependent BDNF expression in the adult, Martinowich et al. (2011a) found that in wild-type animals, sleep deprivation dramatically increased BDNF transcription (primarily promoter I) as well as cortistatin, a neuropeptide expressed in a subset of cortical GABAergic interneurons implicated in sleep homeostasis. Such increases were not observed in BDNF-KIV. Moreover, BDNF-KIV animals exhibited a substantial decrease in the amount of sleeping time, compared to WT animals. Thus, activity-dependent BDNF expression regulates sleep homeostasis possibly through cortistatin-expressing interneurons.

4.2 BDNF-TrkB Controls Network Oscillations Through Regulation of PV Interneurons

Compared with the vast knowledge of BDNF regulation at the cellular (synaptic transmission and plasticity) and behavioral (cognitive functions) levels, only few studies have been conducted to address the role of BDNF in neuronal networks. Neuronal rhythmic activity, particularly γ-oscillations, is thought to be important for neuronal assemblies underlying temporal encoding, binding of sensory features, and memory storage and retrieval (Freeman 1975; Fries 2005; Rodriguez et al. 1999; Singer and Gray 1995; Tallon-Baudry and Bertrand 1999). Several studies have demonstrated that the parvalbumin-expressing, fast-spiking GABAergic interneurons (PV interneuron) are essential for the γ-frequency

synchronization in cortical and hippocampal networks. PV interneuron is a major cell population in the forebrain that expresses the BDNF receptor, TrkB.

To explore the role of BDNF-TrkB signaling in network function in neuronal circuits, a line of mutant mice in which the *TrkB* gene is specifically deleted in PV interneurons (TrkB-PV$^{-/-}$) was generated (Zheng et al. 2011). These mice showed two interesting electrophysiological phenotypes: (1) The inputs and outputs of the PV interneurons, which are reflected by the amplitude of glutamatergic synaptic currents recorded in the PV interneurons and the frequency of GABAergic inputs to the pyramidal cells, respectively, were reduced in the TrkB-PV$^{-/-}$ mice. These results suggest that cortical BDNF-TrkB signaling is critical for the function of PV interneurons. (2) In parallel, the rhythmic network activity in the gamma-frequency range (30–80 Hz) recorded in the CA1 area was found to be dramatically reduced. Further characterization demonstrated that this was due to a reduction as well as desynchronization of action potentials generated in PV interneurons. Taken together, these results demonstrate for the first time a role for BDNF-TrkB signaling in network synchrony. This is another emerging area of BDNF biology that may have significant impact not only in the understanding of network oscillations during memory processes but may also help to understand abnormal or dysfunctional network activities under pathophysiological conditions such as neurological diseases and psychiatric disorders.

Conclusions

With important discoveries continually emerging one after another over the last 2 decades, BDNF regulation of synapses has been one of the most exciting areas in the neurotrophin field. BDNF elicits a wide range of effects: during development and in the adult, on excitatory and inhibitory synapses, regulating synaptic transmission or plasticity, structure or function, with either acute or long-term effects, etc. How does BDNF elicit such an array of pleiotrophic properties? One of the key discoveries was that proBDNF, acting through its preferred receptor p75^{NTR}/sortilin, elicits biologically different and often opposing effects to mBDNF. Thus, conversion of proBDNF to mBDNF through proteolytic cleavage has emerged as an important regulatory mechanism. Indeed, pharmacological and genetic studies have revealed that tPA/plasmin-mediated, extracellular conversion of proBDNF to mBDNF is necessary and sufficient for late-phase LTP. Moreover, proBDNF-p75^{NTR} signaling has been shown to facilitate LTD in young hippocampal slices in vitro and perhaps during stress in adults in vivo. Activity-dependent proBDNF→mBDNF conversion appears to play an important role in synaptic competition/elimination during development. These findings form the foundation for the "Yin-yang" hypothesis. Second major breakthrough is identification of the human val/met polymorphism that impacts selectively on activity-dependent but not constitutive BDNF secretion. This provides an unprecedented opportunity to study the function of BDNF in cognitive function and dysfunction in human. Third, the discovery that BDNF mRNA with short 3′UTR is located in neuronal soma whereas that with long 3′UTR is targeted to distal dendrites has unveiled yet another level of

complexity: compartmentalized regulation of BDNF expression in different parts of the same neurons. Indeed, initial investigations suggest that dendritically localized long 3′UTR BDNF mRNA is quiescent, and its translation is induced by local synaptic activity. Functional study of BDNF mRNA trafficking and its activity-dependent translation has been an emerging area of research likely to generate some new surprises. Fourth, neuronal activity has been shown to regulate BDNF-TrkB signaling through a wide range of mechanisms: insertion and endocytosis of TrkB receptor, translocation into lipid rafts, cAMP gating, and differential signaling kinetics. Fifth, BDNF gene is transcribed through nine different promoters in rodents, giving rise to nine mRNAs coding for the same BDNF protein. Differential regulation of BDNF promoters and its functional consequences represent an exciting area of research with profound implications in both basic neuroscience and various neurological and psychiatric disorders. Finally, BDNF also has been shown to play a significant role in brain network development and in synchronization of network activities resulting in different frequencies of oscillations. This is likely to be an intense area of investigation, especially because it will help bridge the gap between neurophysiological mechanisms to cognitive functions in the whole organism, as well as pave the way for understanding pathophysiological conditions in nervous system disorders.

References

Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T (2007) Mouse and rat BDNF gene structure and expression revisited. J Neurosci Res 85:525–535

Akaneya Y, Tsumoto T, Kinoshita S, Hatanaka H (1997) Brain-derived neurotrophic factor enhances long-term potentiation in rat visual cortex. J Neurosci 17:6707–6716

Alonso M, Vianna MR, Depino AM, Mello e Souza T, Pereira P, Szapiro G, Viola H, Pitossi F, Izquierdo I, Medina JH (2002a) BDNF-triggered events in the rat hippocampus are required for both short- and long-term memory formation. Hippocampus 12:551–560

Alonso M, Vianna MR, Izquierdo I, Medina JH (2002b) Signaling mechanisms mediating BDNF modulation of memory formation in vivo in the hippocampus. Cell Mol Neurobiol 22:663–674

An JJ, Gharami K, Liao GY, Woo NH, Lau AG, Vanevski F, Torre ER, Jones KR, Feng Y, Lu B, Xu B (2008) Distinct role of long 3′ UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell 134:175–187

Antal A, Nitsche MA, Kruse W, Kincses TZ, Hoffmann KP, Paulus W (2004) Direct current stimulation over V5 enhances visuomotor coordination by improving motion perception in humans. J Cogn Neurosci 16:521–527

Arimura N, Kimura T, Nakamuta S, Taya S, Funahashi Y, Hattori A, Shimada A, Menager C, Kawabata S, Fujii K, Iwamatsu A, Segal RA, Fukuda M, Kaibuchi K (2009) Anterograde transport of TrkB in axons is mediated by direct interaction with Slp1 and Rab27. Dev Cell 16:675–686

Assaife-Lopes N, Sousa VC, Pereira DB, Ribeiro JA, Chao MV, Sebastiao AM (2010) Activation of adenosine A2A receptors induces TrkB translocation and increases BDNF- mediated phospho-TrkB localization in lipid rafts: implications for neuromodulation. J Neurosci 30:8468–8480

Ballarini F, Moncada D, Martinez MC, Alen N, Viola H (2009) Behavioral tagging is a general mechanism of long-term memory formation. Proc Natl Acad Sci U S A 106:14599–14604

Barco A, Patterson S, Alarcon JM, Gromova P, Mata-Roig M, Morozov A, Kandel ER (2005) Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. Neuron 48:123–137

Barnes P, Thomas KL (2008) Proteolysis of proBDNF is a key regulator in the formation of memory. PLoS One 3:e3248

Barrett GL, Reid CA, Tsafoulis C, Zhu W, Williams DA, Paolini AG, Trieu J, Murphy M (2009) Enhanced spatial memory and hippocampal long-term potentiation in p75 neurotrophin receptor knockout mice. Hippocampus 20:145–152

Bekinschtein P, Cammarota M, Igaz LM, Bevilaqua LR, Izquierdo I, Medina JH (2007) Persistence of long-term memory storage requires a late protein synthesis- and BDNF-dependent phase in the hippocampus. Neuron 53:261–277

Bredy TW, Wu H, Crego C, Zellhoefer J, Sun YE, Barad M (2007) Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. Learn Mem 14:268–276

Broad KD, Mimmack ML, Keverne EB, Kendrick KM (2002) Increased BDNF and trk-B mRNA expression in cortical and limbic regions following formation of a social recognition memory. Eur J Neurosci 16:2166–2174

Castren E, Pitkanen M, Sirvio J, Parsadanian A, Lindholm D, Thoenen H, Riekkinen PJ (1993) The induction of LTP increases BDNF and NGF mRNA but decreases NT-3 mRNA in the dentate gyrus. Neuroreport 4:895–898

Chen G, Kolbeck R, Barde YA, Bonhoeffer T, Kossel A (1999) Relative contribution of endogenous neurotrophins in hippocampal long-term potentiation. J Neurosci 19:7983–7990

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J Neurosci 24:4401–4411

Chen X, Ye H, Kuruvilla R, Ramanan N, Scangos KW, Zhang C, Johnson NM, England PM, Shokat KM, Ginty DD (2005) A chemical-genetic approach to studying neurotrophin signaling. Neuron 46:13–21

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314:140–143

Chhatwal JP, Stanek-Rattiner L, Davis M, Ressler KJ (2006) Amygdala BDNF signaling is required for consolidation but not encoding of extinction. Nat Neurosci 9:870–872

Chiaruttini C, Vicario A, Li Z, Baj G, Braiuca P, Wu Y, Lee FS, Gardossi L, Baraban JM, Tongiorgi E (2009) Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. Proc Natl Acad Sci U S A 106:16481–16486

Choi DC, Maguschak KA, Ye K, Jang SW, Myers KM, Ressler KJ (2010) Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. Proc Natl Acad Sci U S A 107:2675–2680

Cunha C, Angelucci A, D'Antoni A, Dobrossy MD, Dunnett SB, Berardi N, Brambilla R (2009) Brain-derived neurotrophic factor (BDNF) overexpression in the forebrain results in learning and memory impairments. Neurobiol Dis 33:358–368

Dragunow M, Beilharz E, Mason B, Lawlor P, Abraham W, Gluckman P (1993) Brain-derived neurotrophic factor expression after long-term potentiation. Neurosci Lett 160:232–236

Du J, Feng L, Yang F, Lu B (2000) Activity- and Ca(2+)-dependent modulation of surface expression of brain-derived neurotrophic factor receptors in hippocampal neurons. J Cell Biol 150:1423–1434

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112:257–269

Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B (1996) Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature 381:706–709

Frank L, Ventimiglia R, Anderson K, Lindsay RM, Rudge JS (1996) BDNF down-regulates neurotrophin responsiveness, TrkB protein and TrkB mRNA levels in cultured rat hippocampal neurons. Eur J Neurosci 8:1220–1230

Freeman WJ (1975) Mass action in the nervous system: examination of the neurophysiological basis of adaptive behavior through the EEG. Academic, New York

Fregni F, Boggio PS, Nitsche M, Bermpohl F, Antal A, Feredoes E, Marcolin MA, Rigonatti SP, Silva MT, Paulus W, Pascual-Leone A (2005) Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. Exp Brain Res 166:23–30

Frerking M, Malenka RC, Nicoll RA (1998) Brain-derived neurotrophic factor (BDNF) modulates inhibitory, but not excitatory, transmission in the CA1 region of the hippocampus. J Neurophysiol 80:3383–3386

Frey U, Morris RG (1997) Synaptic tagging and long-term potentiation. Nature 385:533–536

Frey U, Muller M, Kuhl D (1996) A different form of long-lasting potentiation revealed in tissue plasminogen activator mutant mice. J Neurosci 16:2057–2063

Fries P (2005) A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. Trends Cogn Sci 9:474–480

Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, Lu B (2010) Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron 66:198–204

Furini CR, Rossato JI, Bitencourt LL, Medina JH, Izquierdo I, Cammarota M (2009) Beta-adrenergic receptors link NO/sGC/PKG signaling to BDNF expression during the consolidation of object recognition long-term memory. Hippocampus 20:672–683

Gandiga PC, Hummel FC, Cohen LG (2006) Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clin Neurophysiol 117:845–850

Gorski JA, Balogh SA, Wehner JM, Jones KR (2003) Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice. Neuroscience 121:341–354

Govindarajan A, Israely I, Huang SY, Tonegawa S (2011) The dendritic branch is the preferred integrative unit for protein synthesis-dependent LTP. Neuron 69:132–146

Haapasalo A, Sipola I, Larsson K, Akerman KE, Stoilov P, Stamm S, Wong G, Castren E (2002) Regulation of TRKB surface expression by brain-derived neurotrophic factor and truncated TRKB isoforms. J Biol Chem 277:43160–43167

Hall J, Thomas KL, Everitt BJ (2000) Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. Nat Neurosci 3:533–535

Heerssen HM, Segal RA (2002) Location, location, location: a spatial view of neurotrophin signal transduction. Trends Neurosci 25:160–165

Heldt SA, Stanek L, Chhatwal JP, Ressler KJ (2007) Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. Mol Psychiatry 12:656–670

Holm MM, Nieto-Gonzalez JL, Vardya I, Vaegter CB, Nykjaer A, Jensen K (2009) Mature BDNF, but not proBDNF, reduces excitability of fast-spiking interneurons in mouse dentate gyrus. J Neurosci 29:12412–12418

Hong EJ, McCord AE, Greenberg ME (2008) A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. Neuron 60:610–624

Huang EJ, Reichardt LF (2003) Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 72:609–642

Huang YY, Bach ME, Lipp HP, Zhuo M, Wolfer DP, Hawkins RD, Schoonjans L, Kandel ER, Godfraind JM, Mulligan R, Collen D, Carmeliet P (1996) Mice lacking the gene encoding tissue-type plasminogen activator show a selective interference with late-phase long-term

potentiation in both Schaffer collateral and mossy fiber pathways. Proc Natl Acad Sci U S A 93:8699–8704
Huang Y, Ko H, Cheung ZH, Yung KK, Yao T, Wang JJ, Morozov A, Ke Y, Ip NY, Yung WH (2011) Dual actions of brain-derived neurotrophic factor on GABAergic transmission in cerebellar Purkinje neurons. Exp Neurol 233:791–798
Huber KM, Sawtell NB, Bear MF (1998) Brain-derived neurotrophic factor alters the synaptic modification threshold in visual cortex. Neuropharmacology 37:571–579
Hwang JJ, Park MH, Choi SY, Koh JY (2005) Activation of the Trk signaling pathway by extracellular zinc. Role of metalloproteinases. J Biol Chem 280:11995–12001
Iyer MB, Mattu U, Grafman J, Lomarev M, Sato S, Wassermann EM (2005) Safety and cognitive effect of frontal DC brain polarization in healthy individuals. Neurology 64:872–875
Ji Y, Pang PT, Feng L, Lu B (2005) Cyclic AMP controls BDNF-induced TrkB phosphorylation and dendritic spine formation in mature hippocampal neurons. Nat Neurosci 8:164–172
Ji Y, Lu Y, Yang F, Shen W, Tang TT, Feng L, Duan S, Lu B (2010) Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. Nat Neurosci 13:302–309
Jiang B, Akaneya Y, Ohshima M, Ichisaka S, Hata Y, Tsumoto T (2001) Brain-derived neurotrophic factor induces long-lasting potentiation of synaptic transmission in visual cortex in vivo in young rats, but not in the adult. Eur J Neurosci 14:1219–1228
Jiao Y, Zhang Z, Zhang C, Wang X, Sakata K, Lu B, Sun QQ (2011) A key mechanism underlying sensory experience-dependent maturation of neocortical GABAergic circuits in vivo. Proc Natl Acad Sci U S A 108:12131–12136
Kaneko M, Xie Y, An JJ, Stryker MP, Xu B (2012) Dendritic BDNF synthesis is required for late-phase spine maturation and recovery of cortical responses following sensory deprivation. J Neurosci 32:4790–4802
Kanemoto K, Kawasaki J, Tarao Y, Kumaki T, Oshima T, Kaji R, Nishimura M (2003) Association of partial epilepsy with brain-derived neurotrophic factor (BDNF) gene polymorphisms. Epilepsy Res 53:255–258
Kang H, Schuman EM (1995) Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science 267:1658–1662
Kang H, Welcher AA, Shelton D, Schuman EM (1997) Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. Neuron 19:653–664
Keifer J, Sabirzhanov BE, Zheng Z, Li W, Clark TG (2009) Cleavage of proBDNF to BDNF by a tolloid-like metalloproteinase is required for acquisition of in vitro eyeblink classical conditioning. J Neurosci 29:14956–14964
Kelleher RJ 3rd, Govindarajan A, Tonegawa S (2004) Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron 44:59–73
Kesslak JP, So V, Choi J, Cotman CW, Gomez-Pinilla F (1998) Learning upregulates brain-derived neurotrophic factor messenger ribonucleic acid: a mechanism to facilitate encoding and circuit maintenance? Behav Neurosci 112:1012–1019
Klann E, Sweatt JD (2008) Altered protein synthesis is a trigger for long-term memory formation. Neurobiol Learn Mem 89:247–259
Kleim JA, Chan S, Pringle E, Schallert K, Procaccio V, Jimenez R, Cramer SC (2006) BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. Nat Neurosci 9:735–737
Klintsova AY, Dickson E, Yoshida R, Greenough WT (2004) Altered expression of BDNF and its high-affinity receptor TrkB in response to complex motor learning and moderate exercise. Brain Res 1028:92–104
Koponen E, Voikar V, Riekki R, Saarelainen T, Rauramaa T, Rauvala H, Taira T, Castren E (2004) Transgenic mice overexpressing the full-length neurotrophin receptor trkB exhibit increased activation of the trkB-PLCgamma pathway, reduced anxiety, and facilitated learning. Mol Cell Neurosci 26:166–181

Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T (1995) Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc Natl Acad Sci U S A 92:8856–8860

Koshimizu H, Kiyosue K, Hara T, Hazama S, Suzuki S, Uegaki K, Nagappan G, Zaitsev E, Hirokawa T, Tatsu Y, Ogura A, Lu B, Kojima M (2009) Multiple functions of precursor BDNF to CNS neurons: negative regulation of neurite growth, spine formation and cell survival. Mol Brain 2:27

Koshimizu H, Hazama S, Hara T, Ogura A, Kojima M (2010) Distinct signaling pathways of precursor BDNF and mature BDNF in cultured cerebellar granule neurons. Neurosci Lett 473:229–232

Lau AG, Irier HA, Gu J, Tian D, Ku L, Liu G, Xia M, Fritsch B, Zheng JQ, Dingledine R, Xu B, Lu B, Feng Y (2010) Distinct 3′UTRs differentially regulate activity-dependent translation of brain-derived neurotrophic factor (BDNF). Proc Natl Acad Sci U S A 107:15945–15950

Lee KF, Li E, Huber LJ, Landis SC, Sharpe AH, Chao MV, Jaenisch R (1992) Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. Cell 69:737–749

Lee R, Kermani P, Teng KK, Hempstead BL (2001) Regulation of cell survival by secreted proneurotrophins. Science 294:1945–1948

Linnarsson S, Bjorklund A, Ernfors P (1997) Learning deficit in BDNF mutant mice. Eur J Neurosci 9:2581–2587

Liu IY, Lyons WE, Mamounas LA, Thompson RF (2004) Brain-derived neurotrophic factor plays a critical role in contextual fear conditioning. J Neurosci 24:7958–7963

Liu QR, Walther D, Drgon T, Polesskaya O, Lesnick TG, Strain KJ, de Andrade M, Bower JH, Maraganore DM, Uhl GR (2005) Human brain derived neurotrophic factor (BDNF) genes, splicing patterns, and assessments of associations with substance abuse and Parkinson's disease. Am J Med Genet B Neuropsychiatr Genet 134B:93–103

Liu QR, Lu L, Zhu XG, Gong JP, Shaham Y, Uhl GR (2006) Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. Brain Res 1067:1–12

Louhivuori V, Vicario A, Uutela M, Rantamaki T, Louhivuori LM, Castren E, Tongiorgi E, Akerman KE, Castren ML (2010) BDNF and TrkB in neuronal differentiation of Fmr1-knockout mouse. Neurobiol Dis 41:469–480

Lu Y, Ji Y, Ganesan S, Schloesser R, Martinowich K, Sun M, Mei F, Chao MV, Lu B (2011) TrkB as a potential synaptic and behavioral tag. J Neurosci 31:11762–11771

Ma YL, Wang HL, Wu HC, Wei CL, Lee EH (1998) Brain-derived neurotrophic factor antisense oligonucleotide impairs memory retention and inhibits long-term potentiation in rats. Neuroscience 82:957–967

Ma B, Culver BP, Baj G, Tongiorgi E, Chao MV, Tanese N (2010) Localization of BDNF mRNA with the Huntington's disease protein in rat brain. Mol Neurodegener 5:22

Ma L, Wang DD, Zhang TY, Yu H, Wang Y, Huang SH, Lee FS, Chen ZY (2011) Region- specific involvement of BDNF secretion and synthesis in conditioned taste aversion memory formation. J Neurosci 31:2079–2090

Martinowich K, Schloesser RJ, Jimenez DV, Weinberger DR, Lu B (2011a) Activity-dependent brain-derived neurotrophic factor expression regulates cortistatin-interneurons and sleep behavior. Mol Brain 4:11

Martinowich K, Schloesser RJ, Lu Y, Jimenez DV, Paredes D, Greene JS, Greig NH, Manji HK, Lu B (2011b) Roles of p75(NTR), long-term depression, and cholinergic transmission in anxiety and acute stress coping. Biol Psychiatry 71:75–83

Matsumoto T, Rauskolb S, Polack M, Klose J, Kolbeck R, Korte M, Barde YA (2008) Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat Neurosci 11:131–133

Meyer-Franke A, Wilkinson GA, Kruttgen A, Hu M, Munro E, Hanson MG Jr, Reichardt LF, Barres BA (1998) Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. Neuron 21:681–693

Minichiello L, Korte M, Wolfer D, Kuhn R, Unsicker K, Cestari V, Rossi-Arnaud C, Lipp HP, Bonhoeffer T, Klein R (1999) Essential role for TrkB receptors in hippocampus-mediated learning. Neuron 24:401–414

Mizuno M, Yamada K, Olariu A, Nawa H, Nabeshima T (2000) Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. J Neurosci 20:7116–7121

Moncada D, Viola H (2007) Induction of long-term memory by exposure to novelty requires protein synthesis: evidence for a behavioral tagging. J Neurosci 27:7476–7481

Morimoto K, Sato K, Sato S, Yamada N, Hayabara T (1998) Time-dependent changes in neurotrophic factor mRNA expression after kindling and long-term potentiation in rats. Brain Res Bull 45:599–605

Mowla SJ, Farhadi HF, Pareek S, Atwal JK, Morris SJ, Seidah NG, Murphy RA (2001) Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. J Biol Chem 276:12660–12666

Mu JS, Li WP, Yao ZB, Zhou XF (1999) Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. Brain Res 835:259–265

Nagappan G, Zaitsev E, Senatorov VV Jr, Yang J, Hempstead BL, Lu B (2009) Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. Proc Natl Acad Sci U S A 106:1267–1272

Ninan I, Bath KG, Dagar K, Perez-Castro R, Plummer MR, Lee FS, Chao MV (2010) The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the hippocampus. J Neurosci 30:8866–8870

Nitsche MA, Liebetanz D, Antal A, Lang N, Tergau F, Paulus W (2003) Modulation of cortical excitability by weak direct current stimulation – technical, safety and functional aspects. Suppl Clin Neurophysiol 56:255–276

Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, Jacobsen C, Kliemannel M, Schwarz E, Willnow TE, Hempstead BL, Petersen CM (2004) Sortilin is essential for proNGF-induced neuronal cell death. Nature 427:843–848

Olofsdotter K, Lindvall O, Asztely F (2000) Increased synaptic inhibition in dentate gyrus of mice with reduced levels of endogenous brain-derived neurotrophic factor. Neuroscience 101:531–539

Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung WH, Hempstead BL, Lu B (2004) Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 306:487–491

Patterson SL, Grover LM, Schwartzkroin PA, Bothwell M (1992) Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. Neuron 9:1081–1088

Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER (1996) Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron 16:1137–1145

Patterson SL, Pittenger C, Morozov A, Martin KC, Scanlin H, Drake C, Kandel ER (2001) Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. Neuron 32:123–140

Peterson DA, Dickinson-Anson HA, Leppert JT, Lee KF, Gage FH (1999) Central neuronal loss and behavioral impairment in mice lacking neurotrophin receptor p75. J Comp Neurol 404:1–20

Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, Egan MF, Meyer-Lindenberg A, Weinberger DR (2004) The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci 24:10099–10102

Pruunsild P, Kazantseva A, Aid T, Palm K, Timmusk T (2007) Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters. Genomics 90:397–406

Raju CS, Fukuda N, Lopez-Iglesias C, Goritz C, Visa N, Percipalle P (2011) In neurons, activity-dependent association of dendritically transported mRNA transcripts with the transacting factor CBF-A is mediated by A2RE/RTS elements. Mol Biol Cell 22:1864–1877

Reis J, Schambra HM, Cohen LG, Buch ER, Fritsch B, Zarahn E, Celnik PA, Krakauer JW (2009) Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. Proc Natl Acad Sci U S A 106:1590–1595

Righi M, Tongiorgi E, Cattaneo A (2000) Brain-derived neurotrophic factor (BDNF) induces dendritic targeting of BDNF and tyrosine kinase B mRNAs in hippocampal neurons through a phosphatidylinositol-3 kinase-dependent pathway. J Neurosci 20:3165–3174

Rodriguez E, George N, Lachaux JP, Martinerie J, Renault B, Varela FJ (1999) Perception's shadow: long-distance synchronization of human brain activity. Nature 397:430–433

Rosch H, Schweigreiter R, Bonhoeffer T, Barde YA, Korte M (2005) The neurotrophin receptor p75NTR modulates long-term depression and regulates the expression of AMPA receptor subunits in the hippocampus. Proc Natl Acad Sci U S A 102:7362–7367

Saarelainen T, Pussinen R, Koponen E, Alhonen L, Wong G, Sirvio J, Castren E (2000) Transgenic mice overexpressing truncated trkB neurotrophin receptors in neurons have impaired long-term spatial memory but normal hippocampal LTP. Synapse 38:102–104

Sakata K, Woo NH, Martinowich K, Greene JS, Schloesser RJ, Shen L, Lu B (2009) Critical role of promoter IV-driven BDNF transcription in GABAergic transmission and synaptic plasticity in the prefrontal cortex. Proc Natl Acad Sci U S A 106:5942–5947

Simonato M, Bregola G, Armellin M, Del Piccolo P, Rodi D, Zucchini S, Tongiorgi E (2002) Dendritic targeting of mRNAs for plasticity genes in experimental models of temporal lobe epilepsy. Epilepsia 43(Suppl 5):153–158

Singer W, Gray CM (1995) Visual feature integration and the temporal correlation hypothesis. Annu Rev Neurosci 18:555–586

Sommerfeld MT, Schweigreiter R, Barde YA, Hoppe E (2000) Down-regulation of the neurotrophin receptor TrkB following ligand binding. Evidence for an involvement of the proteasome and differential regulation of TrkA and TrkB. J Biol Chem 275:8982–8990

Sun Y, Lim Y, Li F, Liu S, Lu JJ, Haberberger R, Zhong JH, Zhou XF (2012) ProBDNF collapses neurite outgrowth of primary neurons by activating RhoA. PLoS One 7:e35883

Suzuki S, Numakawa T, Shimazu K, Koshimizu H, Hara T, Hatanaka H, Mei L, Lu B, Kojima M (2004) BDNF-induced recruitment of TrkB receptor into neuronal lipid rafts: roles in synaptic modulation. J Cell Biol 167:1205–1215

Szeszko PR, Lipsky R, Mentschel C, Robinson D, Gunduz-Bruce H, Sevy S, Ashtari M, Napolitano B, Bilder RM, Kane JM, Goldman D, Malhotra AK (2005) Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. Mol Psychiatry 10:631–636

Tallon-Baudry C, Bertrand O (1999) Oscillatory gamma activity in humans and its role in object representation. Trends Cogn Sci 3:151–162

Tanaka T, Saito H, Matsuki N (1997) Inhibition of GABAA synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. J Neurosci 17:2959–2966

Timmusk T, Palm K, Metsis M, Reintam T, Paalme V, Saarma M, Persson H (1993) Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron 10:475–489

Tongiorgi E, Baj G (2008) Functions and mechanisms of BDNF mRNA trafficking. Novartis Found Symp 289:136–147, discussion 147–151, 193–195

Tongiorgi E, Righi M, Cattaneo A (1997) Activity-dependent dendritic targeting of BDNF and TrkB mRNAs in hippocampal neurons. J Neurosci 17:9492–9505

Watson FL, Heerssen HM, Moheban DB, Lin MZ, Sauvageot CM, Bhattacharyya A, Pomeroy SL, Segal RA (1999) Rapid nuclear responses to target-derived neurotrophins require retrograde transport of ligand-receptor complex. J Neurosci 19:7889–7900

Watson FL, Heerssen HM, Bhattacharyya A, Klesse L, Lin MZ, Segal RA (2001) Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. Nat Neurosci 4:981–988

Webster BR, Celnik PA, Cohen LG (2006) Noninvasive brain stimulation in stroke rehabilitation. NeuroRx 3:474–481

Woo NH, Teng HK, Siao CJ, Chiaruttini C, Pang PT, Milner TA, Hempstead BL, Lu B (2005) Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat Neurosci 8:1069–1077

Wright JW, Alt JA, Turner GD, Krueger JM (2004) Differences in spatial learning comparing transgenic p75 knockout, New Zealand Black, C57BL/6, and Swiss Webster mice. Behav Brain Res 153:453–458

Wu C, Butz S, Ying Y, Anderson RG (1997) Tyrosine kinase receptors concentrated in caveolae-like domains from neuronal plasma membrane. J Biol Chem 272:3554–3559

Wu H, Tao J, Chen PJ, Shahab A, Ge W, Hart RP, Ruan X, Ruan Y, Sun YE (2010) Genome- wide analysis reveals methyl-CpG-binding protein 2-dependent regulation of microRNAs in a mouse model of Rett syndrome. Proc Natl Acad Sci U S A 107:18161–18166

Wu YC, Williamson R, Li Z, Vicario A, Xu J, Kasai M, Chern Y, Tongiorgi E, Baraban JM (2011) Dendritic trafficking of brain-derived neurotrophic factor mRNA: regulation by translin-dependent and -independent mechanisms. J Neurochem 116:1112–1121

Xu ZQ, Sun Y, Li HY, Lim Y, Zhong JH, Zhou XF (2011) Endogenous proBDNF is a negative regulator of migration of cerebellar granule cells in neonatal mice. Eur J Neurosci 33:1376–1384

Yang F, Je HS, Ji Y, Nagappan G, Hempstead B, Lu B (2009a) Pro-BDNF-induced synaptic depression and retraction at developing neuromuscular synapses. J Cell Biol 185:727–741

Yang J, Siao CJ, Nagappan G, Marinic T, Jing D, McGrath K, Chen ZY, Mark W, Tessarollo L, Lee FS, Lu B, Hempstead BL (2009b) Neuronal release of proBDNF. Nat Neurosci 12:113–115

York RD, Molliver DC, Grewal SS, Stenberg PE, McCleskey EW, Stork PJ (2000) Role of phosphoinositide 3-kinase and endocytosis in nerve growth factor-induced extracellular signal-regulated kinase activation via Ras and Rap1. Mol Cell Biol 20:8069–8083

Zhang Y, Moheban DB, Conway BR, Bhattacharyya A, Segal RA (2000) Cell surface Trk receptors mediate NGF-induced survival while internalized receptors regulate NGF-induced differentiation. J Neurosci 20:5671–5678

Zheng K, An JJ, Yang F, Xu W, Xu ZQ, Wu J, Hokfelt TG, Fisahn A, Xu B, Lu B (2011) TrkB signaling in parvalbumin-positive interneurons is critical for gamma-band network synchronization in hippocampus. Proc Natl Acad Sci U S A 108:17201–17206

Zhou P, Porcionatto M, Pilapil M, Chen Y, Choi Y, Tolias KF, Bikoff JB, Hong EJ, Greenberg ME, Segal RA (2007) Polarized signaling endosomes coordinate BDNF-induced chemotaxis of cerebellar precursors. Neuron 55:53–68

Zweifel LS, Kuruvilla R, Ginty DD (2005) Functions and mechanisms of retrograde neurotrophin signalling. Nat Rev Neurosci 6:615–625

Nerve Growth Factor and Nociception: From Experimental Embryology to New Analgesic Therapy

Gary R. Lewin, Stefan G. Lechner, and Ewan St. John Smith

Abstract

Nerve growth factor (NGF) is central to the development and functional regulation of sensory neurons that signal the first events that lead to pain. These sensory neurons, called nociceptors, require NGF in the early embryo to survive and also for their functional maturation. The long road from the discovery of NGF and its roles during development to the realization that NGF plays a major role in the pathophysiology of inflammatory pain will be reviewed. In particular, we will discuss the various signaling events initiated by NGF that lead to long-lasting thermal and mechanical hyperalgesia in animals and in man. It has been realized relatively recently that humanized function blocking antibodies directed against NGF show remarkably analgesic potency in human clinical trials for painful conditions as varied as osteoarthritis, lower back pain, and interstitial cystitis. Thus, anti-NGF medication has the potential to make a major impact on day-to-day chronic pain treatment in the near future. It is therefore all the more important to understand the precise pathways and mechanisms that are controlled by NGF to both initiate and sustain mechanical and thermal hyperalgesia. Recent work suggests that NGF-dependent regulation of the mechanosensory properties of sensory neurons that signal mechanical pain may open new

G.R. Lewin (✉)
Department of Neuroscience, Molecular Physiology of Somatic Sensation, Max Delbrück Center for Molecular Medicine, Robert-Rössle Str. 10, 13122 Berlin, Germany
e-mail: glewin@mdc-berlin.de

S.G. Lechner
Institute of Pharmacology, Heidelberg University, Im Neuenheimer Feld 366, 69120 Heidelberg, Germany

E.S.J. Smith
Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1PD, UK

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_10,

mechanistic avenues to refine and exploit relevant molecular targets for novel analgesics.

Keywords
NGF • Hyperalgesia • Pain • Inflammation • Mechanotransduction • STOML3 • Sensitization • TRP channel

1 Introduction

Nerve growth factor (NGF) is the founding member of the neurotrophin family. In the last 20 years the link between the biology of NGF and pain has been well established (Heppenstall and Lewin 2000; Pezet and McMahon 2006; Mantyh et al. 2011). At present, there are at least five major pharmaceutical companies running clinical trials of humanized antibodies designed to sequester NGF for the treatment of pain in conditions as varied as osteoarthritis, lower back pain, and interstitial cystitis (Cattaneo 2010; Lane et al. 2010; Evans et al. 2011; Brown et al. 2012, 2013). The first example of an NGF sequestering drug is Tanezumab a humanized monoclonal antibody that potently binds NGF developed by Rinat/Pfizer (Lane et al. 2010). Although the eventual success of an NGF-based drug for pain therapy is far from certain at the present time, the key role played by NGF signaling in pain is not in doubt. In this review we will provide an overview of how the study of NGF graduated from the province of embryologists to be the one of the most exciting drug targets for chronic pain in recent years. Since an NGF signaling axis is undoubtedly important in the etiology of pain, it is important to understand how NGF functions in the context of nociception and above all in the context of inflammatory hyperalgesia. Here we will primarily review the mechanistic basis of how NGF functions in nociception and chronic pain. The further understanding of NGF biology will be extremely important for understanding how best to manipulate NGF signaling to effectively treat chronic pain.

2 Experimental Embryology Leads the Way

In a classic series of experiments performed by Rita Levi-Montalcini and her collaborator Victor Hamburger the activity that was to be identified as NGF was studied using chicken embryos (Hamburger 1993). They described a process, now termed programmed cell death, whereby an overabundance of neurons generated during development, is reduced in number by apoptosis during critical periods. The fact that many, but not all, neurons die during such critical periods raised the question of what are the factors that keep the remaining neurons alive. From these types of experiments came the key insight that led to the eventual identification of NGF. Experiments using limb ablation, or the grafting of supernumerary limbs in embryos during critical stages of development, showed that the number of

surviving motor neurons, sympathetic ganglion neurons, or sensory neurons was dependent on the size of the peripheral target. Hamburger and Levi-Montalcini postulated that some target-derived survival factor synthesized in limiting amounts was responsible for preventing many of the neurons from undergoing programmed cell death. Bueker made the serendipitous discovery that injection of a mouse sarcoma tumor cell line into chick embryos could mimic the survival promoting effects of increased target size (Bueker and Hilderman 1953; Cohen 2008). These experiments eventually led to the identification of a source of this as yet unknown growth factor, namely, the mouse submaxillary gland. Stanley Cohen used this biochemical source to purify NGF and was able to use this purified protein material to generate rabbit polyclonal antibodies which bind to NGF (Cohen 1960; Levi-Montalcini and Booker 1960). This enabled Levi-Montalcini to carry out the first function blocking experiments, which addressed the endogenous function of NGF in the mouse. Thus, the injection of NGF binding antibodies into newborn mice led to a dramatic loss of sympathetic neurons showing that these neurons require NGF for their survival. The antibody approach taken by Levi-Montalcini was based on the idea that sequestration of endogenous NGF by high-affinity antibodies will prevent NGF binding to its endogenous receptors to prevent cell death or promote nerve fiber growth. It is worth noting that therapeutic interventions for the treatment of pain now being pursued 50 years later are based on this very same idea.

The availability of antibody tools to manipulate the endogenous levels of NGF allowed researchers to address the functional consequences of NGF sequestration. Initially, efforts focused on identifying precisely which neuronal populations depend on NGF for survival and when. It is through such experiments and later genetics that we know that NGF is required for the survival of sympathetic ganglion neurons and a large proportion of embryonic sensory neurons that are destined to become nociceptors (Ruit et al. 1990, 1992; Crowley et al. 1994). It appears that both sympathetic and sensory neurons largely lose their absolute dependency on NGF for survival in the postnatal period (Ruit et al. 1990, 1992).

In the 1980s and 1990s the main focus of developmental biologists was the question of whether these neurons required the neurotrophins to live, or otherwise in their absence to die, normally through an active apoptotic program (Lewin and Barde 1996). For example, it was known that all sensory neurons that express the high affinity NGF receptor trkA during embryonic development require NGF to survive, but it is also now clear that this population is not phenotypically homogenous (Crowley et al. 1994; Marmigère and Ernfors 2007). Recently, it has become possible using a nice genetic trick to examine the influence of NGF signaling in the embryo without the confounding effects of cell death. Thus mice lacking the cell death regulator Bax were generated on a genetic background in which the gene encoding NGF was also deleted; in the absence of Bax, neurons cannot execute an apoptotic program and remain alive in the absence of NGF (Patel et al. 2000; Luo et al. 2007). One key finding of such experiments is that NGF signaling is not required for long-distance axonal growth in the embryo, but is required for the terminal branch formation in the skin. However, there are other phenotypic characteristics of developing nociceptors that also require NGF signaling, for example, the expression of nociceptor-specific ion channels like the

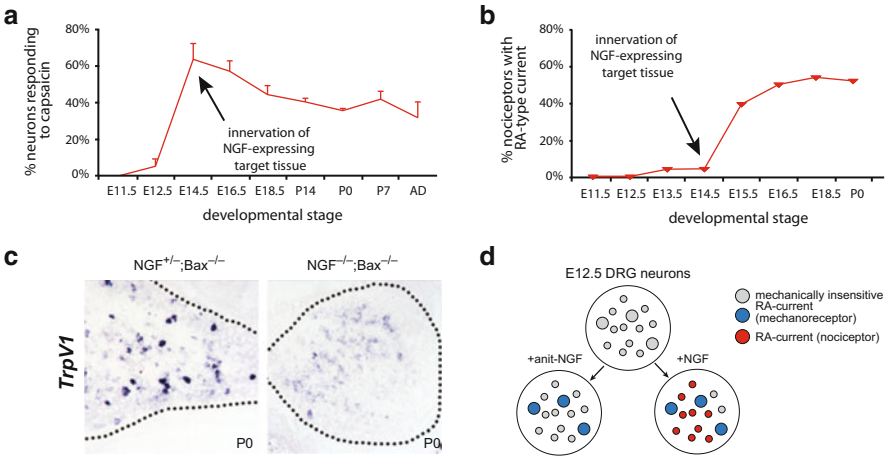

Fig. 1 NGF controls the expression of Trpv1 and mechanically gated ion channels in DRGs during embryonic development. (**a**) and (**b**) show the proportions of nociceptors that respond to the TRPV1 agonist capsaicin (**a**) and to mechanical stimulation (**b**) plotted as a function of developmental stage [data from (Hjerling-Leffler et al. 2007; Lechner et al. 2009)]. Note, both mechanosensitivity and capsaicin sensitivity are acquired at E14.5 when the peripheral projections begin to innervate NGF-expressing target tissues. (**c**) Trvp1 in situ hybridization in DRGs. Note in the absence of NGF (NGF−/−; Bax−/−), TrpV1 is not expressed in DRG neurons (Luo et al. 2007). (**d**) NGF is required for the acquisition of mechanotransduction currents in vitro. DRG neurons from E12.5 Bax−/− mice acquire RA-type mechanotransduction currents when cultured in the presence of NGF, but remain mechano-insensitive in the absence of NGF signaling (anti-NGF) see (Lechner et al. 2009)

capsaicin-activated ion channel TRPV1 and TRPM8 a menthol-gated channel involved in cold sensing (Luo et al. 2007) (Fig. 1a). Functional experiments using calcium imaging techniques also indicated that embryonic sensory neurons begin to respond to capsaicin at embryonic stages coinciding with the innervation of NGF-rich target tissues (Hjerling-Leffler et al. 2007) (Fig. 1a). The vast majority of nociceptors are primarily sensitive to mechanical stimuli and many possess fast activated mechanosensitive currents that are probably the functional basis of their mechanosensitivity (Hu and Lewin 2006; Wetzel et al. 2007). We thus asked when this mechanotransduction apparatus appears during development and if its appearance is regulated by target innervation or neurotrophins (Lechner et al. 2009) (Fig. 1b, d). Interestingly, one key finding of our study was that there are several waves of mechanotransduction induction in the sensory lineage with the first born, low-threshold mechanoreceptors (trkC population) acquiring mechanosensitive currents as soon as they innervate their peripheral targets (Fig. 1b). However, this process appears to be independent of growth factors and is probably regulated by an as yet unknown genetic program, possibly involving C-Maf genes (Lechner et al. 2009; Wende et al. 2012). In contrast, the vast majority of trkA-positive sensory neurons innervate their targets later and here it appears that target-derived NGF is absolutely required for the induction of mechanosensory competence (Lechner et al. 2009) (Fig. 1d). Thus the

physiological properties of developing sensory neurons that are essential for their adult function may already be specified by neurotrophin signaling in the early embryo (Fig. 1).

Despite the fact that NGF is not required for the continued survival of adult sensory neurons, it continues to be synthesized in the peripheral targets into adulthood. Indeed it has long been noted that the levels of NGF in the target correlate very well with the density of sympathetic and sensory innervation (Korsching and Thoenen 1983; Shelton and Reichardt 1984; Lewin and Barde 1996). Studies in the 1980s already showed that it is primarily neuropeptide containing nociceptive sensory neurons in the adult that respond to NGF (Lewin and Barde 1996). Thus the neuropeptide content, primarily substance P and calcitonin gene-related peptide (CGRP), of sensory neurons innervating tissues high in NGF, such as the skin, was observed to be high compared to tissues low in NGF (McMahon et al. 1989). Indeed, Lindsay and Harmar demonstrated that NGF directly upregulates the substance P content of adult sensory neurons (Lindsay and Harmar 1989). TrkA receptor expression is a feature of all developing nociceptors in the embryo, but its expression is extinguished in postnatal, small diameter, non-peptidergic nociceptors (Molliver et al. 1997). The high-affinity trkA receptor is the primary NGF signaling receptor and is co-expressed in neuropeptide-positive nociceptors in adults. In mature animals the peripheral tissue could be shown to influence the chemical composition of sensory afferents. Thus in experiments where a cutaneous nerve was rerouted to the NGF-poor skeletal muscle and a muscle nerve was rerouted to NGF-rich skin, the substance P content changed to match that characteristic of the new target, e.g., muscle nerve innervating skin now had a high substance P content (McMahon and Gibson 1987; McMahon et al. 1989). What was even more striking was the fact that the central connectivity of muscle afferents that had been redirected to skin now resembled that of normal skin afferents (Lewin and McMahon 1991). These results led us to carry out the first serious test of the idea that a neurotrophic factor could regulate synaptic strength in the nervous system. We decided to artificially raise the levels of NGF in the skeletal muscle, in this case the gastrocnemius muscle, by chronically pumping NGF into the muscle for a period of 14 days. By making extracellular recordings from spinal dorsal horn neurons we knew that only very few of these neurons receive strong synaptic drive from afferents innervating skeletal muscle. However, after exposure to NGF skeletal muscle afferents showed a huge increase in their ability to excite dorsal horn neurons and this increase was very large when compared to effects of muscle afferents innervating the contralateral, untreated muscle (Lewin et al. 1992b). This was in all probability the very first demonstration that a neurotrophic factor can modulate synaptic strength. Shortly afterwards, an elegant and more direct proof of this idea came from the lab of Moo Ming Poo, which showed that both neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF) can increase the strength of neuromuscular synapses in vitro, with a surprisingly fast time course in the range of seconds (Lohof et al. 1993). Together these studies provided the foundation of a huge and important area of study, namely, how neurotrophins regulate synapses and synaptic strength in the nervous system (see chapters 9 and 16 from Bai Lui et al. and Boyce and Mendell).

3 NGF and Hyperalgesia: The Linchpin Theory 1993

Hyperalgesia is defined as an increase in the felt intensity of a noxious stimulus, usually following an injury or an inflammatory process. Secondary hyperalgesia is the area of hypersensitivity surrounding an injured area that is not due to peripheral sensitization of the primary afferent, as the afferents that innervate the secondary area cannot be directly sensitized by the injury. This type of hyperalgesia is thought to be due to sensitization of central circuits to afferent input coming from nearby the initial injury site (Treede et al. 1992; Lewin and Moshourab 2004). During the 1980s, it was becoming increasingly clear that the neurobiological basis of secondary hyperalgesia was to a large extent dependent on a phenomenon termed central sensitization (Woolf 1983; McMahon and Wall 1984; Cook et al. 1987; McMahon et al. 1993). Thus strong activation of nociceptors leads to a rapid and long-lasting plasticity at synapses between primary sensory neurons and dorsal horn neurons, and this long-lasting change in synaptic strength can sustain hyperalgesia. Hyperalgesia is induced following injury or inflammation, but can also be produced after skin application of substances that activate or sensitize nociceptors. A classic example of algogen-induced heat and mechanical hyperalgesia is that following the application of capsaicin to the skin (LaMotte et al. 1991). While working on the role of NGF in determining the phenotypic identity of nociceptors in Lorne Mendell's lab (Ritter et al. 1991; Lewin et al. 1992a), Amy Ritter and Gary Lewin noted that rats that had been exposed to daily injections of NGF were behaviorally more sensitive to mechanical and heat stimuli than untreated animals. These observations led them to make a more systematic study of the effects of NGF on nociceptive behaviors in the rat. To their surprise a single systemic injection of NGF (1 mg/kg body weight) produced profound heat and mechanical hyperalgesia, which lasted for several days. Interestingly, heat and mechanical hyperalgesia appeared to be mechanistically distinct as heat hyperalgesia appeared within minutes, whereas mechanical hyperalgesia first became apparent around 7 h after the injection, becoming maximal and sustained at 24 h (Lewin et al. 1993). The fact that a single molecule, NGF, could set into train a series of rapid functional changes with all the hallmarks of hyperalgesia normally seen after sterile inflammation raised the obvious question of whether NGF was necessary for inflammatory hyperalgesia. This question was particularly pertinent in light of data published by Donnerer and colleagues in 1992 showing that NGF was upregulated in the sciatic nerve following inflammation of the skin (Donnerer et al. 1992). It was now an obvious step to use blocking antibodies in vivo to show whether an inflammation-dependent rise in NGF was a necessary first step in producing hyperalgesia. After obtaining preliminary data using NGF blocking antibodies, a new model of inflammatory hyperalgesia was proposed where NGF represents a linchpin molecule that provides the key humoral link between inflammation and the nociceptive sensory neurons that initiate and sustain heat and mechanical hyperalgesia (Lewin and Mendell 1993). The key features of this model are shown in Fig. 2, highlighting the areas of progress that have been made since the discovery that NGF is necessary for inflammatory hyperalgesia. Soon after we reported that NGF could induce

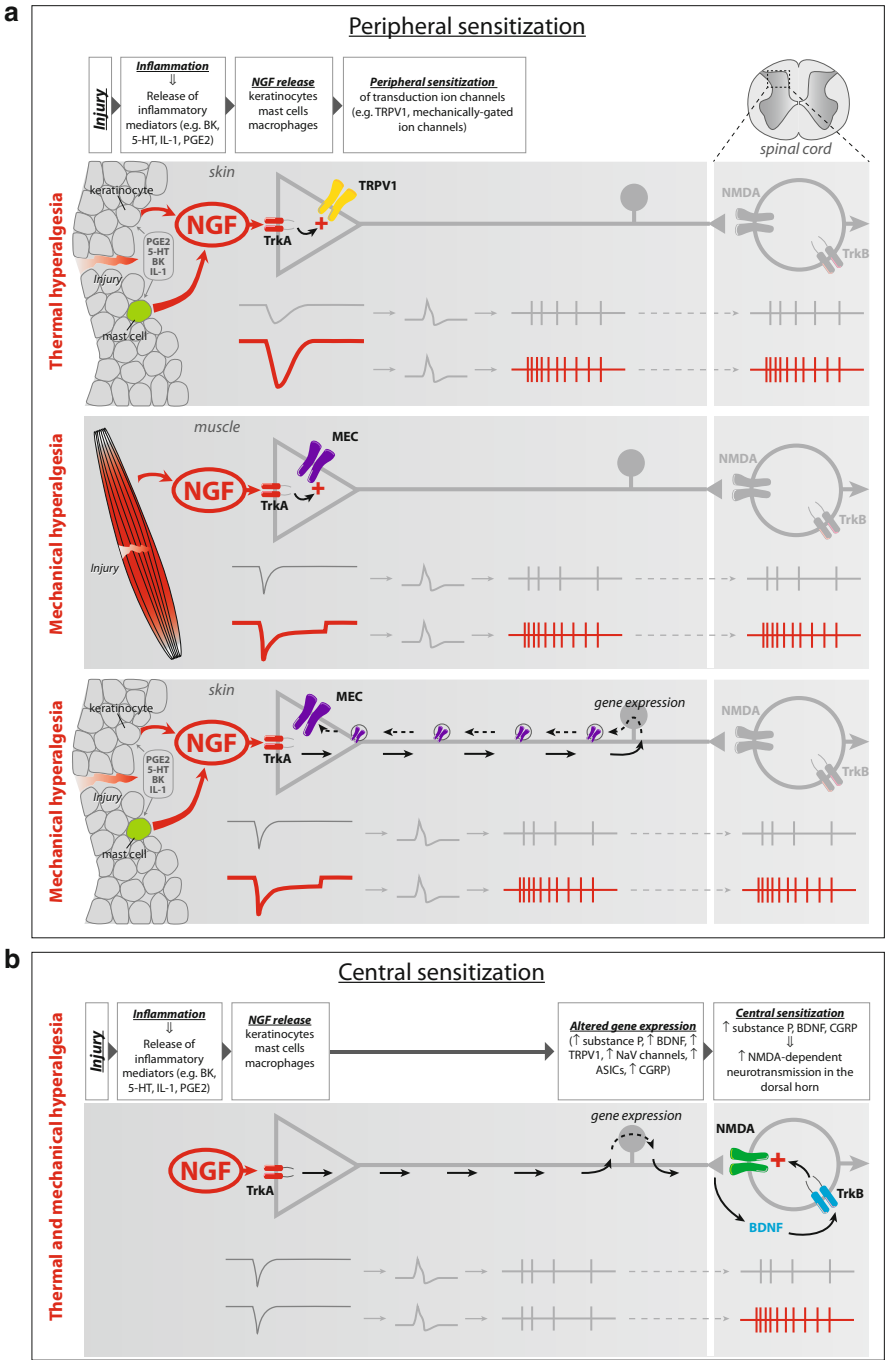

Fig. 2 Mechanisms of peripheral and central sensitization. (**a**) peripheral sensitization may result from posttranslational modifications (*top* and *middle panel*) or from increased gene expression and

hyperalgesia, several groups tested whether NGF blocking antibodies could ameliorate or block heat and mechanical hyperalgesia following inflammation. The first two reports showed that both the heat and mechanical hyperalgesia that follow a complete Freund's adjuvant-induced inflammation could be ameliorated by the administration of NGF blocking antibodies (Lewin et al. 1994; Woolf et al. 1994). Later on, the use of improved molecular tools to sequester NGF, namely, trkA-IgG fusion proteins that specifically bind endogenous NGF, was also shown to be capable of ameliorating heat and mechanical hyperalgesia associated with a carrageenan-evoked inflammation model in rats (McMahon et al. 1995). The key finding that blockade of NGF pain signaling in inflammatory conditions, where NGF is elevated, has a major analgesic effect has now been repeated in many models (Pezet and McMahon 2006; Mantyh et al. 2011).

In 1993, Lewin and Mendell proposed a mechanistic model illustrating the various ways in which increased NGF could produce heat and mechanical hyperalgesia following inflammation. One key feature of this model was the idea that the mechanisms that underlie the NGF-dependent heat hyperalgesia are distinct from those that underlie the mechanical hyperalgesia (Lewin and Mendell 1993; Lewin et al. 1994). We supposed that an important difference was that NGF is capable of inducing extremely rapid changes in the peripheral terminals of C-fibers that sensitizes them to noxious heat stimuli. Mechanical hyperalgesia on the other hand seemed to require the induction of changes in gene expression that eventually leads to central sensitization that maintains mechanical hyperalgesia (Lewin and Mendell 1993; Lewin et al. 1994). In the last 20 years much progress has been made in elucidating the molecular mechanisms that underlie peripheral NGF-dependent heat hyperalgesia. Progress has also been made in understanding NGF-dependent mechanical hyperalgesia and new data indicate that both central and peripheral mechanisms may be important, the molecular basis of which is just beginning to be unraveled.

4 NGF-Dependent Heat Hyperalgesia: Molecular Mechanisms

The availability of NGF in the skin was shown early on to regulate the number of C-fibers that respond to noxious heat. Thus, decreasing NGF levels with blocking antibodies reduced the number of C-fibers that respond to heat and raised NGF levels increased the number of heat-sensitive C-fibers (Lewin and Mendell 1994). These early experiments demonstrated that the molecular basis of noxious heat

Fig. 2 (continued) the insertion of additional mechanically gated ion channels in the plasma membrane of the peripheral nerve terminal (*bottom*). (**b**) NGF signaling induces the release of substance P, BDNF, and CGRP from the central terminals of sensory neurons, which sensitize NMDA receptors in second-order projection neurons resulting in the strengthening of synaptic transmission in the spinal dorsal horn—i.e., central sensitization

transduction was itself a target of regulation by NGF. The regulation of noxious heat transduction in single C-fibers in an inflammatory pain model was also shown to be dependent on NGF (Koltzenburg et al. 1999). The very rapid NGF-induced heat hyperalgesia was shown to be partly mediated by NGF-induced mast cell degranulation, which can in turn release more NGF (Mazurek et al. 1986; Lewin et al. 1994; Andreev et al. 1995). However, subsequent studies have emphasized that most of the rapid heat sensitization initiated by NGF takes place in the nociceptor. An important advance in the field was the discovery that a subpopulation of isolated sensory neurons possesses an ionic inward current directly activated by noxious heat sometimes referred to as I_{heat} (Cesare and McNaughton 1996). The I_{heat} inward current could also be sensitized by algogens like bradykinin and recording from isolated cells has proved to be a useful model to study molecules involved in nociceptor sensitization (Cesare and McNaughton 1996; Cesare et al. 1999). There was great excitement in the field when the capsaicin-gated ion channel TRPV1 was cloned by Julius and colleagues and shown to be gated by heat with an activation threshold similar to that of $I_{heat} \sim 43$ °C (Caterina et al. 1997). Thus the capsaicin receptor and the noxious heat transduction channel appeared to be one and the same thing. It was thus very striking when Mendell and Shu showed that a single short exposure of isolated sensory neurons to NGF (as well as NT-4) greatly potentiated the capsaicin current amplitude measured minutes later (Shu and Mendell 1999). Nerve growth factor-induced heat hyperalgesia was later found to be dependent on the presence of the TRPV1 ion channel as NGF-induced hyperalgesia is not found in TRPV1$^{-/-}$ mice (Chuang et al. 2001); the persistence of NGF-induced heat hyperalgesia in p75$^{-/-}$ mice demonstrates that trkA is probably the necessary receptor for downstream sensitization (Bergmann et al. 1998). The present consensus is that the TRPV1 ion channel is a noxious heat-gated ion channel present in many polymodal, noxious heat-sensitive C-fibers, but its presence does not appear to be necessary for these neurons to respond to noxious heat in vivo (Woodbury et al. 2004). Recent studies have implicated new heat-activated ion channels such as anoctamin-1, a calcium-activated chloride channel, and the TRP channel TRPM3 as being required for heat transduction in nociceptors (Vriens et al. 2011; Cho et al. 2012). However, it is not yet known if NGF-dependent heat hyperalgesia and nociceptor sensitization are dependent on either anoctamin-1 or TRPM3.

The absolute requirement for TRPV1 for NGF-dependent heat hyperalgesia and nociceptor sensitization has led many workers to use increased TRPV1 activity as a molecular surrogate for sensitization. Thus capsaicin has often been used, rather than heat, to activate TRPV1. Initial work using rat DRG neurons identified PKA as responsible for the sensitization brought about by NGF (Shu and Mendell 1999), but later work demonstrated that although protein kinase activity was involved in producing sensitization, it was PKC and PI3K that were responsible (Bonnington and McNaughton 2003). Differences in the sensitization protocol used and the recording method (whole-cell electrophysiology vs. calcium imaging) have been suggested to explain the differences in the results obtained. Whereas PKC acts predominantly via direct phosphorylation of TRPV1 (Numazaki et al. 2002), the

PI3K pathway has multiple steps: following trkA autophosphorylation at Tyr760, PI3K is activated, which in turn activates Src kinase, a non-receptor tyrosine kinase that subsequently phosphorylates Tyr200 on TRPV1 resulting in translocation to the plasma membrane and increased membrane expression (Zhang et al. 2005; Stein et al. 2006). An alternative explanation for NGF-induced heat hyperalgesia has been built on the observation that mutated trkA, which is unable to activate phospholipase C (PLC), fails to mediate NGF-induced sensitization, which the authors suggested was due to the action of PLC liberating TRPV1 from PIP2 inhibition being prevented; antibodies to PIP2 also evoked TRPV1 sensitization (Chuang et al. 2001). However, it has been argued that NGF can exert all its effects in a PIP2-independent manner (Zhang and McNaughton 2006) and later studies have shown that direct application of PIP2 actually potentiates TRPV1 (Stein et al. 2006). The study by Stein and colleagues has, however, recently been challenged by the finding that in artificial liposomes TRPV1 activation by both heat and capsaicin is inhibited by a variety of phosphoinositide lipids interacting with the C terminus of TRPV1 (Cao et al. 2013). Moreover, the authors show that activation threshold is not altered by channel number and therefore conclude that although NGF-dependent increased membrane expression of TRPV1 may account for some of the thermal hypersensitivity observed, it cannot explain decreases in thermal threshold. NGF-induced heat hyperalgesia is rapid in onset in vivo, but is also very long lasting and it has been suggested that NGF can also enhance TRPV1 expression levels via the Ras-MAPK pathway (Ji et al. 2002), which could contribute to the more persistent heat hyperalgesia in the presence of NGF. It should, however, be noted that there is good evidence that persistent heat hyperalgesia following inflammation or NGF elevation may also be dependent on central sensitization (Fig. 2).

The fact that TRPV1 is necessary for sensitization, but not for the transduction of noxious heat by nociceptors, is an important fact that requires further investigation (Woodbury et al. 2004; Koerber et al. 2010). It may be that freshly phosphorylated TRPV1 protein or newly inserted TRPV1 molecules in turn directly interact with candidate heat-gated channels, like anoctamin-1 or TRPM3, to produce sensitization. Alternatively, TRPV1 may itself have a signaling function that is required for the sensitization of heat transduction. In order to answer these questions a definitive identification of the molecule(s) necessary for heat transduction will be required. The signaling pathways that converge onto TRPV1 from trkA activation also appear to be engaged by other growth factor receptors such as c-Ret together with its co-receptors GFRα2 and GFRα3 (Stucky et al. 2002; Malin et al. 2006) that are preferentially activated by neurturin and artemin, respectively (Baloh et al. 2000; Bespalov and Saarma 2007). Neurturin signaling in particular may be like NGF, in the sense that it regulates the number of heat-sensitive neurons amongst the subpopulation of isolectin B4 (IB4)-positive sensory neurons that in the adult lack trkA receptors (Molliver et al. 1997; Stucky and Lewin 1999; Stucky et al. 2002). The receptor tyrosine kinase c-Kit is the receptor for stem cell factor (SCF) and was recently found to be expressed by a subpopulation of noxious heat-sensitive nociceptors (Milenkovic et al. 2007). It was shown that SCF/c-Kit

signaling is necessary to maintain nociceptor heat sensitivity and SCF can, like NGF, sensitize I_{heat} and produce a rapid, but short lasting, heat hyperalgesia in a TRPV1-dependent manner (Milenkovic et al. 2007). Interestingly, in the case of GDNF-like ligands and SCF where heat sensitization has been reported, mechanical hyperalgesia was absent [but see (Albers et al. 2006)].

5 Mechanisms of NGF-Dependent Mechanical Hyperalgesia

Mechanical hyperalgesia is the symptom that most concerns patients with painful conditions caused by inflammation or injury. It was thus very striking to observe that a short burst of elevated NGF can be sufficient to induce mechanical hyperalgesia that can last for days or even weeks in rodents and humans (Lewin et al. 1993; Petty et al. 1994). Systemic or local injection of NGF is unlikely to lead to sustained trkA activation because this small polypeptide would be rapidly degraded by extracellular proteases after injection. Thus, a pulse of NGF is sufficient to set in train a series of events that sustain mechanical hyperalgesia, often for days. Early pharmacological experiments already indicated that long-lasting NGF-induced heat hyperalgesia, but not mechanical hyperalgesia, is sustained by a central sensitization that requires NMDA receptors (Lewin et al. 1994) (Fig. 2). NGF can produce long-lasting changes in gene expression in adult sensory neurons and the first genes shown to be controlled by NGF were substance P and CGRP (Lindsay and Harmar 1989). Release of neuropeptides from sensory neurons may modulate the strength of spinal cord synapses (Seybold 2009); however, mice with a targeted mutation of the tachykinin-1 gene coding for the substance P peptide do not show deficits in inflammation-induced mechanical hyperalgesia (Cao et al. 1998). Thus considering that NGF is required for inflammation-induced mechanical hyperalgesia, it appears to be unlikely that substance P is a major central mediator. In contrast, studies on mice lacking a second major neuropeptide, CGRP expressed in trkA-positive sensory neurons (Molliver et al. 1997), have indicated broad deficits in inflammatory hyperalgesia including mechanical hyperalgesia (Salmon et al. 2001). One unusual rodent species, the naked mole rat, completely lacks both substance P and CGRP in cutaneous nociceptors, but exhibits a similar degree of mechanical hyperalgesia following complete Freund's adjuvant to that seen in mice (Park et al. 2008). Interestingly, however, NGF injected into naked mole rats does not produce heat hyperalgesia and this may be due to the presence of a hypo-functional trkA receptor in this species (Park et al. 2008; Smith et al. 2012).

Neurotrophins were traditionally thought of as being produced by the targets of sensory neurons, but it became apparent from developmental studies that many sensory neurons actually express and produce neurotrophins (Ernfors et al. 1990). It was therefore striking, when it was discovered that BDNF is normally produced by a subset of trkA-positive nociceptors and that the number of trkA neurons making this factor is dramatically increased by increased NGF (Apfel et al. 1996; Michael et al. 1997). Indeed, BDNF could be shown to be released by activity in sensory neurons and its release is enhanced by elevated NGF levels that follow

inflammation (Balkowiec and Katz 2000; Lever et al. 2001). Thus, increased peripheral NGF leads to increased production and release of BDNF from the central synapses of nociceptors in the spinal cord, which may be critical for certain central sensitization events, especially those involving NMDA receptors (Kerr et al. 1999). Direct electrophysiological evidence demonstrating that BDNF can rapidly potentiate transmission at synapses formed by nociceptors was provided by Mendell and colleagues (Garraway et al. 2003). The effects of mature BDNF on spinal synapses are rapid and probably occur via both pre- and postsynaptic trkB receptors and the potentiation observed is because of phosphorylation of NMDA receptor subunits (Kerr et al. 1999; Heppenstall and Lewin 2001; Garraway et al. 2003) (Fig. 2).

One complication of examining the central sensitization effects of BDNF is that this factor is also produced within the brain and spinal cord. Furthermore, the production and release of BDNF may be controlled by many factors. For example, it has been proposed that, when activated, spinal microglia cells may release BDNF, which in turn can modulate the excitability of dorsal horn neurons. The modulation of the anion gradient in lamina I projection neurons, possibly via the modulation of KCC2 (a potassium chloride co-transporter), can lead to a shift in the reversal potential for anions like chloride which makes normally hyperpolarizing inputs from inhibitory interneurons either ineffective or even depolarizing (Coull et al. 2005). This type of BDNF effect is thought to be particularly relevant for sustaining neuropathic pain. Other work, notably from Mendell's group, has also shown how BDNF can have highly synapse-specific effects in the spinal cord (Mendell and Arvanian 2002).

Global deletion of the BDNF gene leads to early postnatal lethality which has made the study of BDNF's role in the adult nervous system more difficult (Carroll et al. 1998). Nevertheless, studies using isolated spinal cords from young neurotrophin gene mutant mice have shown that the plasticity of ventral root potentials, which reflects C-fiber drive flexion reflexes, is selectively attenuated in the absence of BDNF, but not in the absence of NT-4 (Heppenstall and Lewin 2001). A systematic examination of pain-related behaviors in BDNF heterozygote mutant mice also indicated that even reduced gene dosage of this important factor can lead to deficits in acute noxious heat sensitivity and reduced pain behaviors, e.g., in the formalin test (MacQueen et al. 2001). An elegant genetic study using mice in which the BDNF gene was selectively deleted in nociceptive sensory neurons showed that BDNF is required for normal heat hyperalgesia following inflammation (Zhao et al. 2006). Although the authors did not definitively address the question of whether NGF-induced mechanical hyperalgesia depends on sensory neuron-derived BDNF, direct injection of NGF into skeletal muscle did not provoke mechanical hyperalgesia in this model, which in common with other studies suggests that elevated muscle NGF provokes central sensitization (Lewin et al. 1992b; Zhao et al. 2006). In summary, it seems that at least a proportion of the sustained heat hyperalgesia initiated by NGF may be sustained by central sensitization driven by BDNF and subsequent phosphorylation of postsynaptic NMDA receptors (Lewin et al. 1994; Zhao et al. 2006). However, it remains unclear whether the long-lasting mechanical hyperalgesia initiated by increased NGF is primarily dependent on peripheral or central mechanisms.

The long-lasting changes initiated by NGF may also affect the electrical properties of the primary afferent axons that transfer noxious information to the central nervous system. Nociceptors possess an array of voltage-gated sodium channels (Na_Vs) that are in some cases selectively expressed in these cells and strongly implicated in painful conditions. Nociceptors possess TTX-resistant and TTX-sensitive Na_Vs, which are carried primarily by $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ channels (Momin and Wood 2008); the modulation of such channels has been proposed to play a role in sensitization processes (England et al. 1996; Gold et al. 1996). Action potential initiation, voltage threshold, and sustained firing are dependent on the activation properties of Na_V channels (Blair and Bean 2002). It is therefore of interest that the availability of NGF can indeed modulate the action potential shape of nociceptors, both in culture as well as in vivo. Nociceptors have unusually broad action potentials with a prominent hump on their falling phase (Lechner et al. 2009). It is possible to identify nociceptors in cultures of adult sensory neurons that do not respond to NGF, as these can be live stained with fluorescently conjugated IB4. Interestingly, the density of TTX-sensitive sodium currents is actually less in NGF-sensitive nociceptors compared to IB4-positive NGF-insensitive neurons, which also display broader action potentials (Stucky and Lewin 1999). In vivo experiments have shown that chronically increasing the availability of NGF is associated with a broadening of the action potentials of identified Aδ nociceptors; conversely NGF deprivation is associated with a narrowing of the action potential in the same neurons (Ritter and Mendell 1992; Fang et al. 2005). The expression of TTX-resistant Na_Vs can be regulated by NGF and so it is conceivable that changes in action potential properties partly result from such regulation (Fjell et al. 1999). Genetic ablation of different Na_V genes in the sensory ganglia offers an opportunity to more directly assess their relative contributions to NGF-dependent sensitization events. Using mutant $Na_V1.8$ mice it was shown that the induction of NGF-dependent heat hyperalgesia requires the presence of $Na_V1.8$ channels (Kerr et al. 2001). However, heat hyperalgesia following carrageenan inflammation was only moderately delayed after genetic ablation of $Na_V1.8$ (Akopian et al. 1999) and was not affected in mice in which $Na_V1.8$ was inhibited in a cell autonomous manner (Stürzebecher et al. 2010). It is known that TTX-resistant Na_V currents can be measured very close to the spike initiation zone of peripheral nociceptors (Brock et al. 1998). It is therefore possible that a TRPV1-dependent sensitization process takes place in animals with ablated or attenuated $Na_V1.8$ channels, but that the increased activity of heat-sensitive nociceptors is not relayed to the CNS. The $Na_V1.7$ sodium channel plays an important role in setting the action potential threshold as well as amplifying subthreshold depolarization's to bring these neurons to fire (Dib-Hajj et al. 2013). Genetic ablation of this channel in mice and nonsense mutations in humans lead to a profound loss of pain sensation (Nassar et al. 2004; Cox et al. 2006; Momin and Wood 2008). NGF-dependent heat hyperalgesia is also essentially absent in mice with a sensory neuron-specific deletion of the SCN9A gene encoding $Na_V1.7$ channels (Nassar et al. 2004). Mechanical pain behavior is strongly attenuated in mice lacking $Na_V1.7$ in sensory neurons, which is consistent with a critical role for

this channel in sustaining nociceptor AP propagation (Nassar et al. 2004; Minett et al. 2012). There is, however, as yet only little direct evidence that the primary consequence of $Na_V1.7$ loss is an attenuation of the ability of somatic C-fibers to conduct action potentials (Wilson et al. 2011). For example there are, as yet, no reports in which this issue has been directly addressed using electrophysiological methods in somatic C-fibers; however, shRNA-mediated knockdown of $Na_V1.7$ in the vagus nerve has demonstrated a loss of sustained firing (Muroi et al. 2011). $Na_V1.7$ is an a important channel in olfactory sensory neurons (OSNs) and here it appears to be primarily required for the transfer of sensory information from OSN to second-order neurons in the olfactory bulb (Weiss et al. 2011). This has led to speculation that the primary mechanism leading to the spectacular loss of pain phenotypes in humans lacking $Na_V1.7$ channels is a block of information transfer from primary afferent C-fibers at their central synapses in the dorsal horn (Black et al. 2012; Minett et al. 2012). If the expression or subcellular distribution of $Na_V1.7$ channels is controlled by NGF availability (Gould et al. 2000; Diss et al. 2008), then it is conceivable that anti-NGF drugs work in an $Na_V1.7$-dependent manner.

A key difference between NGF-induced heat and mechanical hyperalgesia is the often radically different times courses that these phenomena display. Pure NGF-dependent hyperalgesia has in the last few years been increasingly studied in human subjects, as the injection of small amounts of NGF into the muscle or skin offers an excellent model for both short- and long-term sensitization, whilst bypassing inflammatory processes. During the first phase I safety trials of recombinant human NGF (rhNGF), it was quickly realized that human subjects experienced local soreness as well as a very long-lasting deep tissue hyperalgesia or myalgia following rhNGF injection (Petty et al. 1994). In this first human study a dose-dependent myalgia and hyperalgesia was observed to last for up to 7 weeks following a single injection. As in animal models, the mechanisms by which NGF produces mechanical hyperalgesia in humans will probably differ between very early phases and later phases following a transient increase in NGF. One early study noted signs of mechanical hyperalgesia within 6 h of an injection of rhNGF into the skin (Dyck et al. 1997). However, later studies using the same approach in humans showed that hyperalgesia, as measured using pressure pain threshold or pinprick sensitivity, first appears after 7 days and peaks 21 days after an intradermal rhNGF injection (Rukwied et al. 2010, 2013; Obreja et al. 2011a; Weinkauf et al. 2012, 2013). This discrepancy could be explained by spillover of injected NGF into underlying muscle tissue in humans, as well as in animal models. Thus, pronounced hyperalgesia has been noted following injection of rhNGF into human muscles or muscle fascia (Svensson et al. 2003, 2008; Andersen et al. 2008; Deising et al. 2012), but this hypersensitivity differs in several important respects from the NGF-induced mechanical hyperalgesia observed in the skin. First, mechanical hyperalgesia is observed within a few hours of the injection and the pressure pain hypersensitivity extends well beyond the area of the initial injection (Svensson et al. 2003, 2008; Andersen et al. 2008; Deising et al. 2012). As a rule, the muscle hypersensitivity following rhNGF injection is also observed to subside within a few

days of the NGF injection, in marked contrast to the very long-lasting hyperalgesia that follows a skin injection. In the skin model the available studies have noted that the mechanical hyperalgesia remains strictly restricted to the area of the initial rhNGF injection (Rukwied et al. 2010; Obreja et al. 2011a; Weinkauf et al. 2012), a strong indicator that a peripheral sensitization process may be involved (Treede et al. 1992; Lewin and Moshourab 2004).

In animal models a systemic injection of NGF provoked mechanical hyperalgesia, which first appears between an hour and several hours after the injection and persists for days (Lewin et al. 1993, 1994; Thompson et al. 1995). One group has, however, claimed to observe mechanical hyperalgesia minutes after the injection (Malik-Hall et al. 2005). As in humans, local skin injection of NGF in rats also provokes a localized mechanical hyperalgesia that persists for days (Mills et al. 2013). It appears that elevated NGF in skeletal muscle can sensitize muscle afferents to mechanical stimuli, but the evidence from human and animal studies suggests that secondary hyperalgesia is a prominent feature of this model, which involves central sensitization (Lewin et al. 1992b; Hoheisel et al. 2007, 2013). The observation that elevated NGF in the skin does not appear to provoke secondary mechanical hyperalgesia suggests that nociceptor sensitization plays a prominent role in this model. In general, it has been remarkably difficult to convincingly demonstrate nociceptor sensitization to mechanical stimuli in a variety of inflammatory models as conflicting results have been published (Andrew and Greenspan 1999; Lewin and Moshourab 2004; Milenkovic et al. 2008; Lennertz et al. 2012), Indeed, initial studies failed to detect prominent mechanical sensitization of nociceptors after acute or long-term NGF exposure (Lewin et al. 1993, 1994; Lewin and Mendell 1994; Obreja et al. 2011b).

The UV-B sunburn model is an interesting system to study peripheral mechanisms of mechanical hyperalgesia, as there is convincing evidence that central mechanisms do not play a prominent role in this model (Bishop et al. 2009, 2010). Recordings from nociceptors innervating UV-B-sensitized skin have demonstrated alterations in their firing rates to suprathreshold mechanical stimulation (Bishop et al. 2010). However, although some fiber types like C-fiber mechanonociceptors lacking noxious heat sensitivity (C-Ms) showed increased suprathreshold responses to intense mechanical stimulation, other fiber types like A-δ mechanonociceptors displayed reduced responses (Bishop et al. 2010). The complex changes in coding properties of different nociceptor subclasses in the UV-B model raise the possibility that mechanical hyperalgesia may be signaled to the spinal circuits by altered patterns of afferent activation dispersed across two or more nociceptor classes. Clear, direct evidence that cutaneous nociceptors are sensitized to mechanical stimuli after exposure to elevated NGF in vivo has been missing, until recently (Hirth et al. 2013). Many nociceptors are polymodal, meaning that they are activated by more than one modality of noxious stimulus, e.g., C-fibers activated by noxious mechanical and heat stimuli are termed C-mechanoheat units (C-MH). Using this classification scheme it is possible to record the following additional types of nociceptors in human skin using microneurography techniques: C-mechanosensitive (C-M), C-mechanosensitive and cold (C-MC), C-mechanosensitive heat and cold (C-MHC), C-mechano-insensitive and

heat-insensitive (C-MiHi), C-mechanosensitive and heat (C-MH), C-fiber heat only (C-H), and finally C-low-threshold mechanoreceptors (C-LT) (Lewin and Moshourab 2004). Broadly, the same types of nociceptors have been recorded in the skin of rats and mice (Lewin and Mendell 1994; Koltzenburg et al. 1997), but there appear to be consistent species differences, particularly in the incidence of each fiber type. In particular, C-MiHi fibers, identified in subhuman primates as mechanically insensitive afferents (MIAs), appear to be rare in rodents (Handwerker et al. 1991; Meyer et al. 1991; Kress et al. 1992; Lewin and Mendell 1994), but are relatively common in human hairy skin (Schmidt et al. 1995; Weidner et al. 1999). Several studies have strongly implicated C-MiHi fibers in peripheral sensitization processes; thus these fibers can rapidly acquire mechanosensitivity when stimulated with strong algogens. Recent studies by Schmelz and colleagues have shown that C-MiHi units are also observed in the skin of the pig, which they have claimed may be a more suitable animal model for human nociceptors (Obreja and Schmelz 2010). One feature of C-MiHi fibers recorded in humans and in pigs is that they display a very strong and prominent activity-dependent slowing of their conduction velocity (Weidner et al. 1999; Obreja et al. 2011b; Hirth et al. 2013). Thus, the higher the firing rate the longer it takes for the action potentials to reach the first spinal synapses. Strikingly, cutaneous NGF elevation in pigs selectively reduced the magnitude of activity-dependent slowing, as well as reducing the number of conduction failures at a moderate stimulation frequency of 2 Hz (Obreja et al. 2011a, b). The authors have named this phenomenon axonal sensitization as it is postulated that reduced slowing and more reliable following of electrical stimuli could underpin mechanical hyperalgesia. Moreover patients experienced more pain when cutaneous electrical stimuli were employed at the height of the hyperalgesia induced by local intradermal injection of rhNGF. The more reliable initiation and propagation of action potentials in nociceptors under these circumstances may be physiologically relevant as electrical stimulation could be seen as analogous to the driving depolarization produced by opening of transduction channels. However, the same authors failed to find very marked signs of nociceptor sensitization to natural mechanical stimuli in initial studies (Obreja et al. 2011a, b). It is clear that the "axonal sensitization" that they observed is probably caused by changes in the distribution or physiological properties of ion channels that regulate conduction. Obvious candidates are $Na_V1.7$ and $Na_V1.8$, which have indeed been implicated as targets of NGF signaling (Fjell et al. 1999; Gould et al. 2000; Fang et al. 2005; Diss et al. 2008). Nevertheless, there are other channels that regulate membrane excitability in nociceptors that could also be targets of NGF in this model, for example, hyperpolarization-activated cyclic nucleotide-gated cation channels like HCN2 (Emery et al. 2011; Mazo et al. 2013).

In a very recent study Hirth and colleagues actually provide good evidence for local nociceptor sensitization that is robust only 21 days after the initial NGF injection in a pig model (Hirth et al. 2013). Essentially, the authors show that at this point a significant and large proportion of formerly C-MiHi fibers are now very sensitive to mechanical stimuli; however, the suprathreshold coding properties of these fibers were not examined. There are a couple of interesting features of these findings, one is that the extremely long period of time it apparently takes before

sensitization of nociceptors is overt following local NGF exposure. Second, why does it takes so long for NGF-mediated signaling to induce an unmasking of mechanosensitivity whereby acute exposure to strong algogens can unsilence C-Mis with a very rapid time course (Schmidt et al. 1995). The human psychophysical data is clear about the fact that acute elevation of NGF in muscle, as opposed to skin, can produce a rapid sensitization, but even here there is little data to indicate why this may be the case. In one study in rats, NGF was injected directly into the muscle and led to an apparent activation of C-fibers afferents in the muscle, but did not lead to an acute sensitization of muscle C-fibers to mechanical stimuli (Hoheisel et al. 2005). In common with the innervation of the viscera (McMahon and Koltzenburg 1990), normal skeletal muscle is innervated by a large number of C-fibers that are insensitive to mechanical stimuli (Jankowski et al. 2013). It is not clear at the present time whether NGF can also lead to unmasking of mechanosensitivity in deep tissue nociceptors such as those innervating skeletal muscle (Fig. 2).

Although sensitization of nociceptors to mechanical stimuli has been observed and studied for many years, the molecular basis of the sensitization process is poorly understood. It has long been thought that one mechanism underlying sensitization may be the induction of excitability changes in nociceptor axons as has been discussed above. However, it is difficult to argue that such a sensitization process should be specific to mechanical stimuli as is often observed. The molecular mechanisms by which nociceptors actually detect mechanical stimuli are only just beginning to be unraveled and it is this transduction process that is likely to be a target for inflammatory factors like NGF. Mechanical stimuli are likely transduced directly at the sensory endings of nociceptors and this process probably involves the direct gating of a mechanosensitive ion channel by force or displacement (Hu et al. 2006). There are enormous technical challenges to overcome before it is possible to make direct recordings of mechanosensitive currents at the endings of nociceptors in situ. However, acutely cultured sensory neurons possess mechanosensitive ion channels that are directly gated by mechanical stimuli (McCarter et al. 1999; Drew et al. 2004, 2007; Hu and Lewin 2006; Lechner et al. 2009; Hu et al. 2010). It is now clear that there are at least two, and maybe three, biophysically distinct mechanosensitive conductances present in sensory neurons (Poole et al. 2011). Mechanosensitive currents in sensory neurons have been classified according to their inactivation kinetics: currents that inactivate very rapidly ($\tau_1 < 5$ ms) are termed rapidly adapting, RA-type; intermediately adapting ($\tau_1 < 50$ ms); and IA-type and slowly adapting (no adaptation during a 230-ms stimulus), SA-type. In the mouse the RA-type currents are sodium selective with a linear current–voltage relation and reversal potential >30 mV (Hu and Lewin 2006; Lechner et al. 2009). The RA-type current was not blocked by ruthinium red, but displays much slowed kinetics in the presence of benzamil, a broad range ENaC/Deg family channel blocker (Hu and Lewin 2006). The slowly adapting current is found exclusively in nociceptors, is a nonselective conductance, and appears much later in the development of sensory neurons (Hu and Lewin 2006; Lechner

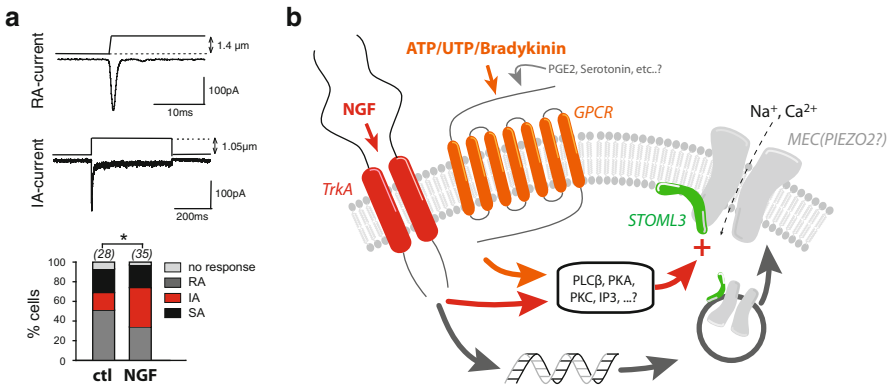

Fig. 3 Sensitization of mechanotransduction currents. (**a**) NGF increases the amplitude and slows the inactivation kinetics of RA- and IA-type currents, reproduced from (Lechner et al. 2009). (**b**) illustrates possible signaling cascades that may underlie the sensitization of mechanotransduction currents. NGF- and bradykinin-induced sensitization requires activation of PKA and PKC (Di Castro et al. 2006). NGF-induced sensitization was further shown to require transcription of new channels (Di Castro et al. 2006)

et al. 2009; Hu et al. 2010). There is now solid evidence that mechanosensitive currents found in cultured sensory neurons are indeed the in vitro counterparts of the transduction current in vivo. Thus manipulations that abolish or reduce the activity of mechanosensitive currents in vitro, such as removal of the essential mechanotransduction protein STOML3 or toxin-mediated block of these channels, also block mechanosensitivity in vivo (Drew et al. 2007; Wetzel et al. 2007; Hu et al. 2010). Agents that sensitize C-fibers in vivo, such as high concentrations of ATP, also rapidly and selectively sensitize the RA- and IA-type currents found in nociceptors (Lechner and Lewin 2009). Thus, within a few seconds of activation of the Gq-coupled $P2Y_2$ receptors by UTP or ATP, the amplitude of RA-type and IA-type currents was elevated and the inactivation time slowed so that each mechanical stimulus evoked a larger charge transfer through transduction channels. This effect leads to a clear increase in mechanically evoked action potential firing both in vitro and in vivo (Lechner and Lewin 2009). The principal sensitization mechanism via $P2Y_2$ receptor activation was to increase the charge transfer by slowing RA- and IA-type current inactivation kinetics; interestingly very similar effects of exposure to NGF have been reported for mechanosensitive currents in sensory neurons (Di Castro et al. 2006; Lechner et al. 2009). However, in contrast to the G-protein-mediated effects of UTP, the NGF effects required several hours to appear to be mediated by protein kinase C and may be due to the insertion of new mechanosensitive channels into the membrane (Di Castro et al. 2006). It is of course difficult to study the detailed molecular mechanism of such effects when the identities of the mechanosensitive channel(s) are unknown (Fig. 3).

Models of mechanotransduction have been very well developed in the *Caenorhabditis elegans* nematode worm model as here most of the molecular

players have been identified using reverse genetic approaches (Lewin and Moshourab 2004; Arnadóttir and Chalfie 2010; Poole et al. 2011; Geffeney and Goodman 2012). Interestingly, in worm touch receptors the ion channel is composed of the MEC-4 and MEC-10 proteins, which are worm orthologs of the acid sensing ion channels (ASICs, all members of the ENaC/Deg family) and the ASIC proteins have also been implicated as regulators of mechanosensitivity in sensory neurons. Thus deletion of the *ASIC2* and *ASIC3* genes, but not the *ASIC1* gene, leads to clear deficits in the mechanosensitivity of cutaneous mechanoreceptors and nociceptors (Price et al. 2000, 2001; Page et al. 2004; Moshourab et al. 2013). However, it appears very unlikely that ASIC subunits are in fact necessary for the formation of a mechanosensitive current in DRG neurons as these appear unaltered following *ASIC* gene deletion (Drew et al. 2004; Lechner et al. 2009). However, another *mec* gene identified in *C. elegans* is the stomatin domain protein MEC-2, which has at least two functional orthologs in mammals, stomatin and STOML3 (stomatin-like protein 3) (Lapatsina et al. 2012a). Both MEC-2 and STOML3 are required for the normal function of mechanotransduction in *C. elegans* and in the mouse, respectively (O'Hagan et al. 2005; Wetzel et al. 2007; Moshourab et al. 2013). Mutant mice lacking the *Stoml3* gene have severe deficits in mechanoreceptor and nociceptor function in that a large proportion of these cutaneous sensory neurons are mechanically insensitive. Indeed a much larger proportion of thinly myelinated nociceptors innervating the hairy skin lack mechanosensitivity in STOML3 mutant mice, a phenotype that is reminiscent of the mechanically insensitive nociceptors identified in normal human and pig skin (Weidner et al. 1999; Hirth et al. 2013). Stomatin-domain proteins like STOML3 and stomatin modulate the proton gating of ASIC2 and ASIC3 proteins and some of the structural motifs of the stomatin domains required for this modulation were recently identified (Price et al. 2004; Brand et al. 2012; Lapatsina et al. 2012b). In this context, it is interesting that deletion of *stomatin* or *stoml3* genes, together with the *Asic3* or *Asic2* genes, leads to a dramatic loss of mechanosensitivity in nociceptors, especially those with thinly myelinated Aδ axons (Moshourab et al. 2013). Although the ASIC proteins probably do not form part of the mechanotransducer, their presence or absence together with stomatin-domain proteins in sensory endings could be a molecular substrate to regulate mechanosensitivity in so-called "silent" nociceptors. The expression of ASIC proteins in sensory neurons is in fact controlled in part by neurotrophin signaling (Mamet et al. 2002; McIlwrath et al. 2005). It has been shown that pro-inflammatory mediators, including NGF, are involved in upregulating ASIC mRNAs and that NGF moderately increases the density of ASIC currents in cultured sensory neurons (Mamet et al. 2002). At the present time, however, it is not clear whether the presence of any of the ASIC proteins in the DRG is required for full-blown NGF-induced hyperalgesia. Acid is itself a potent activator and modulator of muscle nociceptors (Mense 2009), and ASIC3 proteins play a prominent role in muscle hyperalgesia (Sluka et al. 2003). In humans it was recently shown that acid-induced pain is significantly enhanced, even up to 14 days after a single injection of NGF into the muscle fascia of the back. The time course of the enhanced acid pain roughly paralleled the course of the mechanical

hyperalgesia (Deising et al. 2012). The parallel nature of mechanical and acid hypersensitivity in the muscle fascia model could mean that ASIC3, together with stomatin-domain proteins (Moshourab et al. 2013), is involved in regulating the mechanosensitivity of muscle nociceptors, but as yet there is no direct evidence to support this speculation. It is thought that ASIC3 and TRPV1 are the main ion channels that drive nociceptor activation after exposure to physiological tissue acidity observed after inflammation (Smith and Lewin 2009). Recently, we have shown that acid-evoked depolarization via TRPV1 and ASICs is potently counteracted by proton inhibition of Na_Vs, in particular $Na_V1.7$ in nociceptors (Smith et al. 2011). The inhibition of $Na_V1.7$ in nociceptors from naked mole-rats is so potent that it can abolish both the acid-induced activation of nociceptors and the accompanying sensitization of nociceptors to mechanical stimuli (Smith et al. 2011). Since NGF may also regulate $Na_V1.7$, and its presence can put a break on acid nociception, it is conceivable that a cell-specific regulation of this channel might contribute physiological differences between the acid sensitivity of cutaneous and deep tissue nociceptors.

Recently, two proteins were identified as bona fide stretch-activated ion channels, Piezo1 and Piezo2, and are widely expressed in both neuronal and non-neuronal tissues, as well as in sensory neurons (Coste et al. 2010, 2012). RNAi-mediated knockdown of Piezo2 in sensory neurons has implicated this stretch-activated channel as contributing to RA-type mechanosensitive currents (Coste et al. 2010). However, Piezo2 currents are nonselective and when measured in N2a neuroblastoma cells they are blocked by ruthenium red, both features not matching those of native sensory neuron RA currents (Hu and Lewin 2006; Lechner et al. 2009; Coste et al. 2010). Genetic evidence that Piezo1 or 2 are pore-forming mechanotransduction channels in sensory neurons is, however, still lacking.

There is a highly controversial literature on the possible involvement of the mustard oil-activated Trp channel TRPA1 in mechanotransduction (Patel et al. 2010; Nilius et al. 2012). The TRPA1 channel undoubtedly plays an important role governing the chemosensitivity of nociceptive afferents and is required for normal inflammatory pain behaviors in mice (Bautista et al. 2006; Kwan et al. 2006; Macpherson et al. 2007; McNamara et al. 2007). Recent studies have implicated TRPA1 as a contributor to mechanosensitive conductances found in sensory neurons (Vilceanu and Stucky 2010; Brierley et al. 2011); however, although these studies show a diminution of mechanosensitive channel activity, it is very difficult to differentiate between direct and indirect effects of TRPA1 gene deletion or pharmacological blockade. This is especially the case for TRPA1 which is a calcium-permeable ion channel, which itself can also be activated by the elevation of intracellular calcium (Zurborg et al. 2007). Thus, since mechanosensitive channels are calcium permeable it is possible that ion fluxes generated by transducing currents could be rapidly amplified by activating TRPA1 channels (Brierley et al. 2011). Similarly to ASIC proteins there is some evidence that TRPA1 channels are regulated by NGF availability (Malin et al. 2011), and deletion of the TRPA1 gene leads to complex changes in the mechanosensitivity of identified C-fiber afferents innervating the hairy skin (Kwan et al. 2009). There is solid

pharmacological evidence that TRPA1 blockade can prevent the moderate sensitization of C-fibers to suprathreshold mechanical stimulation following complete Freund's adjuvant inflammation (Lennertz et al. 2012). However, it is unclear if the presence of TRPA1 channels is required for NGF-induced mechanical hyperalgesia.

6 Cell Biology of Long-Lasting Sensitization Induced by NGF

The cell biology of DRG sensory neurons is unusual; these neurons accomplish two fundamentally different tasks at their central and peripheral endings that are separated by an enormous distance. Synaptic transmission and precise connectivity are established at the spinal cord end and transduction is accomplished at specialized endings in the periphery. In between, located about two-thirds of the distance between these points is the cell body, which must provide specialized proteins, membranes, and organelles that are sometimes differentially distributed between the peripheral and central branch (García-Añoveros et al. 2001). The retrograde and local signal transduction events initiated by NGF have been studied for decades and it is clear that NGF can exert some effects locally in the periphery and many effects are transported and propagated to the cell body via the so-called signaling endosome (Campenot and MacInnis 2004). However, in the periphery of sensory axons there exists a robust and stable transduction apparatus equipped to transduce mechanical signals in different ways in different sensory subtypes. Indeed, there are now examples of ion channel proteins that are specifically targeted to the peripheral endings of specific mechanoreceptor types, e.g., the potassium channel KCNQ4 in rapidly adapting mechanoreceptors (Heidenreich et al. 2012). How is this exquisite spatial and functional segregation achieved? The transport of proteins involved in the transduction and transformation of sensory signals at the peripheral endings of sensory neurons is very poorly understood, but represents a clear potential target for NGF modulation of afferent mechanosensitivity. Since STOML3 is the only protein known to participate directly in fast mechanotransduction it was of interest to examine how this membrane protein is trafficked within sensory neurons. We found that STOML3 is localized to a highly mobile and molecularly distinct transport vesicle within cultured sensory neuron axons (Lapatsina et al. 2012b). These vesicles are capable of co-transporting the related stomatin-domain protein, stomatin, together with each of the ASIC family members found in the DRG. Members of the Rab GTPase family of protein are involved in controlling the organization and identity of different membranous compartments within cells and neurons. For example, the Rab5 and Rab7 proteins are localized to signaling endosomes that are thought to retrogradely transport neurotrophin signals from the periphery to the cell body (Deinhardt et al. 2006). Interestingly, the STOML3 containing vesicles are not part of the signaling endosome pool as they are Rab5 negative, but are Rab11 positive. Rab11-positive vesicles have been characterized as composing a slowly recycling endocytic compartment and may be transported predominantly anterogradely in sensory neurons (Ascaño et al. 2009;

Eva et al. 2010). Indeed gain- or loss-of-function Rab11 mutants radically change vesicle behavior, but these compartments still contain STOML3 (Lapatsina et al. 2012b). The STOML3 vesicle is obviously enriched in proteins that are destined to function in transduction at the peripheral endings of sensory neurons and so we have proposed to name these vesicles "transducosomes." Indeed uncoupling of the "transducosome" from microtubules leads to rapid incorporation into the plasma membrane with an accompanying increase in acid-gated currents (Lapatsina et al. 2012b). Ex vivo recordings from sensory afferents innervating the skin have demonstrated that transduction of mechanical stimuli at the peripheral endings of sensory neurons is very stable for many hours in the absence of a connection to the cell body. Indeed early nerve injury experiments provided evidence that anterogradely transported proteins are first incorporated into cut endings to confer mechanosensitivity at a speed which is consistent with their transport distally via fast axonal transport (Koschorke et al. 1994). The stability of the transduction complexes at sensory endings is likely to be a function of three main factors: the number of "transducosomes" that arrive per unit of time, the propensity of such vesicles to fuse with the membrane and deliver functional transduction proteins, and finally the stability of existing transduction complexes. If this model is correct it is obvious that the ability of a sensory neuron to become sensitized to mechanical stimuli or indeed to become newly mechanically sensitive can be regulated at the levels of vesicle transport, fusion, or endocytosis of or recovery of spent transduction complexes. It is clear from the time course of fast mechanical hyperalgesia (hours) that local action of NGF might regulate the steps outlined above, but the molecular details are still completely unclear. Long-lasting mechanical hyperalgesia could be sustained by signals that are carried by signaling endosomes to initiate a cell body response, which may or may not include new gene expression, but would change the transduction process via the transport of novel, perhaps modulatory, subunits to the mechanotransducer. We recently identified a large extracellular tether protein that appears to be required for efficient and fast transduction in mechanoreceptors and many nociceptors (Hu et al. 2010). It is obvious that the transport of this protein could provide a way to "unsilence" nociceptors, but this hypothesis can only be tested once the identity of this protein is known.

7 The NGF Nexus of Pain

It is clear that NGF elevation that accompanies inflammation initiates a complex series of events, some of which are local and fast and others are global and long lasting. Anti-NGF therapy is remarkably effective in a broad variety of pain conditions ranging from muscle pain to bone cancer pain (Mantyh et al. 2010, 2011; Jimenez-Andrade et al. 2011). This remarkable efficacy of anti-NGF probably arises through the broad range of molecular events that are set into motion by elevated NGF levels in a variety of different tissues. In this review we have concentrated on molecular targets in the sensory innervation of skin and skeletal

muscle, but there is now abundant evidence that NGF may influence post-inflammatory events in other deep tissues. It follows that the diverse molecular changes initiated by NGF all serve to promote hyperalgesia, and we have discussed many individual examples in this review. Although both heat and mechanical hyperalgesia may be sustained, at least in part by synaptic changes in the spinal cord, there is increasing evidence that peripheral mechanisms that are very long lasting could also be specific targets of NGF signaling. For example, sensory mechanotransduction itself may be controlled by NGF signaling in a cell-specific manner. The molecular dissection of such effects will depend on identifying more of the key molecular players in mechanotransduction. It should also be noted that more knowledge on the downstream targets of NGF could eventually lead to the development of next generation pharmaceuticals that target these downstream players directly without the need to alter NGF availability.

Acknowledgments The authors' work was supported by grants from the Deutsche Forschungsgemeinshaft collaborative research centers 665 and 958 and a senior European Research Council grant. EStJS was supported by fellowship from the Alexander von Humboldt foundation.

References

Akopian AN, Souslova V, England S, Okuse K, Ogata N, Ure J, Smith A, Kerr BJ, McMahon SB, Boyce S, Hill R, Stanfa LC, Dickenson AH, Wood JN (1999) The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. Nat Neurosci 2:541–548

Albers KM, Woodbury CJ, Ritter AM, Davis BM, Koerber HR (2006) Glial cell-line-derived neurotrophic factor expression in skin alters the mechanical sensitivity of cutaneous nociceptors. J Neurosci 26:2981–2990

Andersen H, Arendt-Nielsen L, Svensson P, Danneskiold-Samsøe B, Graven-Nielsen T (2008) Spatial and temporal aspects of muscle hyperalgesia induced by nerve growth factor in humans. Exp Brain Res 191:371–382

Andreev NY, Dimitrieva N, Koltzenburg M, McMahon SB (1995) Peripheral administration of nerve growth factor in the adult rat produces a thermal hyperalgesia that requires the presence of sympathetic post-ganglionic neurones. Pain 63:109–115

Andrew D, Greenspan JD (1999) Mechanical and heat sensitization of cutaneous nociceptors after peripheral inflammation in the rat. J Neurophysiol 82:2649–2656

Apfel SC, Wright DE, Wiideman AM, Dormia C, Snider WD, Kessler JA (1996) Nerve growth factor regulates the expression of brain-derived neurotrophic factor mRNA in the peripheral nervous system. Mol Cell Neurosci 7:134–142

Arnadóttir J, Chalfie M (2010) Eukaryotic mechanosensitive channels. Annu Rev Biophys 39: 111–137

Ascaño M, Richmond A, Borden P, Kuruvilla R (2009) Axonal targeting of Trk receptors via transcytosis regulates sensitivity to neurotrophin responses. J Neurosci 29:11674–11685

Balkowiec A, Katz DM (2000) Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA in situ. J Neurosci 20: 7417–7423

Baloh RH, Enomoto H, Johnson EM Jr, Milbrandt J (2000) The GDNF family ligands and receptors – implications for neural development. Curr Opin Neurobiol 10:103–110

Bautista DM, Jordt S-E, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124:1269–1282

Bergmann I, Reiter R, Toyka KV, Koltzenburg M (1998) Nerve growth factor evokes hyperalgesia in mice lacking the low-affinity neurotrophin receptor p75. Neurosci Lett 255:87–90
Bespalov MM, Saarma M (2007) GDNF family receptor complexes are emerging drug targets. Trends Pharmacol Sci 28:68–74
Bishop T, Ballard A, Holmes H, Young AR, McMahon SB (2009) Ultraviolet-B induced inflammation of human skin: characterisation and comparison with traditional models of hyperalgesia. Eur J Pain 13:524–532
Bishop T, Marchand F, Young AR, Lewin GR, McMahon SB (2010) Ultraviolet-B-induced mechanical hyperalgesia: a role for peripheral sensitisation. Pain 150:141–152
Black JA, Frézel N, Dib-Hajj SD, Waxman SG (2012) Expression of Nav1.7 in DRG neurons extends from peripheral terminals in the skin to central preterminal branches and terminals in the dorsal horn. Mol Pain 8:82
Blair NT, Bean BP (2002) Roles of tetrodotoxin (TTX)-sensitive Na+ current, TTX-resistant Na+ current, and Ca2+ current in the action potentials of nociceptive sensory neurons. J Neurosci 22:10277–10290
Bonnington JK, McNaughton PA (2003) Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. J Physiol 551:433–446
Brand J, Smith ESJ, Schwefel D, Lapatsina L, Poole K, Omerbašić D, Kozlenkov A, Behlke J, Lewin GR, Daumke O (2012) A stomatin dimer modulates the activity of acid-sensing ion channels. EMBO J 31:3635–3646
Brierley SM, Castro J, Harrington AM, Hughes PA, Page AJ, Rychkov GY, Blackshaw LA (2011) TRPA1 contributes to specific mechanically activated currents and sensory neuron mechanical hypersensitivity. J Physiol 589:3575–3593
Brock JA, McLachlan EM, Belmonte C (1998) Tetrodotoxin-resistant impulses in single nociceptor nerve terminals in guinea-pig cornea. J Physiol 512(Pt 1):211–217
Brown MT, Murphy FT, Radin DM, Davignon I, Smith MD, West CR (2012) Tanezumab reduces osteoarthritic knee pain: results of a randomized, double-blind, placebo-controlled phase III trial. J Pain 13:790–798
Brown MT, Murphy FT, Radin DM, Davignon I, Smith MD, West CR (2013) Tanezumab reduces osteoarthritic hip pain: results of a randomized, double-blind, placebo-controlled phase 3 trial. Arthritis Rheum 65(7):1795–1803
Bueker ED, Hilderman HL (1953) Growth-stimulating effects of mouse sarcomas I, 37, and 180 on spinal and sympathetic ganglia of chick embryos as contrasted with effects of other tumors. Cancer 6:397–415
Campenot RB, MacInnis BL (2004) Retrograde transport of neurotrophins: fact and function. J Neurobiol 58:217–229
Cao YQ, Mantyh PW, Carlson EJ, Gillespie A-M, Epstein CJ, Basbaum AI (1998) Primary afferent tachykinins are required to experience moderate to intense pain. Nature 392:390–394
Cao E, Cordero-Morales JF, Liu B, Qin F, Julius D (2013) TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. Neuron 77:667–679
Carroll P, Lewin GR, Koltzenburg M, Toyka KV, Thoenen H (1998) A role for BDNF in mechanosensation. Nat Neurosci 1:42–46
Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824
Cattaneo A (2010) Tanezumab, a recombinant humanized mAb against nerve growth factor for the treatment of acute and chronic pain. Curr Opin Mol Ther 12:94–106
Cesare P, McNaughton P (1996) A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin. Proc Natl Acad Sci USA 93:15435–15439
Cesare P, Dekker LV, Sardini A, Parker PJ, McNaughton PA (1999) Specific involvement of PKC-epsilon in sensitization of the neuronal response to painful heat. Neuron 23:617–624
Cho H, Yang YD, Lee J, Lee B, Kim T, Jang Y, Back SK, Na HS, Harfe BD, Wang F, Raouf R, Wood JN, Oh U (2012) The calcium-activated chloride channel anoctamin 1 acts as a heat sensor in nociceptive neurons. Nat Neurosci 15:1015–1021

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 411:957–962

Cohen S (1960) Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum*. Proc Natl Acad Sci USA 46:302–311

Cohen S (2008) Origins of growth factors: NGF and EGF. J Biol Chem 283:33793–33797

Cook AJ, Woolf CJ, Wall PD, McMahon SB (1987) Dynamic receptive field plasticity in rat spinal cord dorsal horn following C-primary afferent input. Nature 325:151–153

Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A (2010) Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 330:55–60

Coste B, Xiao B, Santos JS, Syeda R, Grandl J, Spencer KS, Kim SE, Schmidt M, Mathur J, Dubin AE, Montal M, Patapoutian A (2012) Piezo proteins are pore-forming subunits of mechanically activated channels. Nature 483:176–181

Coull JAM, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y (2005) BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 438:1017–1021

Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, Karbani G, Jafri H, Mannan J, Raashid Y, Al-Gazali L, Hamamy H, Valente EM, Gorman S, Williams R, McHale DP, Wood JN, Gribble FM, Woods CG (2006) An SCN9A channelopathy causes congenital inability to experience pain. Nature 444:894–898

Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S, Armanini MP, Ling LH, McMahon SB, Shelton DL, Levinson AD (1994) Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 76:1001–1011

Deinhardt K, Salinas S, Verastegui C, Watson R, Worth D, Hanrahan S, Bucci C, Schiavo G (2006) Rab5 and Rab7 control endocytic sorting along the axonal retrograde transport pathway. Neuron 52:293–305

Deising S, Weinkauf B, Blunk J, Obreja O, Schmelz M, Rukwied R (2012) NGF-evoked sensitization of muscle fascia nociceptors in humans. Pain 153:1673–1679

Di Castro A, Drew LJ, Wood JN, Cesare P (2006) Modulation of sensory neuron mechanotransduction by PKC- and nerve growth factor-dependent pathways. Proc Natl Acad Sci USA 103:4699–4704

Dib-Hajj SD, Yang Y, Black JA, Waxman SG (2013) The Na(V)1.7 sodium channel: from molecule to man. Nat Rev Neurosci 14:49–62

Diss JKJ, Calissano M, Gascoyne D, Djamgoz MBA, Latchman DS (2008) Identification and characterization of the promoter region of the Nav1.7 voltage-gated sodium channel gene (SCN9A). Mol Cell Neurosci 37:537–547

Donnerer J, Schuligoi R, Stein C (1992) Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor in vivo. Neuroscience 49:693–698

Drew LJ, Rohrer DK, Price MP, Blaver KE, Cockayne DA, Cesare P, Wood JN (2004) Acid-sensing ion channels ASIC2 and ASIC3 do not contribute to mechanically activated currents in mammalian sensory neurones. J Physiol 556:691–710

Drew LJ, Rugiero F, Cesare P, Gale JE, Abrahamsen B, Bowden S, Heinzmann S, Robinson M, Brust A, Colless B, Lewis RJ, Wood JN (2007) High-threshold mechanosensitive ion channels blocked by a novel conopeptide mediate pressure-evoked pain. PLoS One 2:e515

Dyck PJ, Peroutka S, Rask C, Burton E, Baker MK, Lehman KA, Gillen DA, Hokanson JL, O'Brien PC (1997) Intradermal recombinant human nerve growth factor induces pressure allodynia and lowered heat-pain threshold in humans. Neurology 48:501–505

Emery EC, Young GT, Berrocoso EM, Chen L, McNaughton PA (2011) HCN2 ion channels play a central role in inflammatory and neuropathic pain. Science 333:1462–1466

England S, Bevan S, Docherty RJ (1996) PGE2 modulates the tetrodotoxin-resistant sodium current in neonatal rat dorsal root ganglion neurones via the cyclic AMP-protein kinase A cascade. J Physiol 495(Pt 2):429–440

Ernfors P, Wetmore C, Olson L, Persson H (1990) Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. Neuron 5:511–526

Eva R, Dassie E, Caswell PT, Dick G, Ffrench-Constant C, Norman JC, Fawcett JW (2010) Rab11 and its effector Rab coupling protein contribute to the trafficking of beta 1 integrins during axon growth in adult dorsal root ganglion neurons and PC12 cells. J Neurosci 30:11654–11669

Evans RJ, Moldwin RM, Cossons N, Darekar A, Mills IW, Scholfield D (2011) Proof of concept trial of tanezumab for the treatment of symptoms associated with interstitial cystitis. J Urol 185:1716–1721

Fang X, Djouhri L, McMullan S, Berry C, Okuse K, Waxman SG, Lawson SN (2005) TrkA is expressed in nociceptive neurons and influences electrophysiological properties via Nav1.8 Expression in rapidly conducting nociceptors. J Neurosci 25:4868–4878

Fjell J, Cummins TR, Fried K, Black JA, Waxman SG (1999) In vivo NGF deprivation reduces SNS expression and TTX-R sodium currents in IB4-negative DRG neurons. J Neurophysiol 81:803–810

García-Añoveros J, Samad TA, Zuvela-Jelaska L, Woolf CJ, Corey DP (2001) Transport and localization of the DEG/ENaC ion channel BNaC1alpha to peripheral mechanosensory terminals of dorsal root ganglia neurons. J Neurosci 21:2678–2686

Garraway SM, Petruska JC, Mendell LM (2003) BDNF sensitizes the response of lamina II neurons to high threshold primary afferent inputs. Eur J Neurosci 18:2467–2476

Geffeney SL, Goodman MB (2012) How we feel: ion channel partnerships that detect mechanical inputs and give rise to touch and pain perception. Neuron 74:609–619

Gold MS, Reichling DB, Shuster MJ, Levine JD (1996) Hyperalgesic agents increase a tetrodotoxin-resistant Na+ current in nociceptors. Proc Natl Acad Sci USA 93:1108–1112

Gould HJ 3rd, Gould TN, England JD, Paul D, Liu ZP, Levinson SR (2000) A possible role for nerve growth factor in the augmentation of sodium channels in models of chronic pain. Brain Res 854:19–29

Hamburger V (1993) The history of the discovery of the nerve growth factor. J Neurobiol 24: 893–897

Handwerker HO, Kilo S, Reeh PW (1991) Unresponsive afferent nerve fibres in the sural nerve of the rat. J Physiol 435:229–242

Heidenreich M, Lechner SG, Vardanyan V, Wetzel C, Cremers CW, De Leenheer EM, Aránguez G, Moreno-Pelayo M, Jentsch TJ, Lewin GR (2012) KCNQ4 K(+) channels tune mechanoreceptors for normal touch sensation in mouse and man. Nat Neurosci 15:138–145

Heppenstall PA, Lewin GR (2000) Neurotrophins, nociceptors and pain. Curr Opin Anaesthesiol 13:573–576

Heppenstall PA, Lewin GR (2001) BDNF but not NT-4 is required for normal flexion reflex plasticity and function. Proc Natl Acad Sci USA 98:8107–8112

Hirth M, Rukwied R, Gromann A, Turnquist B, Weinkauf B, Francke K, Albrecht P, Rice F, Hägglöf B, Ringkamp M, Engelhardt M, Schultz C, Schmelz M, Obreja O (2013) Nerve growth factor induces sensitization of nociceptors without evidence for increased intraepidermal nerve fiber density. Pain 154(11):2500–2511

Hjerling-Leffler J, Alqatari M, Ernfors P, Koltzenburg M (2007) Emergence of functional sensory subtypes as defined by transient receptor potential channel expression. J Neurosci 27:2435–2443

Hoheisel U, Unger T, Mense S (2005) Excitatory and modulatory effects of inflammatory cytokines and neurotrophins on mechanosensitive group IV muscle afferents in the rat. Pain 114:168–176

Hoheisel U, Unger T, Mense S (2007) Sensitization of rat dorsal horn neurons by NGF-induced subthreshold potentials and low-frequency activation. A study employing intracellular recordings in vivo. Brain Res 1169:34–43

Hoheisel U, Reuter R, de Freitas MF, Treede R-D, Mense S (2013) Injection of nerve growth factor into a low back muscle induces long-lasting latent hypersensitivity in rat dorsal horn neurons. Pain 154(10):1953–1960

Hu J, Lewin GR (2006) Mechanosensitive currents in the neurites of cultured mouse sensory neurones. J Physiol 577:815–828

Hu J, Milenkovic N, Lewin GR (2006) The high threshold mechanotransducer: a status report. Pain 120:3–7

Hu J, Chiang L-Y, Koch M, Lewin GR (2010) Evidence for a protein tether involved in somatic touch. EMBO J 29:855–867

Jankowski MP, Rau KK, Ekmann KM, Anderson CE, Koerber HR (2013) Comprehensive phenotyping of group III and IV muscle afferents in mouse. J Neurophysiol 109:2374–2381

Ji R-R, Samad TA, Jin S-X, Schmoll R, Woolf CJ (2002) p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron 36:57–68

Jimenez-Andrade JM, Ghilardi JR, Castañeda-Corral G, Kuskowski MA, Mantyh PW (2011) Preventive or late administration of anti-NGF therapy attenuates tumor-induced nerve sprouting, neuroma formation, and cancer pain. Pain 152:2564–2574

Kerr BJ, Bradbury EJ, Bennett DLH, Trivedi PM, Dassan P, French J, Shelton DB, McMahon SB, Thompson SWN (1999) Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord. J Neurosci 19:5138–5148

Kerr BJ, Souslova V, McMahon SB, Wood JN (2001) A role for the TTX-resistant sodium channel Nav 1.8 in NGF-induced hyperalgesia, but not neuropathic pain. Neuroreport 12:3077–3080

Koerber HR, McIlwrath SL, Lawson JJ, Malin SA, Anderson CE, Jankowski MP, Davis BM (2010) Cutaneous C-polymodal fibers lacking TRPV1 are sensitized to heat following inflammation, but fail to drive heat hyperalgesia in the absence of TPV1 containing C-heat fibers. Mol Pain 6:58

Koltzenburg M, Stucky CL, Lewin GR (1997) Receptive properties of mouse sensory neurons innervating hairy skin. J Neurophysiol 78:1841–1850

Koltzenburg M, Bennett DL, Shelton DL, McMahon SB (1999) Neutralization of endogenous NGF prevents the sensitization of nociceptors supplying inflamed skin. Eur J Neurosci 11:1698–1704

Korsching S, Thoenen H (1983) Nerve growth factor in sympathetic ganglia and corresponding target organs of the rat: correlation with density of sympathetic innervation. Proc Natl Acad Sci USA 80:3513–3516

Koschorke GM, Meyer RA, Campbell JN (1994) Cellular components necessary for mechano-electrical transduction are conveyed to primary afferent terminals by fast axonal transport. Brain Res 641:99–104

Kress M, Koltzenburg M, Reeh PW, Handwerker HO (1992) Responsiveness and functional attributes of electrically localized terminals of cutaneous C-fibers in vivo and in vitro. J Neurophysiol 68:581–595

Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang D-S, Woolf CJ, Corey DP (2006) TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 50:277–289

Kwan KY, Glazer JM, Corey DP, Rice FL, Stucky CL (2009) TRPA1 modulates mechanotransduction in cutaneous sensory neurons. J Neurosci 29:4808–4819

LaMotte RH, Shain CN, Simone DA, Tsai EF (1991) Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. J Neurophysiol 66:190–211

Lane NE, Schnitzer TJ, Birbara CA, Mokhtarani M, Shelton DL, Smith MD, Brown MT (2010) Tanezumab for the treatment of pain from osteoarthritis of the knee. N Engl J Med 363: 1521–1531

Lapatsina L, Brand J, Poole K, Daumke O, Lewin GR (2012a) Stomatin-domain proteins. Eur J Cell Biol 91:240–245

Lapatsina L, Jira JA, Smith ESJ, Poole K, Kozlenkov A, Bilbao D, Lewin GR, Heppenstall PA (2012b) Regulation of ASIC channels by a stomatin/STOML3 complex located in a mobile vesicle pool in sensory neurons. Open Biol 2:120096

Lechner SG, Lewin GR (2009) Peripheral sensitisation of nociceptors via G-protein-dependent potentiation of mechanotransduction currents. J Physiol 587:3493–3503

Lechner SG, Frenzel H, Wang R, Lewin GR (2009) Developmental waves of mechanosensitivity acquisition in sensory neuron subtypes during embryonic development. EMBO J 28:1479–1491

Lennertz RC, Kossyreva EA, Smith AK, Stucky CL (2012) TRPA1 mediates mechanical sensitization in nociceptors during inflammation. PLoS One 7:e43597

Lever IJ, Bradbury EJ, Cunningham JR, Adelson DW, Jones MG, McMahon SB, Marvizón JC, Malcangio M (2001) Brain-derived neurotrophic factor is released in the dorsal horn by distinctive patterns of afferent fiber stimulation. J Neurosci 21:4469–4477

Levi-Montalcini R, Booker B (1960) Destruction of the sympathetic ganglia in mammals by an antiserum to a nerve-growth protein. Proc Natl Acad Sci USA 46:384–391

Lewin GR, Barde YA (1996) Physiology of the neurotrophins. Annu Rev Neurosci 19:289–317

Lewin GR, McMahon SB (1991) Dorsal horn plasticity following re-routeing of peripheral nerves: evidence for tissue-specific neurotrophic influences from the periphery. Eur J Neurosci 3: 1112–1122

Lewin GR, Mendell LM (1993) Nerve growth factor and nociception. Trends Neurosci 16:353–359

Lewin GR, Mendell LM (1994) Regulation of cutaneous C-fiber heat nociceptors by nerve growth factor in the developing rat. J Neurophysiol 71:941–949

Lewin GR, Moshourab R (2004) Mechanosensation and pain. J Neurobiol 61:30–44

Lewin GR, Ritter AM, Mendell LM (1992a) On the role of nerve growth factor in the development of myelinated nociceptors. J Neurosci 12:1896–1905

Lewin GR, Winter J, McMahon SB (1992b) Regulation of afferent connectivity in the adult spinal cord by nerve growth factor. Eur J Neurosci 4:700–707

Lewin GR, Ritter AM, Mendell LM (1993) Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. J Neurosci 13:2136–2148

Lewin GR, Rueff A, Mendell LM (1994) Peripheral and central mechanisms of NGF-induced hyperalgesia. Eur J Neurosci 6:1903–1912

Lindsay RM, Harmar AJ (1989) Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. Nature 337:362–364

Lohof AM, Ip NY, Poo MM (1993) Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. Nature 363:350–353

Luo W, Wickramasinghe SR, Savitt JM, Griffin JW, Dawson TM, Ginty DD (2007) A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpeptidergic DRG neurons. Neuron 54:739–754

Macpherson LJ, Xiao B, Kwan KY, Petrus MJ, Dubin AE, Hwang S, Cravatt B, Corey DP, Patapoutian A (2007) An ion channel essential for sensing chemical damage. J Neurosci 27: 11412–11415

MacQueen GM, Ramakrishnan K, Croll SD, Siuciak JA, Yu G, Young LT, Fahnestock M (2001) Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. Behav Neurosci 115:1145–1153

Malik-Hall M, Dina OA, Levine JD (2005) Primary afferent nociceptor mechanisms mediating NGF-induced mechanical hyperalgesia. Eur J Neurosci 21:3387–3394

Malin SA, Molliver DC, Koerber HR, Cornuet P, Frye R, Albers KM, Davis BM (2006) Glial cell line-derived neurotrophic factor family members sensitize nociceptors in vitro and produce thermal hyperalgesia in vivo. J Neurosci 26:8588–8599

Malin S, Molliver D, Christianson JA, Schwartz ES, Cornuet P, Albers KM, Davis BM (2011) TRPV1 and TRPA1 function and modulation are target tissue dependent. J Neurosci 31:10516–10528

Mamet J, Baron A, Lazdunski M, Voilley N (2002) Proinflammatory mediators, stimulators of sensory neuron excitability via the expression of acid-sensing ion channels. J Neurosci 22: 10662–10670

Mantyh WG, Jimenez-Andrade JM, Stake JI, Bloom AP, Kaczmarska MJ, Taylor RN, Freeman KT, Ghilardi JR, Kuskowski MA, Mantyh PW (2010) Blockade of nerve sprouting and neuroma formation markedly attenuates the development of late stage cancer pain. Neuroscience 171: 588–598

Mantyh PW, Koltzenburg M, Mendell LM, Tive L, Shelton DL (2011) Antagonism of nerve growth factor-TrkA signaling and the relief of pain. Anesthesiology 115:189–204

Marmigère F, Ernfors P (2007) Specification and connectivity of neuronal subtypes in the sensory lineage. Nat Rev Neurosci 8:114–127

Mazo I, Rivera-Arconada I, Roza C (2013) Axotomy-induced changes in activity-dependent slowing in peripheral nerve fibres: role of hyperpolarization-activated/HCN channel current. Eur J Pain 17:1281–1290

Mazurek N, Weskamp G, Erne P, Otten U (1986) Nerve growth factor induces mast cell degranulation without changing intracellular calcium levels. FEBS Lett 198:315–320

McCarter GC, Reichling DB, Levine JD (1999) Mechanical transduction by rat dorsal root ganglion neurons in vitro. Neurosci Lett 273:179–182

McIlwrath SL, Hu J, Anirudhan G, Shin J-B, Lewin GR (2005) The sensory mechanotransduction ion channel ASIC2 (acid sensitive ion channel 2) is regulated by neurotrophin availability. Neuroscience 131:499–511

McMahon SB, Gibson S (1987) Peptide expression is altered when afferent nerves reinnervate inappropriate tissue. Neurosci Lett 73:9–15

McMahon SB, Koltzenburg M (1990) Novel classes of nociceptors: beyond Sherrington. Trends Neurosci 13:199–201

McMahon SB, Wall PD (1984) Receptive fields of rat lamina 1 projection cells move to incorporate a nearby region of injury. Pain 19:235–247

McMahon SB, Lewin GR, Anand P, Ghatei MA, Bloom SR (1989) Quantitative analysis of peptide levels and neurogenic extravasation following regeneration of afferents to appropriate and inappropriate targets. Neuroscience 33:67–73

McMahon SB, Lewin GR, Wall PD (1993) Central hyperexcitability triggered by noxious inputs. Curr Opin Neurobiol 3:602–610

McMahon SB, Bennett DL, Priestley JV, Shelton DL (1995) The biological effects of endogenous nerve growth factor on adult sensory neurons revealed by a trkA-IgG fusion molecule. Nat Med 1:774–780

McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, Hayward NJ, Chong JA, Julius D, Moran MM, Fanger CM (2007) TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci USA 104:13525–13530

Mendell LM, Arvanian VL (2002) Diversity of neurotrophin action in the postnatal spinal cord. Brain Res Brain Res Rev 40:230–239

Mense S (2009) Algesic agents exciting muscle nociceptors. Exp Brain Res 196:89–100

Meyer RA, Davis KD, Cohen RH, Treede RD, Campbell JN (1991) Mechanically insensitive afferents (MIAs) in cutaneous nerves of monkey. Brain Res 561:252–261

Michael GJ, Averill S, Nitkunan A, Rattray M, Bennett DL, Yan Q, Priestley JV (1997) Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. J Neurosci 17:8476–8490

Milenkovic N, Frahm C, Gassmann M, Griffel C, Erdmann B, Birchmeier C, Lewin GR, Garratt AN (2007) Nociceptive tuning by stem cell factor/c-Kit signaling. Neuron 56:893–906

Milenkovic N, Wetzel C, Moshourab R, Lewin GR (2008) Speed and temperature dependences of mechanotransduction in afferent fibers recorded from the mouse saphenous nerve. J Neurophysiol 100:2771–2783

Mills CD, Nguyen T, Tanga FY, Zhong C, Gauvin DM, Mikusa J, Gomez EJ, Salyers AK, Bannon AW (2013) Characterization of nerve growth factor-induced mechanical and thermal hypersensitivity in rats. Eur J Pain 17:469–479

Minett MS, Nassar MA, Clark AK, Passmore G, Dickenson AH, Wang F, Malcangio M, Wood JN (2012) Distinct Nav1.7-dependent pain sensations require different sets of sensory and sympathetic neurons. Nat Commun 3:791

Molliver DC, Wright DE, Leitner ML, Parsadanian AS, Doster K, Wen D, Yan Q, Snider WD (1997) IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. Neuron 19:849–861

Momin A, Wood JN (2008) Sensory neuron voltage-gated sodium channels as analgesic drug targets. Curr Opin Neurobiol 18:383–388

Moshourab RA, Wetzel C, Martinez-Salgado C, Lewin GR (2013) Stomatin-domain protein interactions with acid sensing ion channels modulate nociceptor mechanosensitivity. J Physiol 591:5555–5574

Muroi Y, Ru F, Kollarik M, Canning BJ, Hughes SA, Walsh S, Sigg M, Carr MJ, Undem BJ (2011) Selective silencing of NaV1.7 decreases excitability and conduction in vagal sensory neurons. J Physiol 589:5663–5676

Nassar MA, Stirling LC, Forlani G, Baker MD, Matthews EA, Dickenson AH, Wood JN (2004) Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. Proc Natl Acad Sci USA 101:12706–12711

Nilius B, Appendino G, Owsianik G (2012) The transient receptor potential channel TRPA1: from gene to pathophysiology. Pflugers Arch 464:425–458

Numazaki M, Tominaga T, Toyooka H, Tominaga M (2002) Direct phosphorylation of capsaicin receptor VR1 by protein kinase Cepsilon and identification of two target serine residues. J Biol Chem 277:13375–13378

O'Hagan R, Chalfie M, Goodman MB (2005) The MEC-4 DEG/ENaC channel of Caenorhabditis elegans touch receptor neurons transduces mechanical signals. Nat Neurosci 8:43–50

Obreja O, Schmelz M (2010) Single-fiber recordings of unmyelinated afferents in pig. Neurosci Lett 470:175–179

Obreja O, Kluschina O, Mayer A, Hirth M, Schley M, Schmelz M, Rukwied R (2011a) NGF enhances electrically induced pain, but not axon reflex sweating. Pain 152:1856–1863

Obreja O, Ringkamp M, Turnquist B, Hirth M, Forsch E, Rukwied R, Petersen M, Schmelz M (2011b) Nerve growth factor selectively decreases activity-dependent conduction slowing in mechano-insensitive C-nociceptors. Pain 152:2138–2146

Page AJ, Brierley SM, Martin CM, Martinez-Salgado C, Wemmie JA, Brennan TJ, Symonds E, Omari T, Lewin GR, Welsh MJ, Blackshaw LA (2004) The ion channel ASIC1 contributes to visceral but not cutaneous mechanoreceptor function. Gastroenterology 127:1739–1747

Park TJ, Lu Y, Jüttner R, Smith ESJ, Hu J, Brand A, Wetzel C, Milenkovic N, Erdmann B, Heppenstall PA, Laurito CE, Wilson SP, Lewin GR (2008) Selective inflammatory pain insensitivity in the African naked mole-rat (Heterocephalus glaber). PLoS Biol 6:e13

Patel TD, Jackman A, Rice FL, Kucera J, Snider WD (2000) Development of sensory neurons in the absence of NGF/TrkA signaling in vivo. Neuron 25:345–357

Patel A, Sharif-Naeini R, Folgering JRH, Bichet D, Duprat F, Honoré E (2010) Canonical TRP channels and mechanotransduction: from physiology to disease states. Pflugers Arch 460:571–581

Petty BG, Cornblath DR, Adornato BT, Chaudhry V, Flexner C, Wachsman M, Sinicropi D, Burton LE, Peroutka SJ (1994) The effect of systemically administered recombinant human nerve growth factor in healthy human subjects. Ann Neurol 36:244–246

Pezet S, McMahon SB (2006) Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci 29:507–538

Poole K, Lechner SG, Lewin GR (2011) The handbook of touch: the molecular and genetic basis of touch. Springer, New York, NY

Price MP, Lewin GR, McIlwrath SL, Cheng C, Xie J, Heppenstall PA, Stucky CL, Mannsfeldt AG, Brennan TJ, Drummond HA, Qiao J, Benson CJ, Tarr DE, Hrstka RF, Yang B, Williamson RA,

Welsh MJ (2000) The mammalian sodium channel BNC1 is required for normal touch sensation. Nature 407:1007–1011
Price MP, McIlwrath SL, Xie J, Cheng C, Qiao J, Tarr DE, Sluka KA, Brennan TJ, Lewin GR, Welsh MJ (2001) The DRASIC cation channel contributes to the detection of cutaneous touch and acid stimuli in mice. Neuron 32:1071–1083
Price MP, Thompson RJ, Eshcol JO, Wemmie JA, Benson CJ (2004) Stomatin modulates gating of acid-sensing ion channels. J Biol Chem 279:53886–53891
Ritter AM, Mendell LM (1992) Somal membrane properties of physiologically identified sensory neurons in the rat: effects of nerve growth factor. J Neurophysiol 68:2033–2041
Ritter AM, Lewin GR, Kremer NE, Mendell LM (1991) Requirement for nerve growth factor in the development of myelinated nociceptors in vivo. Nature 350:500–502
Ruit KG, Osborne PA, Schmidt RE, Johnson EM Jr, Snider WD (1990) Nerve growth factor regulates sympathetic ganglion cell morphology and survival in the adult mouse. J Neurosci 10:2412–2419
Ruit KG, Elliott JL, Osborne PA, Yan Q, Snider WD (1992) Selective dependence of mammalian dorsal root ganglion neurons on nerve growth factor during embryonic development. Neuron 8:573–587
Rukwied R, Mayer A, Kluschina O, Obreja O, Schley M, Schmelz M (2010) NGF induces non-inflammatory localized and lasting mechanical and thermal hypersensitivity in human skin. Pain 148:407–413
Rukwied RR, Main M, Weinkauf B, Schmelz M (2013) NGF sensitizes nociceptors for cowhage- but not histamine-induced itch in human skin. J Invest Dermatol 133:268–270
Salmon A-M, Damaj MI, Marubio LM, Epping-Jordan MP, Merlo-Pich E, Changeux J-P (2001) Altered neuroadaptation in opiate dependence and neurogenic inflammatory nociception in αCGRP-deficient mice. Nat Neurosci 4:357–358
Schmidt R, Schmelz M, Forster C, Ringkamp M, Torebjörk E, Handwerker H (1995) Novel classes of responsive and unresponsive C nociceptors in human skin. J Neurosci 15:333–341
Seybold VS (2009) The role of peptides in central sensitization. Handb Exp Pharmacol 194:451–491
Shelton DL, Reichardt LF (1984) Expression of the beta-nerve growth factor gene correlates with the density of sympathetic innervation in effector organs. Proc Natl Acad Sci USA 81:7951–7955
Shu X, Mendell LM (1999) Nerve growth factor acutely sensitizes the response of adult rat sensory neurons to capsaicin. Neurosci Lett 274:159–162
Sluka KA, Price MP, Breese NM, Stucky CL, Wemmie JA, Welsh MJ (2003) Chronic hyperalgesia induced by repeated acid injections in muscle is abolished by the loss of ASIC3, but not ASIC1. Pain 106:229–239
Smith ESJ, Lewin GR (2009) Nociceptors: a phylogenetic view. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 195:1089–1106
Smith ESJ, Omerbašić D, Lechner SG, Anirudhan G, Lapatsina L, Lewin GR (2011) The molecular basis of acid insensitivity in the African naked mole-rat. Science 334:1557–1560
Smith ESJ, Purfürst B, Grigoryan T, Park TJ, Bennett NC, Lewin GR (2012) Specific paucity of unmyelinated C-fibers in cutaneous peripheral nerves of the African naked-mole rat: comparative analysis using six species of Bathyergidae. J Comp Neurol 520:2785–2803
Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE (2006) Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. J Gen Physiol 128:509–522
Stucky CL, Lewin GR (1999) Isolectin B(4)-positive and -negative nociceptors are functionally distinct. J Neurosci 19:6497–6505
Stucky CL, Rossi J, Airaksinen MS, Lewin GR (2002) GFR alpha2/neurturin signalling regulates noxious heat transduction in isolectin B4-binding mouse sensory neurons. J Physiol 545:43–50
Stürzebecher AS, Hu J, Smith ESJ, Frahm S, Santos-Torres J, Kampfrath B, Auer S, Lewin GR, Ibañez-Tallon I (2010) An in vivo tethered toxin approach for the cell-autonomous inactivation of voltage-gated sodium channel currents in nociceptors. J Physiol 588:1695–1707

Svensson P, Cairns BE, Wang K, Arendt-Nielsen L (2003) Injection of nerve growth factor into human masseter muscle evokes long-lasting mechanical allodynia and hyperalgesia. Pain 104:241–247

Svensson P, Wang K, Arendt-Nielsen L, Cairns BE (2008) Effects of NGF-induced muscle sensitization on proprioception and nociception. Exp Brain Res 189:1–10

Thompson SW, Dray A, McCarson KE, Krause JE, Urban L (1995) Nerve growth factor induces mechanical allodynia associated with novel A fibre-evoked spinal reflex activity and enhanced neurokinin-1 receptor activation in the rat. Pain 62:219–231

Treede RD, Meyer RA, Raja SN, Campbell JN (1992) Peripheral and central mechanisms of cutaneous hyperalgesia. Prog Neurobiol 38:397–421

Vilceanu D, Stucky CL (2010) TRPA1 mediates mechanical currents in the plasma membrane of mouse sensory neurons. PLoS One 5:e12177

Vriens J, Owsianik G, Hofmann T, Philipp SE, Stab J, Chen X, Benoit M, Xue F, Janssens A, Kerselaers S, Oberwinkler J, Vennekens R, Gudermann T, Nilius B, Voets T (2011) TRPM3 Is a nociceptor channel involved in the detection of noxious heat. Neuron 70:482–494

Weidner C, Schmelz M, Schmidt R, Hansson B, Handwerker HO, Torebjörk HE (1999) Functional attributes discriminating mechano-insensitive and mechano-responsive C nociceptors in human skin. J Neurosci 19:10184–10190

Weinkauf B, Obreja O, Schmelz M, Rukwied R (2012) Differential effects of lidocaine on nerve growth factor (NGF)-evoked heat- and mechanical hyperalgesia in humans. Eur J Pain 16:543–549

Weinkauf B, Main M, Schmelz M, Rukwied R (2013) Modality-specific nociceptor sensitization following UV-B irradiation of human skin. J Pain 14:739–746

Weiss J, Pyrski M, Jacobi E, Bufe B, Willnecker V, Schick B, Zizzari P, Gossage SJ, Greer CA, Leinders-Zufall T, Woods CG, Wood JN, Zufall F (2011) Loss-of-function mutations in sodium channel Nav1.7 cause anosmia. Nature 472:186–190

Wende H, Lechner SG, Cheret C, Bourane S, Kolanczyk ME, Pattyn A, Reuter K, Munier FL, Carroll P, Lewin GR, Birchmeier C (2012) The transcription factor c-Maf controls touch receptor development and function. Science 335:1373–1376

Wetzel C, Hu J, Riethmacher D, Benckendorff A, Harder L, Eilers A, Moshourab R, Kozlenkov A, Labuz D, Caspani O, Erdmann B, Machelska H, Heppenstall PA, Lewin GR (2007) A stomatin-domain protein essential for touch sensation in the mouse. Nature 445:206–209

Wilson MJ, Yoshikami D, Azam L, Gajewiak J, Olivera BM, Bulaj G, Zhang M-M (2011) μ-Conotoxins that differentially block sodium channels NaV1.1 through 1.8 identify those responsible for action potentials in sciatic nerve. Proc Natl Acad Sci USA 108:10302–10307

Woodbury CJ, Zwick M, Wang S, Lawson JJ, Caterina MJ, Koltzenburg M, Albers KM, Koerber HR, Davis BM (2004) Nociceptors lacking TRPV1 and TRPV2 have normal heat responses. J Neurosci 24:6410–6415

Woolf CJ (1983) Evidence for a central component of post-injury pain hypersensitivity. Nature 306:686–688

Woolf CJ, Safieh-Garabedian B, Ma Q-P, Crilly P, Winter J (1994) Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. Neuroscience 62:327–331

Zhang X, McNaughton PA (2006) Why pain gets worse: the mechanism of heat hyperalgesia. J Gen Physiol 128:491–493

Zhang X, Huang J, McNaughton PA (2005) NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J 24:4211–4223

Zhao J, Seereeram A, Nassar MA, Levato A, Pezet S, Hathaway G, Morenilla-Palao C, Stirling C, Fitzgerald M, McMahon SB, Rios M, Wood JN, London Pain Consortium (2006) Nociceptor-derived brain-derived neurotrophic factor regulates acute and inflammatory but not neuropathic pain. Mol Cell Neurosci 31:539–548

Zurborg S, Yurgionas B, Jira JA, Caspani O, Heppenstall PA (2007) Direct activation of the ion channel TRPA1 by Ca2+. Nat Neurosci 10:277–279

Neurotrophins and the Regulation of Energy Balance and Body Weight

M. Rios

Abstract

Complex interactions between the brain and peripheral tissues mediate the effective control of energy balance and body weight. Hypothalamic and hindbrain neural circuits integrate peripheral signals informing the nutritional status of the animal and in response regulate nutrient intake and energy utilization. Obesity and its many medical complications emerge from the dysregulation of energy homeostasis. Excessive weight gain might also arise from alterations in reward systems of the brain that drive consumption of calorie dense, palatable foods in the absence of an energy requirement. Several neurotrophins, most notably brain-derived neurotrophic factor, have been implicated in the molecular and cellular processes underlying body weight regulation. Here, we review investigations interrogating their roles in energy balance and reward centers of the brain impacting feeding behavior and energy expenditure.

Keywords

Neurotrophins • Hypothalamus • Hindbrain • Energy balance • Mesolimbic • Dopamine • Food intake • Energy expenditure • Obesity • BDNF • NT-4 • GDNF • CNTF

1 Introduction

Energy homeostasis, the finely regulated equilibrium between caloric intake and energy expenditure, is fundamental for animal survival because it safeguards essential energy stores. It is regulated by short-term mechanisms that control food intake based on the immediate nutritional requirements of the animal and long-term mechanisms that protect energy reserves and body weight (Dietrich and Horvath 2009).

M. Rios (✉)
Department of Neuroscience, Tufts University School of Medicine, Boston, MA 02111, USA
e-mail: maribel.rios@tufts.edu

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_11,

Neuropeptidergic circuits in the hypothalamus and hindbrain contribute to the homeostatic control of food intake and energy utilization by integrating hunger, satiety, and body adiposity cues from the periphery. Eating can also be driven by the motivational and pleasurable aspects of palatable foods in the absence of a homeostatic requirement (Berridge 2009). Brain systems involved in motivated and reward-seeking behaviors, including the mesolimbic dopamine pathway, are involved in this form of hedonic feeding. Perturbations in homeostatic and reward neural circuits in the brain have been linked to the etiology of excessive feeding and obesity.

Several neurotrophins have been implicated in the central mechanisms influencing food intake and body weight and in disease processes leading to obesity. Among those, brain-derived neurotrophic factor (BDNF) has been studied far more extensively in this context and thus will be discussed in more detail. Roles for nerve growth factor (NGF), neurotrophin-3 (NT-3) and 4 (NT-4), ciliary neurotrophic factor (CNTF), and glial-derived neurotrophic factor (GDNF) in feeding behavior have also been suggested and will be reviewed here. First, we describe the neural circuits involved in the regulation of feeding behavior and then discuss findings informing the role of neurotrophins in brain systems impacting food consumption and body weight.

2 Brain Circuits Regulating Feeding Behavior

The hypothalamus plays a critical part in the regulation of homeostatic feeding. It integrates acute satiety and hunger cues and long-term adiposity signals from the periphery and responds by regulating the expression and secretion of selective intra- and extra-hypothalamic peptides and neurotransmitters that influence feeding responses and energy expenditure (Dietrich and Horvath 2009; Simpson et al. 2009). For example, postpandrial satiety is mediated by elevated levels of nutrients and peripheral appetite-suppressing hormones released into the circulation, which directly affect hypothalamic neurons to increase the anorexigenic tone. Adipocyte-derived leptin, pancreatic insulin, and the gut hormones, glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY), are some of the peripheral factors that are present in excess in the fed state and act in the hypothalamus to reduce food intake and increase energy expenditure. Under conditions of negative energy balance, levels of satiety factors are reduced and gastric secretion of the orexigenic ghrelin is increased, leading to the activation of hypothalamic signaling cascades that drive eating and reduce energy expenditure (Dietrich and Horvath 2009; Simpson et al. 2009). Several interconnected hypothalamic regions are involved in the regulation of energy homeostasis, including the arcuate nucleus (Arc), paraventricular nucleus (PVN), ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), and lateral hypothalamus (LH).

The Arc has close access to nutritional signals released into the circulation due to its proximity to fenestrated capillaries at the base of the hypothalamus (Cone et al. 2001). It contains two functionally distinct populations of neurons that express

the potent orexigenic neuropeptide Y (NPY) or proopiomelanocortin (POMC), a precursor for α-melanocyte stimulating hormone (α-MSH), the anorexigenic ligand of melanocortin receptor 4 (MC4-R) (Dietrich and Horvath 2009; Simpson et al. 2009). NPY cells co-express agouti-related protein (AgRP), an endogenous antagonist of MC4-R. POMC-containing neurons also synthesize the satiety factor cocaine and amphetamine-regulated transcript (CART). Levels of activity of NPY and POMC cells are associated with the metabolic state of the animal. Low energy levels lead to activation of NPY neurons and elevated NPY and AgRP expression and secretion, ultimately resulting in increased feeding and reduced energy expenditure. Conversely, positive energy balance is ensued by increased POMC neuron activity and α-MSH secretion, facilitating satiety and increased energy expenditure. NPY and POMC neurons are key cellular targets of leptin and other peripheral metabolic signals and project to intra- and extra-hypothalamic regions (Schwartz et al. 1996, 1997).

The PVN is a primary target of NPY and POMC neurons. This hypothalamic region contains cells expressing thyrotropin releasing hormone (TRH), corticotropin releasing hormone (CRH), urocortin, and oxytocin, all of which are involved in the regulation of energy homeostasis (Antoni et al. 1983; Kublaoui et al. 2008; Toni and Lechan 1993). The VMH and LH are also targets of NPY^+ and $POMC^+$ fibers and play paramount roles in appetite regulation. Whereas lesions to the VMH result in hyperphagia and obesity, destruction of the LH elicits hypophagia and weight loss (Anand and Brobeck 1951; Penicaud et al. 1983). VMH neurons project within the hypothalamus and to other brain regions including the bed nucleus of the stria terminalis and the amygdala (Canteras et al. 1994). Cells in the LH project to the DMH, VMH, and Arc and outside the hypothalamus including the ventral tegmental area (Leinninger et al. 2009; Saper et al. 1979). Similar to the Arc, cells in the VMH and LH contain receptors for nutritional signals, including leptin and ghrelin (Hakansson et al. 1998; Harrold et al. 2008). The LH also contains cells that synthesize the orexigenic factors melanin concentrating hormone (MCH) and hypocretin (Date et al. 1999; Zamir et al. 1986). Finally, the DMH has connections with the Arc, PVN, LHA, and VMH and contains both orexigenic and anorexigenic systems (Luiten and Room 1980).

The dorsal vagal complex (DVC) also participates in the homeostatic control of feeding. It is located in the caudal brain stem and comprises the area postrema, the nucleus of the solitary tract (NTS), and the dorsal motor nucleus of the vagus. The DVC interprets mechanosensory, chemosensory, and hormonal signals communicated by vagal nerve afferents from the gut, which primarily innervate the NTS to inform gastric distention and gut hormone and nutrient levels (Schwartz 2000). The DVC represents an alternate route for communicating energy status signals to the hypothalamus, with which it has reciprocal connections. It contains glucose-sensing mechanisms and receptors for leptin, insulin, α-MSH, and the gut peptide cholecystokinin (CCK), which mediates the acute inhibition of feeding (Williams et al. 2009). Melanocortin and leptin signaling in the DVC contribute to the control of food intake (Grill et al. 2002; Williams et al. 2000; Zheng et al. 2005).

In the absence of a homeostatic requirement, hedonic feeding can be driven by the highly rewarding qualities of palatable foods rich in sugar or fat. Hedonic feeding is controlled at least in part by the mesolimbic dopamine pathway, a regulator of motivated and reward-seeking behaviors. The mesolimbic system is composed of dopamine (DA) neurons in the ventral tegmental area (VTA) and their projections to the nucleus accumbens (NAc), medial prefrontal cortex (mPFC), and amygdala. This neural circuitry is a critical anatomical substrate for the behavioral effects of drugs of abuse and natural rewards such as food (Bassareo et al. 2002; Hernandez and Hoebel 1988; Rada et al. 2005). Indeed, ingestion of palatable food or sucrose increases DA transmission in the NAc and PFC (Bassareo and Di Chiara 1997, 1999; Ghiglieri et al. 1997). Moreover, novel palatable food consumption increases phosphorylation of dopamine and cAMP-regulated phosphoprotein (DARPP-32), an effect prevented by administration of D1 receptor antagonists (Rauggi et al. 2005).

Homeostatic and reward circuits in the brain can act in concert to impact appetitive behaviors. Indeed, caloric restriction augments the incentive salience and rewarding properties of food (Berthoud 2004). Their interactions are facilitated by reciprocal neural connections. Whereas GABAergic neurons in the NAc project to the LH and regulate hypocretin^{+} and MCH^{+} cells there, MCH neurons innervate cells in the NAc and regulate dopamine signaling (Baldo et al. 2004; Bittencourt et al. 1992; Pissios et al. 2008; Sears et al. 2010; Zheng et al. 2003). Additionally, neurons that contain leptin receptors in the LH directly innervate the VTA and influence activity of the mesolimbic dopamine pathway (Leinninger et al. 2009). Thus, reward and homeostatic systems in the brain are distinct yet interrelated pathways that interact to orchestrate feeding responses. Below we discuss how several neurotrophins act in these circuits to influence appetitive responses and energy expenditure.

3 BDNF

BDNF is a highly conserved member of the family of neurotrophins expressed in the developing and mature central nervous system. This multifunctional growth factor signals through the tropomyosin-related kinase B (TrkB) receptor and activates phospholipase C gamma (PLC-γ), mitogen-activated protein kinase (MAPK), and phosphatidylinositol-3 kinase (PI3-K) intracellular signaling cascades (Patapoutian and Reichardt 2001; Reichardt 2006). It plays essential roles in the differentiation, survival, and synaptic plasticity of several classes of neurons. A role of BDNF in the control of feeding behavior was first suggested by early rodent studies showing that chronic intracerebroventricular (ICV) delivery of BDNF induced reductions in body weight gain (Lapchak and Hefti 1992; Martin-Iverson et al. 1994; Pelleymounter et al. 1995). Subsequent investigations showed that BDNF$^{+/-}$ mutant mice are hyperphagic and obese, indicating the necessity of BDNF in appetite control (Kernie et al. 2000; Lyons et al. 1999). Similarly, mice that contain 25 % of normal TrkB levels due to carrying hypomorphic TrkB alleles

display excessive feeding (Xu et al. 2003). A closer examination of the meal microstructure of $BDNF^{+/-}$ mice revealed that their elevated body weights were related to increased meal number but normal meal size under standard chow conditions and increased meal size when administered a high fat diet (Fox and Byerly 2004). In addition to increases in body weight, BDNF mutant mice develop other aspects of the metabolic syndrome including leptin and insulin resistance, dyslipidemia, and hyperglycemia (Kernie et al. 2000; Rios et al. 2001). However, these metabolic parameters can be normalized when mutants attain normal body weights through food restriction, indicating that central BDNF is not required for glucose or lipid metabolism. Because selective depletion of BDNF in the mouse brain elicits hyperphagia and dramatic obesity (Rios et al. 2001), it is clear that this neurotrophin regulates appetitive behaviors by acting on central feeding circuits.

Findings from human studies also support a chief role of the BDNF/TrkB pathway in energy balance regulation. For example, a de novo missense mutation, Y722C, in the *TrkB* gene that impairs BDNF-induced MAP kinase activation was identified in an individual exhibiting severe hyperphagia and obesity (Yeo et al. 2004). Elevated levels of food intake and body weight were also reported in an 8-year-old female with monoallelic BDNF expression due to a de novo chromosomal inversion (Gray et al. 2006). Additional evidence comes from investigations of individuals afflicted with Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) syndrome due to large truncations within chromosome 11, which contains the human *Bdnf* gene. They showed that 100 % of WAGR patients rendered BDNF haploinsufficient by truncations encompassing the *Bdnf* gene were obese by 10 years of age (Han et al. 2008). In contrast, only 20 % of WAGR patients with intact *Bdnf* alleles developed obesity. Finally, several studies have linked the functional *Bdnf*Val66Met polymorphism to higher body mass index in humans (Beckers et al. 2008; Skledar et al. 2012; Speliotes et al. 2010; Thorleifsson et al. 2009). This highly prevalent mutation (Shimizu et al. 2004) impedes activity-dependent secretion and signaling of BDNF (Chen et al. 2006). These investigations include a recent association meta-analysis of nearly 250,000 individuals that identified *Bdnf* as a genetic locus linked to obesity susceptibility in humans (Speliotes et al. 2010).

Recent investigations have shed light onto the neural substrates mediating the appetite-suppressing effects of BDNF. In the hypothalamus, the VMH appears to be a critical target. Hypothalamic expression of BDNF is highest in this region, spans the dorsomedial, medial, and ventrolateral aspects of this nucleus, and is robustly regulated by energy status (Unger et al. 2007; Xu et al. 2003). Indeed, prolonged fasting results in a vast depletion of BDNF transcripts in this region. Moreover, glucose, a caloric signal, acts centrally to induce rapid elevations in BDNF and TrkB mRNA content in the VMH. Metabolic signals appear to preferentially influence BDNF expression in the VMH directed by promoter I and promoters II and IV to a lesser degree (Tran et al. 2006; Unger et al. 2007). Expression of BDNF mRNA in the VMH is also positively regulated by leptin and steroidogenic factor 1 (SF-1) (Komori et al. 2006; Tran et al. 2006). Leptin, the principal adipostatic hormone, reduces food intake and augments energy expenditure through activation

of the Janus kinase 2/STAT3 pathway in specific regions of the brain. Intravenous administration of leptin induces expression of BDNF mRNA primarily in the dorsomedial division of the VMH (Komori et al. 2006). SF-1 is a member of the NR5A subfamily of nuclear receptors and a transcription factor essential for VMH development and organization. It induces BDNF expression through interactions with *Bdnf* promoters I and IV (Tran et al. 2006), and its pattern of expression significantly overlaps that of BDNF in the VMH, particularly in the anterior portion of this nucleus (Tran et al. 2003). Neuronal precursors in the VMH of SF-1 null mice fail to terminally differentiate, resulting in aberrant afferent connections and cytoarchitecture (Tran et al. 2003, 2006). Because SF-1 mutants have deficient expression of BDNF in the VMH and in light of the well-demonstrated roles of BDNF in neuronal survival, differentiation, and synaptic connectivity, it is plausible that this neurotrophin mediates some of the effects of SF-1 in the developing VMH. Consistent with a supportive developmental role, BDNF transcripts are highly expressed in the rat fetal VMH starting at embryonic day 17 with expression peaking at postnatal day 4 (Sugiyama et al. 2003; Tran et al. 2003). BDNF mRNA content in the VMH then gradually decreases during the first postnatal week until reaching adult expression levels (Sugiyama et al. 2003).

It is clear that independently from effects that it might exert on developing feeding circuits, BDNF also contributes prominently to the control of appetite in the mature animal. In agreement, central and systemic administration of BDNF mitigated body weight gain and improved glucose metabolism in various models of obesity including leptin (*ob*/*ob*) and leptin receptor (*db*/*db*)-deficient mice (Nakagawa et al. 2000; Tonra et al. 1999). Furthermore, BDNF infusion into the VMH of adult wild-type rats resulted in decreased food intake and body weight (Wang et al. 2007c). Finally, mice that had intact levels of BDNF throughout development but deletion of *Bdnf* in the VMH in adulthood exhibited increases in standard chow intake and body weight (Unger et al. 2007). These findings indicate that BDNF acts as a required satiety factor in the adult brain and that the VMH is an essential source of this neurotrophin for food intake control.

The melanocortin system is a predominant mediator of leptin signaling and plays an instrumental part in the regulation of energy balance through actions in the hypothalamus. In the VMH, the anorexigenic effects of this signaling pathway are partly mediated by BDNF. Indeed, α-MSH-containing fibers originating in the Arc terminate in the VMH, where they positively regulate BDNF expression via activation of MC4-R (Xu et al. 2003). Accordingly, mouse models of obesity due to impaired MC4-R signaling, including A^y lethal yellow and MC4-R null mice, exhibit reduced BDNF mRNA expression in the VMH (Xu et al. 2003). Moreover, application of the selective MC4-R agonist, MK1, to isolated rat hypothalamus induced BDNF secretion, and this effect was blocked by a MC4-R antagonist (Nicholson et al. 2007). In vivo studies indicate the functional relevance of BDNF in the anorexigenic effects of melanocortin signaling. For example, ICV infusion of BDNF mitigated the hyperphagia, and excessive body weight gain in MC4-R-deficient mice administered a high fat diet (Xu et al. 2003). Additionally, ICV administration of an anti-BDNF antibody counteracted the satiety effect of

peripheral administration of MK1 (Nicholson et al. 2007). The facilitative effect of BDNF on melanocortin signaling has clinical relevance as MC4-R mutations are a frequent cause of morbid obesity in humans, accounting for up to 5 % of cases (Farooqi et al. 2000; Vaisse et al. 2000).

The PVN contains BDNF and TrkB (Xu et al. 2003; Yan et al. 1997) and is also a substrate for the anorexigenic actions of this neurotrophin signaling pathway. Evidentiary is the finding that selective delivery of BDNF to the PVN in rats reduced food intake by 32 % compared to vehicle administration (Wang et al. 2010b). The effects of BDNF in this region appear to involve the CRH pathway, which is known to suppress feeding and increase sympathetic activity, which positively impacts energy expenditure. Supporting evidence includes the findings that TrkB receptors co-localize with CRH and that ICV infusion of BDNF results in elevated levels of CRH in the PVN (Toriya et al. 2010). Notably, coadministration of the CRH receptor 1 and 2 antagonist, α-helical-$CRH_{9\text{-}41}$, abrogated the satiety effects of BDNF. Moreover, reductions in subcutaneous, perirenal, mesenteric, and epididymal fat pad weights and serum triglyceride levels induced by BDNF treatment were also abolished by co-delivery of α-helical-$CRH_{9\text{-}41}$. BDNF also increases expression of PVN urocortin, a member of the CRH family that suppresses appetite and reduces body weight more potently than CRH (Toriya et al. 2010). This observation suggests that BDNF might recruit the urocortin pathway to inhibit feeding. Consistent with this idea, BDNF-induced anorexia was significantly attenuated by blockade of CRH R2, which has high affinity for urocortin but low affinity for CRH (Toriya et al. 2010). BDNF also appears to influence PVN oxytocin-containing neurons that also play a part in energy balance regulation (Kublaoui et al. 2008). In vitro studies involving isolated hypothalamic oxytocinergic neurons demonstrated that BDNF promotes their survival and triggers oxytocin release (Kusano et al. 1999; Moreno et al. 2011). BDNF synthesized in the PVN could act in TrkB receptors expressed locally to regulate CRH, urocortin, and oxytocin signaling pathways. Alternatively, BDNF synthesized in the VMH could act as an anterograde signal in the PVN, which contains VMH fibers.

Cells in the Arc do not synthesize BDNF, but TrkB receptors and BDNF-containing nerve fibers are present in this region (Xu et al. 2003; Yan et al. 1997), suggestive of neurotrophin signaling involvement in local processes underlying appetitive responses. The role of BDNF there, however, remains elusive. Immunohistochemical studies of NPY and POMC-containing neurons in the Arc of mice centrally depleted of BDNF ($BDNF^{2L/2LCk\text{-}cre}$) showed no gross differences compared to wild-type animals (Rios et al. 2001). However, studies by Wang et al. suggest that BDNF might regulate NPY neurons in the Arc. These investigators showed that BDNF infusion into the PVN prevents elevations in NPY expression in the Arc induced by fasting and notably reduces NPY-induced feeding (Wang et al. 2007b). Of note, the density of excitatory and inhibitory inputs onto NPY and POMC neurons in the Arc is dynamically regulated in opposite ways by the metabolic state of the animal (Pinto et al. 2004; Sternson et al. 2005). For example, the density and strength of excitatory inputs from the VMH to appetite-inhibiting

POMC neurons in the Arc is reduced in the fasted state and restored following refeeding (Sternson et al. 2005). BDNF is a known facilitator of structural and synaptic plasticity in the cerebral cortex, hippocampus, and cerebellum (Carter et al. 2002; Korte et al. 1995; McAllister et al. 1997). Therefore, it is plausible that it might also participate in synaptic remodeling processes in the Arc that promote satiety. This possibility warrants further examination.

Studies assessing the role of BDNF in the LH and DMH, both of which express BDNF and TrkB, are rather limited but seem to suggest that these are not critical substrates for the satiety effects of this neurotrophin. Immunohistochemical studies in our laboratory indicate that the MCH and hypocretin neuronal populations in the LH of $BDNF^{2L/2LCk\text{-}cre}$ mutant mice are not significantly altered (Rios et al. 2001), suggesting that BDNF is not required for their survival or maturation. Furthermore, Wang et al. (2007b) reported that a single injection of BDNF to the LH did not affect food intake or body weight. Because the effect of chronic BDNF delivery has not been investigated, the possibility remains that BDNF might influence feeding through long-term mechanisms acting in the LH. In the DMH, expression of leptin receptors and CART mRNA appears intact in BDNF haploinsufficient mice (Kernie et al. 2000). Furthermore, in contrast to the VMH and PVN, expression of BDNF mRNA in the DMH is not influenced by caloric signals (Unger et al. 2007).

In addition to controlling caloric intake, the hypothalamus regulates efferent autonomic pathways that impact metabolism and energy expenditure. For example, cells in the VMH and PVN project to sympathetic and parasympathetic areas of the medulla and spinal cord that regulate autonomic nervous system function. The role of hypothalamic BDNF in the regulation of components of energy expenditure, including basal metabolic rate and thermogenesis, remains somewhat unclear. Because pair feeding is sufficient to normalize body weights of $BDNF^{+/-}$ mutants and mice with depletion of BDNF in the VMH (Coppola and Tessarollo 2004; Unger et al. 2007), alterations in energy expenditure do not appear to contribute to the obesity of BDNF mutants. However, consistent with a role in energy expenditure, selective BDNF administration into the VMH or PVN resulted in elevated basal metabolic rate (Wang et al. 2007a, 2010a). Other studies implicate BDNF in central processes enhancing thermogenesis. Normally, uncoupling protein 1 (UCP1) in brown adipose tissue uncouples fatty acid oxidation from ATP production, dissipating energy in the form of heat (Enerback et al. 1997). It was reported that ICV BDNF administration in obese *db*/*db* mice enhanced norepinephrine turnover and UCP1 expression in brown adipose tissue, suggesting positive regulation of thermogenesis and energy expenditure (Nonomura et al. 2001; Tsuchida et al. 2001). Furthermore, Cao et al. (2011) showed that overexpression of BDNF in the rodent hypothalamus resulted in the activation of brown fat transcriptional programs in white fat cells, inducing a phenotypic white to brown cellular switch that promoted energy expenditure and resistance to diet-induced obesity.

While considering the conflicting evidence pertaining to the role of BDNF in energy expenditure, it is important to note that the reported elevated level of locomotor activity in $BDNF^{+/-}$ and $BDNF^{2L/2LCk\text{-}cre}$ mutant mice (Kernie et al. 2000;

Rios et al. 2001) is a confounding factor. Deficits in the basal metabolic rate of these mutants might be masked by increases in locomotor activity that also contribute to energy expenditure. However, this does not appear to be the case for mice with selective BDNF depletion in the VMH as they show normal levels of activity and normalized body weights when pair fed with control mice (Unger et al. 2007). After considering the cumulative evidence, it is reasonable to conclude that in the hypothalamus, BDNF plays a critical part in the control of homeostatic food intake and an important but likely nonessential role in the regulation of energy expenditure.

The satiety effects of BDNF are not restricted to the hypothalamus and also involve the DVC. Whereas BDNF-containing cell bodies and fibers are present in the NTS, $TrkB^+$ cells are located in the NTS and area postrema (Conner et al. 1997; Yan et al. 1997). Consistent with a role in local processes impacting appetite control, BDNF protein levels in the DVC are reduced by fasting and induced by refeeding (Bariohay et al. 2005). Importantly, infusion of this neurotrophin into the DVC of rats significantly decreased daily food intake and cumulative body weight gain (Bariohay et al. 2005). As it is the case in the ventromedial hypothalamus, BDNF appears to be a downstream effector of melanocortin signaling in the DVC. Accordingly, MC4-R stimulation induces BDNF expression in this region, and pharmacological blockade of TrkB abolishes the anorexigenic effect of MC4R agonists in the DVC (Bariohay et al. 2005, 2009). Notably, the hyperphagia induced by MC4-R blockade via delivery of antagonists to the fourth ventricle can be prevented by coadministration of BDNF. It is also important to note that CCK and leptin, which promote satiety through interactions with the melanocortin system in the DVC (Fan et al. 2004; Matson et al. 1997), also induce BDNF expression in this region (Bariohay et al. 2009), suggesting that BDNF might mediate their effects.

Obesity is associated with the development of insulin resistance and type 2 diabetes. Lipid accumulation in nonadipose tissues such as liver, muscle, and pancreas is believed to greatly contribute to this outcome. BDNF has been shown to alleviate obesity-related metabolic disease independently of mechanisms that mediate appetite suppression. For example, chronic subcutaneous administration of BDNF in obese *db*/*db* mice triggered significant reductions in serum concentrations of non-esterified free fatty acid, total cholesterol, and phospholipids compared to pair-fed, vehicle-treated *db*/*db* mice (Tsuchida et al. 2002). Furthermore, liver triglyceride content, an indicator of hepatic fat accumulation, and hepatomegaly were also significantly reduced in BDNF-treated *db*/*db* mice. In addition to its beneficial effects on lipid metabolism, BDNF also improves glucose homeostasis in animal models of obesity including *ob*/*ob* and *db*/*db* mice, and this effect persists for weeks after BDNF treatment cessation (Tonra et al. 1999). The mechanisms underlying the facilitative effects of BDNF on glucose and lipid homeostasis remain to be fully elucidated. However, central neural circuits appear to be involved because infusion of BDNF directly into the brain is also efficacious in improving blood glucose control (Nonomura et al. 2001). The effects of BDNF are also mediated by increases in insulin sensitivity in the liver (Kuroda et al. 2003),

a tissue highly susceptible to chronic increases in dietary fat intake, which elicits hepatic steatosis, reduced hepatic insulin sensitivity, and a concomitant failure to suppress liver glucose output. The pattern of BDNF and TrkB expression suggests neurotrophic support of the autonomic innervation of the liver. In adult mice, whereas BDNF mRNA is expressed in mouse hepatocytes (Lommatzsch et al. 1999), TrkB is expressed by periductal nerve fibers innervating the liver (Garcia-Suarez et al. 2006).

As noted earlier, food intake is a complex behavior governed not only by homeostatic systems but also by reward-related processes that promote intake of palatable food with high caloric content. BDNF and TrkB participate in the regulation of hedonic feeding through the positive regulation of the mesolimbic dopamine pathway. BDNF is expressed in dopamine neurons in the VTA and in pyramidal neurons in the mPFC from which it is anterogradely transported to the NAc, a region with minimal BDNF expression (Numan et al. 1998; Numan and Seroogy 1999; Okazawa et al. 1992). TrkB is expressed in VTA dopaminergic neurons, mPFC, and GABAergic medium spiny-projection neurons in the NAc (Freeman et al. 2003; Numan et al. 1998; Numan and Seroogy 1999; Yan et al. 1997). Similar to their behavior under standard chow conditions, $BDNF^{2L/2LCk\text{-}cre}$ mutant mice exhibit a twofold increase in food intake when they have free access to a palatable high fat diet (Cordeira et al. 2010). Interestingly, their hyperphagia is further exacerbated by intermittent access to high fat food. Alterations in the mesolimbic reward pathway accompany the abnormal eating behavior of these BDNF mutant mice. For example, amperometric recordings in acute brain slices revealed deficient evoked DA release in the NAc shell but not in the NAc core in BDNF mutants. This deficit persisted in the presence of nomifensine, a dopamine transporter inhibitor, indicating that this alteration was due to reduced dopamine secretion rather than increased reuptake of dopamine.

Diminished mesolimbic dopamine transmission and signaling underlies the overeating of high fat food triggered by depleted BDNF signal in the brain. In support of this assertion, stimulation of dopamine 1 (D1) receptors with a selective agonist in $BDNF^{2L/2LCk\text{-}cre}$ mutants completely normalized their high fat food intake (Cordeira et al. 2010). Notably, mice with selective deletion of *Bdnf* in the adult VTA became hyperphagic and obese when they had ad libitum access to palatable high fat food (Cordeira et al. 2010). In contrast, they showed no significant changes in food intake when fed a standard chow diet compared to controls. The diet-specific effects of deleting *Bdnf* in the VTA suggest that BDNF signaling in the mesolimbic system is essential for the regulation of hedonic but not of homeostatic feeding.

Hypoactivity of the mesolimbic dopamine system was associated previously with excessive food reward seeking. For example, hyperphagic and obese *ob*/*ob* mice also exhibit decreased extracellular levels of dopamine in the NAc (Fulton et al. 2006). Moreover, significant reductions in their food intake and body weight were observed following administration of D1 receptor agonists (Bina and Cincotta 2000). Additional evidence comes from a human study showing that obese subjects exhibited decreased striatal activity compared to lean subjects in response to

consumption of palatable food as measured by functional MRI (Stice et al. 2008). Based on these and other findings, it has been proposed that decreased dopamine tone might produce a reward deficiency syndrome that, behaviorally, manifests as compensatory overeating to boost dopamine transmission (Blum et al. 2000). In agreement, chronic high fat food consumption was sufficient to normalize decreased dopamine signaling in the NAc of Δ-FosB overexpressing mice, which also exhibited increased instrumental responding to food reward (Teegarden et al. 2008).

The disease mechanisms leading to reduced mesolimbic dopamine secretion in BDNF mutants remain unclear. BDNF is not essential for the survival of VTA dopamine neurons (Baquet et al. 2005) or for dopamine synthesis in these cells (Cordeira et al. 2010). Because expression of TrkB mRNA in the VTA of sated wild-type mice is increased by intake of palatable high fat food (Cordeira et al. 2010), it is plausible that BDNF might act to regulate the excitability of VTA dopamine neurons during food reward-related processes. In agreement with this idea, Pu et al. (2006) showed that BDNF is essential for the potentiation of excitatory synapses onto VTA dopamine neurons following cocaine withdrawal in rats. Moreover, chronic food restriction in rats results in both decreased TrkB protein expression in the VTA and reduced glutamatergic transmission in VTA dopamine neurons (Pan et al. 2011). It is worth noting that food restriction decreases mesolimbic dopamine secretion in rodents (Pothos et al. 1995a, b) and is considered a high risk factor for eating disorders in humans (Herman and Polivy 1990; Ledoux et al. 1993). Thus, the findings described above raise the interesting possibility that perturbed BDNF signaling underlies decreases in dopamine tone induced by food restriction that might lead to maladaptive behaviors, including disordered eating. Interestingly, a recent study involving adolescent girls showed that carriers of the *Bdnf*Val66Met allele that engaged in food restriction were more likely to exhibit binge eating than wild-type carriers (Akkermann et al. 2011). These data suggest an interaction of food restriction and diminished BDNF signaling in the emergence of eating disorders. A role of BDNF in binge eating is also suggested by a reported significant association of the *Bdnf*Val66Met allele with increased frequency and severity of bingeing in a population of Caucasian females diagnosed with bulimia nervosa or binge eating disorder (Monteleone et al. 2006).

In summary, the collective data indicate that central BDNF plays fundamental roles in the regulation of feeding behavior and body weight. It acts in energy balance regulatory centers in the hypothalamus and DVC to provide essential regulatory signals influencing homeostatic feeding. Furthermore, it regulates the mesolimbic dopamine pathway to control hedonic feeding.

4 NGF, NT-3, and NT-4

NGF, NT-3, and NT-4 are also members of the family of neurotrophins and are structurally related to BDNF. Their effects are mediated by their high affinity tyrosine kinase receptors TrkA, TrkC, and TrkB, respectively, and the low affinity

receptor p75. Even though their roles in body weight regulation have been under studied, some reports implicate them in the regulation of food intake. For example, excessive weight gain in adult rats with altered cholinergic activity due to partial fimbrial transections was ameliorated by NGF treatment (Lapchak and Hefti 1992). Moreover, ICV NGF infusion induced hypophagia and weight loss in a dose-dependent manner in non-obese mice (Williams 1991). However, a conflicting study showed that ICV administration of NGF in $BDNF^{+/-}$ mice was ineffective in reducing their elevated body weights (Kernie et al. 2000). In humans, a single nucleotide polymorphism (SNP) within the NGF gene was not significantly associated with eating disorders in families of Spanish, French, and German origin (Mercader et al. 2008). However, the risk of developing eating disorders in individuals with a TrkC SNP was significantly increased if they also carried the NGF SNP, suggesting an epistatic interaction between these genes in disease mechanisms relevant to disordered eating.

Rodent studies indicate that NT-3/TrkC signaling does not play an essential role in energy balance regulation. Accordingly, mice with central depletion of this neurotrophin or with overexpression of TrkC exhibit normal body weights (Bates et al. 1999; Dierssen et al. 2006). However, as noted above, a SNP in the TrkC gene located in intron 8 and predicted to result in lower TrkC expression was significantly associated with eating disorders (Mercader et al. 2008). Moreover, elevated levels of TrkC expression were observed in the hypothalamus of the *anx*/*anx* mouse model of anorexia (Mercader et al. 2008). It is important to note that eating disorders are multifactorial psychiatric afflictions, the foundation of which might not exactly parallel that of energy balance disorders. In that context, it is interesting to note that features such as anxiety and depression, which have been associated with altered NT-3 function (Amador-Arjona et al. 2010; Dierssen et al. 2006; Hock et al. 2000), are comorbid with eating disorders.

The effects of NT-4 on feeding behavior are slightly clearer. Data supporting a role in the underlying physiological processes include its ability to mitigate the hyperphagia and excessive body weight gain in $BDNF^{+/-}$ mice following intracerebral infusion into the third ventricle (Kernie et al. 2000). Similarly, daily intravenous delivery of NT-4 in a mouse model of diet-induced obesity or central administration in rhesus monkeys reduced food intake and body weight (Tsao et al. 2008). NT-4-induced anorexia is not leptin-dependent as this neurotrophin was equally efficacious in obese *db*/*db* mice, which lack leptin receptors (Tsao et al. 2008). The weight loss of NT-4-treated mice could not be solely attributed to reduced food intake. Indeed, vehicle-treated obese mice that were pair fed with NT-4 treated animals did not exhibit the same amplitude of weight loss as the latter, suggesting that NT-4 also influences energy expenditure.

Albeit sharing the TrkB receptor with BDNF, NT-4 exhibits distinct effects on food intake and body weight and does not play an essential role in the regulation of energy balance. In contrast to the dramatic levels of obesity exhibited by BDNF and TrkB mutant mice, NT-4 null mutants have normal body weights (Fox et al. 2001). However, it is worth noting that a close examination of their meal microstructure revealed longer meal duration when administered a standard chow diet

(Fox et al. 2001). This alteration does not impact overall food intake or body weight because it is accompanied by compensatory behaviors, including reduced meal frequency and decreased average rate of food intake. However, intermittent access to high fat food can override these compensatory changes, ultimately resulting in long-term increases in total food intake (Byerly and Fox 2006). Because direct application of NT-4 to the hypothalamus also resulted in appetite suppression (Tsao et al. 2008), alterations in hypothalamic feeding circuits might be responsible for the observed changes in eating behavior. Alternatively, increased feeding in NT-4 mutant mice might be associated with identified deficits in vagal intraganglionic mechanoreceptors in the small intestine (Fox et al. 2001). This defect is potentially relevant because alterations in vagal sensory neurons innervating the gastrointestinal tract have been implicated in obesity and eating disorders (Faris et al. 2000; Schwartz 2000). Mechanoreceptors in the vagal sensory pathway are thought to sense peristaltic contractions induced by food accumulation in the duodenum and subsequently provide negative feedback to energy balance regulating centers in the hindbrain to mediate meal termination. A study directly interrogated the negative feedback action of gastrointestinal vagal afferents in NT-4 mutant mice in response to fat and carbohydrates. These investigations revealed that in response to lipid intragastric infusion, NT-4 mutant mice had smaller reductions in standard chow intake compared to wild-type mice, indicating attenuated satiation due to reduced negative feedback signaling in response to macronutrients in the GI tract (Chi and Powley 2007).

The distinct effects of BDNF and NT-4 might be explained by their unique patterns of expression in energy balance and reward centers of the brain. Alternatively, they might diverge in the conformational changes they induce in TrkB following binding, effectively influencing the strength of downstream signaling in pathways recruited during feeding-related processes. In agreement with the first scenario, knocking NT-4 into the *Bdnf* locus resulted in mice with a 30 % decrease in body weight compared to littermate wild-type controls (Fan et al. 2000). In agreement with the alternative model, TrkB-Shc signaling appears to be more critical for NT-4 mediated functions than for BDNF effects (Fan et al. 2000). Nonetheless, it is clear that endogenous NT-4 is not sufficient to overcome the hyperphagia and obesity triggered by deficient BDNF signaling in the brain. Together, the cumulative data indicate significant but nonessential roles of NT-4 in the regulation of body weight.

5 CNTF

CNTF is a neurocytokine from the interleukin 6 family expressed both in the peripheral and central nervous systems during development and in adulthood (Ip and Yancopoulos 1996). It supports the survival of several types of neurons and glia. The actions of CNTF are mediated by a tripartite receptor complex comprising gp130, the LIF β receptor, and the CNTF-α receptor. Together, they activate signaling cascades involving protein tyrosine kinases of the Jak family and

STAT transcription factors. The distribution of the CNTFR-α-gp130-β complex is similar to that of the leptin receptor including expression in the Arc and PVN (MacLennan et al. 1996). Moreover, signaling pathways downstream of CNTF and leptin are similar, most notably the STAT3 pathway, which plays a critical part in the regulation of caloric intake and expenditure (Xu et al. 2007). These parallels prompted investigations interrogating the role of CNTF in the central regulation of body weight. They revealed that chronic CNTF treatment was efficacious in ameliorating several aspects of the metabolic syndrome in *ob*/*ob* and *db*/*db* mice including their hyperphagia, increased adiposity, and hyperinsulinemia (Gloaguen et al. 1997). The satiety effects of CNTF are partly mediated by the negative regulation of NPY in the Arc (Xu et al. 1998). Accordingly, the increase in hypothalamic NPY expression normally induced by fasting was absent in animals treated with CNTF. Moreover, CNTF administration markedly reduced NPY-induced feeding. Thus, the data indicate a double action of CNTF that prevents both NPY expression and events downstream of NPY that promote eating.

Because the appetite-suppressing effects of CNTF persist long after cessation of treatment, Kokoeva et al. sought to investigate whether this neurotrophin had long lasting effects on hypothalamic cells. They discovered that similar to the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricles, there is persistent neurogenesis albeit at lower levels, in the adult hypothalamus (Kokoeva et al. 2005). Notably, they uncovered a potent mitogenic effect of CNTF in this process. Specifically, they found that when the cell proliferation marker bromodeoxyuridine (BrdU) was chronically co-delivered with CNTF into the lateral ventricles, there was a dramatic increase of newborn cells as marked by BrdU immunoreactivity in the hypothalamus compared to vehicle treatment. These adult-generated cells express the CNTF receptor, arise from the hypothalamic parenchyma, and migrate to the base of the third ventricle, a region that includes the Arc. A significant proportion of new cells follow a neuronal fate differentiation pathway, and some express the orexigenic factor NPY or the anorexigenic peptide POMC (Kokoeva et al. 2005). A subset of them is responsive to leptin, suggesting that they might be recruited into functional feeding circuits in the hypothalamus. Because elevated levels of hypothalamic $BrdU^+$ cells persist weeks after CNTF treatment cessation, it is possible that the facilitative effect of CNTF on adult hypothalamic neurogenesis is a mechanism underlying its prolonged effects on appetite suppression. In agreement with this idea, ICV co-delivery of the antimitotic drug, cytosine-β-δ-arabinofuranoside (Ara-C), with CNTF, abrogated the long-term suppression of appetite by this neurotrophin without affecting induced acute decreases in food intake (Kokoeva et al. 2005). The effects of Ara-C did not appear to be related to neurotoxicity but to suppression of cell proliferation.

Similar to BDNF, CNTF can facilitate metabolism in obesity-related disorders. It acts in the periphery to reverse fatty acid-induced insulin resistance. This is achieved by increasing fatty acid oxidation and reducing insulin resistance in skeletal muscle by activating AMP-activated protein kinase (Watt et al. 2006). In the *db*/*db* model of obesity and diabetes, CNTF treatment significantly improved glucose and lipid metabolism (Sleeman et al. 2003). CNTF-treated *db*/*db* mice

showed reduced hepatic steatosis, improved liver function, and enhanced hepatic responsiveness to insulin. These metabolic improvements were accompanied by changes in expression of genes involved in lipid metabolism including reduced expression of SCD-1, a rate limiting enzyme in lipid synthesis, and increased expression of CPT-1, which facilitates lipid oxidation (Sleeman et al. 2003). The beneficial effects of CNTF on metabolic disease do not emerge exclusively from reduced food intake and body weight as pair fed vehicle-treated *db*/*db* mice did not exhibit the same level of metabolic improvement as CNTF-treated mutants.

The long lasting anorexigenic actions of CNTF and its ability to alleviate obesity-related metabolic disease have made it an attractive target for drug development. An analog of CNTF, Axokine (Regeneron), underwent Phase III clinical trials for the treatment of obesity. A modest effect on weight loss was observed compared to placebo. However, a majority of treated individuals developed neutralizing antibodies against Axokine, and the drug was not commercialized. Nonetheless, the cumulative evidence is consistent with an important role of CNTF in central mechanisms mediating satiety and body weight control.

6 GDNF

GDNF supports the survival and maturation of central dopamine neurons (REF). It binds GFR-α receptors to form a signaling complex that recruits and activates the receptor tyrosine kinase, rearranged during transfection (Ret). Studies involving chronic central delivery of GDNF in rodents, monkeys, and humans serendipitously found that this neurotrophic factor induced weight loss (Aoi et al. 2000; Hoane et al. 1999; Lapchak et al. 1997). Follow-up investigations showed that viral-mediated delivery of GDNF to the hypothalamus in rats also promoted body weight reduction (Tumer et al. 2006), suggesting that this brain region was a relevant substrate for the actions of GDNF. Weight and adipocity loss in GDNF-treated rats was associated with reduced food intake and increased energy expenditure, but these effects were transient. The effects of GDNF were not associated with altered dopamine levels in the hypothalamus, a region with low levels of expression of components of the GDNF signaling complex, Ret and GFR-α1. This observation raised the possibility that excess GDNF signal in hypothalamic neurons of AAV-GDNF-treated rats acted in extra hypothalamic areas where these cells project, including midbrain dopamine neurons. Consistent with this idea, AAV-GDNF delivery to the midbrain (substantia nigra) elicited decreases in food intake and body weight that were more robust than those induced by delivery to the hypothalamus (Manfredsson et al. 2009). Furthermore, a single injection of GDNF into the substantia nigra had an anorexigenic effect that lasted 7–10 days (Hudson et al. 1995). This effect was associated with neurite sprouting in tyrosine hydroxylase (TH)-containing neurons in this region and increased TH immunoreactivity in the striatum. Finally, intranigrally or intraventricularly administered GDNF partially rescued the weight gain induced by 6-hydroxydopamine-induced lesions in rats (Lapchak and Hefti 1992).

The studies outlined above implicate the nigrostriatal pathway in the anorexigenic actions of GDNF. However, it is important to note that in at least one of the studies involving AAV-GDNF delivery to the midbrain, elevated levels of dopamine were detected in the NAc (Manfredsson et al. 2009). Therefore, a contribution of the mesolimbic dopamine pathway cannot be ruled out.

The effects of GDNF on feeding might be related to its ability to potentiate midbrain dopamine circuits. It increases the excitability of dopamine neurons in vitro by inhibition of A-type potassium channels (Yang et al. 2001). Moreover, GDNF synthesized in the NAc acts as a retrograde signal for dopamine neurons in the VTA, where it positively regulates spontaneous firing activity through a mechanism involving the mitogen-activated protein kinase (MAPK) pathway (Wang et al. 2010c). This GDNF-mediated facilitation results in an increase in dopamine secretion in the NAc. Consistent with a critical role in dopamine tone, Ret ablation results in decreased evoked release of dopamine (Kramer et al. 2007). Clearly, much remains to be unraveled regarding the effects of GDNF. For now, we can conclude that this neurotrophin participates in the regulation of feeding behavior likely through mechanisms involving dopamine transmission.

7 Summary

Obesity is a pervasive disorder reaching epidemic proportions that contributes to the burden of chronic disease and disability. It can arise from alterations in central neural circuits that promote positive energy balance and weight gain. Several neurotrophins have emerged as chief players in the complex mechanisms regulating food intake and energy expenditure and in candidate disease processes driving obesity. BDNF, for example, is a required satiety factor that participates in homeostatic mechanisms in the hypothalamus and hindbrain balancing nutritional requirements and energy status. This neurotrophin also inhibits hedonic feeding via the positive regulation of the mesolimbic dopamine reward pathway. NT-4, which shares the TrkB receptor with BDNF, also influences feeding responses but is not essential for maintaining normal body weight, illustrating the complexity of neurotrophin signaling and food intake regulation. CNTF and GDNF, for their part, also play important roles in the regulation of appetitive behaviors, and the hypothalamic and midbrain dopamine neurons, respectively, appear to be substrates for their anorexigenic actions. The molecular and cellular underpinnings of neurotrophin action influencing energy balance and body weight are critical but largely uncharted research areas that require further investigation. Attaining this mechanistic understanding will potentially uncover novel therapeutic avenues for the treatment and prevention of obesity and its associated medical afflictions.

References

Akkermann K, Hiio K, Villa I, Harro J (2011) Food restriction leads to binge eating dependent upon the effect of the brain-derived neurotrophic factor Val66Met polymorphism. Psychiatry Res 185:39–43

Amador-Arjona A, Delgado-Morales R, Belda X, Gagliano H, Gallego X, Keck ME, Armario A, Dierssen M (2010) Susceptibility to stress in transgenic mice overexpressing TrkC, a model of panic disorder. J Psychiatr Res 44:157–167

Anand BK, Brobeck JR (1951) Localization of a "feeding center" in the hypothalamus of the rat. Proc Soc Exp Biol Med 77:323–324

Antoni FA, Palkovits M, Makara GB, Linton EA, Lowry PJ, Kiss JZ (1983) Immunoreactive corticotropin-releasing hormone in the hypothalamoinfundibular tract. Neuroendocrinology 36:415–423

Aoi M, Date I, Tomita S, Ohmoto T (2000) Single or continuous injection of glial cell line-derived neurotrophic factor in the striatum induces recovery of the nigrostriatal dopaminergic system. Neurol Res 22:832–836

Baldo BA, Gual-Bonilla L, Sijapati K, Daniel RA, Landry CF, Kelley AE (2004) Activation of a subpopulation of orexin/hypocretin-containing hypothalamic neurons by GABAA receptor-mediated inhibition of the nucleus accumbens shell, but not by exposure to a novel environment. Eur J Neurosci 19:376–386

Baquet ZC, Bickford PC, Jones KR (2005) Brain-derived neurotrophic factor is required for the establishment of the proper number of dopaminergic neurons in the substantia nigra pars compacta. J Neurosci 25:6251–6259

Bariohay B, Lebrun B, Moyse E, Jean A (2005) Brain-derived neurotrophic factor plays a role as an anorexigenic factor in the dorsal vagal complex. Endocrinology 146:5612–5620

Bariohay B, Roux J, Tardivel C, Trouslard J, Jean A, Lebrun B (2009) Brain-derived neurotrophic factor/tropomyosin-related kinase receptor type B signaling is a downstream effector of the brainstem melanocortin system in food intake control. Endocrinology 150:2646–2653

Bassareo V, Di Chiara G (1997) Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. J Neurosci 17:851–861

Bassareo V, Di Chiara G (1999) Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. Eur J Neurosci 11:4389–4397

Bassareo V, De Luca MA, Di Chiara G (2002) Differential expression of motivational stimulus properties by dopamine in nucleus accumbens shell versus core and prefrontal cortex. J Neurosci 22:4709–4719

Bates B, Rios M, Trumpp A, Chen C, Fan G, Bishop JM, Jaenisch R (1999) Neurotrophin-3 is required for proper cerebellar development. Nat Neurosci 2:115–117

Beckers S, Peeters A, Zegers D, Mertens I, Van Gaal L, Van Hul W (2008) Association of the BDNF Val66Met variation with obesity in women. Mol Genet Metab 95:110–112

Berridge KC (2009) 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. Physiol Behav 97:537–550

Berthoud HR (2004) Neural control of appetite: cross-talk between homeostatic and non-homeostatic systems. Appetite 43:315–317

Bina KG, Cincotta AH (2000) Dopaminergic agonists normalize elevated hypothalamic neuropeptide Y and corticotropin-releasing hormone, body weight gain, and hyperglycemia in ob/ob mice. Neuroendocrinology 71:68–78

Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, Vale W, Sawchenko PE (1992) The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. J Comp Neurol 319:218–245

Blum K, Braverman ER, Holder JM, Lubar JF, Monastra VJ, Miller D, Lubar JO, Chen TJ, Comings DE (2000) Reward deficiency syndrome: a biogenetic model for the diagnosis and

treatment of impulsive, addictive, and compulsive behaviors. J Psychoactive Drugs 32 Suppl: i–iv, 1–112

Byerly MS, Fox EA (2006) High-fat hyperphagia in neurotrophin-4 deficient mice reveals potential role of vagal intestinal sensory innervation in long-term controls of food intake. Neurosci Lett 400:240–245

Canteras NS, Simerly RB, Swanson LW (1994) Organization of projections from the ventromedial nucleus of the hypothalamus: a Phaseolus vulgaris-leucoagglutinin study in the rat. J Comp Neurol 348:41–79

Cao L, Choi EY, Liu X, Martin A, Wang C, Xu X, During MJ (2011) White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. Cell Metab 14:324–338

Carter AR, Chen C, Schwartz PM, Segal RA (2002) Brain-derived neurotrophic factor modulates cerebellar plasticity and synaptic ultrastructure. J Neurosci 22:1316–1327

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314:140–143

Chi MM, Powley TL (2007) NT-4-deficient mice lack sensitivity to meal-associated preabsorptive feedback from lipids. Am J Physiol Regul Integr Comp Physiol 292:R2124–R2135

Cone RD, Cowley MA, Butler AA, Fan W, Marks DL, Low MJ (2001) The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. Int J Obes Relat Metab Disord 25 (Suppl 5):S63–S67

Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S (1997) Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. J Neurosci 17:2295–2313

Coppola V, Tessarollo L (2004) Control of hyperphagia prevents obesity in BDNF heterozygous mice. Neuroreport 15:2665–2668

Cordeira JW, Frank L, Sena-Esteves M, Pothos EN, Rios M (2010) Brain-derived neurotrophic factor regulates hedonic feeding by acting on the mesolimbic dopamine system. J Neurosci 30: 2533–2541

Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, Sakurai T, Yanagisawa M, Nakazato M (1999) Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. Proc Natl Acad Sci U S A 96: 748–753

Dierssen M, Gratacos M, Sahun I, Martin M, Gallego X, Amador-Arjona A, Martinez de Lagran M, Murtra P, Marti E, Pujana MA, Ferrer I, Dalfo E, Martinez-Cue C, Florez J, Torres-Peraza JF, Alberch J, Maldonado R, Fillat C, Estivill X (2006) Transgenic mice overexpressing the full-length neurotrophin receptor TrkC exhibit increased catecholaminergic neuron density in specific brain areas and increased anxiety-like behavior and panic reaction. Neurobiol Dis 24:403–418

Dietrich MO, Horvath TL (2009) Feeding signals and brain circuitry. Eur J Neurosci 30: 1688–1696

Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP (1997) Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 387: 90–94

Fan G, Egles C, Sun Y, Minichiello L, Renger JJ, Klein R, Liu G, Jaenisch R (2000) Knocking the NT4 gene into the BDNF locus rescues BDNF deficient mice and reveals distinct NT4 and BDNF activities. Nat Neurosci 3:350–357

Fan W, Ellacott KL, Halatchev IG, Takahashi K, Yu P, Cone RD (2004) Cholecystokinin-mediated suppression of feeding involves the brainstem melanocortin system. Nat Neurosci 7:335–336

Faris PL, Kim SW, Meller WH, Goodale RL, Oakman SA, Hofbauer RD, Marshall AM, Daughters RS, Banerjee-Stevens D, Eckert ED, Hartman BK (2000) Effect of decreasing afferent

vagal activity with ondansetron on symptoms of bulimia nervosa: a randomised, double-blind trial. Lancet 355:792–797

Farooqi IS, Yeo GS, Keogh JM, Aminian S, Jebb SA, Butler G, Cheetham T, O'Rahilly S (2000) Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. J Clin Invest 106:271–279

Fox EA, Byerly MS (2004) A mechanism underlying mature-onset obesity: evidence from the hyperphagic phenotype of brain-derived neurotrophic factor mutants. Am J Physiol Regul Integr Comp Physiol 286:R994–R1004

Fox EA, Phillips RJ, Baronowsky EA, Byerly MS, Jones S, Powley TL (2001) Neurotrophin-4 deficient mice have a loss of vagal intraganglionic mechanoreceptors from the small intestine and a disruption of short-term satiety. J Neurosci 21:8602–8615

Freeman AY, Soghomonian JJ, Pierce RC (2003) Tyrosine kinase B and C receptors in the neostriatum and nucleus accumbens are co-localized in enkephalin-positive and enkephalin-negative neuronal profiles and their expression is influenced by cocaine. Neuroscience 117:147–156

Fulton S, Pissios P, Manchon RP, Stiles L, Frank L, Pothos EN, Maratos-Flier E, Flier JS (2006) Leptin regulation of the mesoaccumbens dopamine pathway. Neuron 51:811–822

Garcia-Suarez O, Gonzalez-Martinez T, Perez-Perez M, Germana A, Blanco-Gelaz MA, Monjil DF, Ciriaco E, Silos-Santiago I, Vega JA (2006) Expression of the neurotrophin receptor TrkB in the mouse liver. Anat Embryol (Berl) 211:465–473

Ghiglieri O, Gambarana C, Scheggi S, Tagliamonte A, Willner P, De Montis MG (1997) Palatable food induces an appetitive behaviour in satiated rats which can be inhibited by chronic stress. Behav Pharmacol 8:619–628

Gloaguen I, Costa P, Demartis A, Lazzaro D, Di Marco A, Graziani R, Paonessa G, Chen F, Rosenblum CI, Van der Ploeg LH, Cortese R, Ciliberto G, Laufer R (1997) Ciliary neurotrophic factor corrects obesity and diabetes associated with leptin deficiency and resistance. Proc Natl Acad Sci U S A 94:6456–6461

Gray J, Yeo GS, Cox JJ, Morton J, Adlam AL, Keogh JM, Yanovski JA, El Gharbawy A, Han JC, Tung YC, Hodges JR, Raymond FL, O'Rahilly S, Farooqi IS (2006) Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. Diabetes 55:3366–3371

Grill HJ, Schwartz MW, Kaplan JM, Foxhall JS, Breininger J, Baskin DG (2002) Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. Endocrinology 143:239–246

Hakansson ML, Brown H, Ghilardi N, Skoda RC, Meister B (1998) Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J Neurosci 18:559–572

Han JC, Liu QR, Jones M, Levinn RL, Menzie CM, Jefferson-George KS, Adler-Wailes DC, Sanford EL, Lacbawan FL, Uhl GR, Rennert OM, Yanovski JA (2008) Brain-derived neurotrophic factor and obesity in the WAGR syndrome. N Engl J Med 359:918–927

Harrold JA, Dovey T, Cai XJ, Halford JC, Pinkney J (2008) Autoradiographic analysis of ghrelin receptors in the rat hypothalamus. Brain Res 1196:59–64

Herman CP, Polivy J (1990) From dietary restraint to binge eating: attaching causes to effects. Appetite 14:123–125, discussion 142–123

Hernandez L, Hoebel BG (1988) Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life Sci 42:1705–1712

Hoane MR, Gulwadi AG, Morrison S, Hovanesian G, Lindner MD, Tao W (1999) Differential in vivo effects of neurturin and glial cell-line-derived neurotrophic factor. Exp Neurol 160: 235–243

Hock C, Heese K, Muller-Spahn F, Huber P, Riesen W, Nitsch RM, Otten U (2000) Increased cerebrospinal fluid levels of neurotrophin 3 (NT-3) in elderly patients with major depression. Mol Psychiatry 5:510–513

Hudson J, Granholm AC, Gerhardt GA, Henry MA, Hoffman A, Biddle P, Leela NS, Mackerlova L, Lile JD, Collins F et al (1995) Glial cell line-derived neurotrophic factor augments midbrain dopaminergic circuits in vivo. Brain Res Bull 36:425–432

Ip NY, Yancopoulos GD (1996) The neurotrophins and CNTF: two families of collaborative neurotrophic factors. Annu Rev Neurosci 19:491–515

Kernie SG, Liebl DJ, Parada LF (2000) BDNF regulates eating behavior and locomotor activity in mice. EMBO J 19:1290–1300

Kokoeva MV, Yin H, Flier JS (2005) Neurogenesis in the hypothalamus of adult mice: potential role in energy balance. Science 310:679–683

Komori T, Morikawa Y, Nanjo K, Senba E (2006) Induction of brain-derived neurotrophic factor by leptin in the ventromedial hypothalamus. Neuroscience 139:1107–1115

Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T (1995) Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc Natl Acad Sci U S A 92:8856–8860

Kramer ER, Aron L, Ramakers GM, Seitz S, Zhuang X, Beyer K, Smidt MP, Klein R (2007) Absence of Ret signaling in mice causes progressive and late degeneration of the nigrostriatal system. PLoS Biol 5:e39

Kublaoui BM, Gemelli T, Tolson KP, Wang Y, Zinn AR (2008) Oxytocin deficiency mediates hyperphagic obesity of Sim1 haploinsufficient mice. Mol Endocrinol 22:1723–1734

Kuroda A, Yamasaki Y, Matsuhisa M, Kubota M, Nakahara I, Nakatani Y, Hoshi A, Gorogawa S, Umayahara Y, Itakura Y, Nakagawa T, Taiji M, Kajimoto Y, Hori M (2003) Brain-derived neurotrophic factor ameliorates hepatic insulin resistance in Zucker fatty rats. Metabolism 52: 203–208

Kusano K, House SB, Gainer H (1999) Effects of osmotic pressure and brain-derived neurotrophic factor on the survival of postnatal hypothalamic oxytocinergic and vasopressinergic neurons in dissociated cell culture. J Neuroendocrinol 11:145–152

Lapchak PA, Hefti F (1992) BDNF and NGF treatment in lesioned rats: effects on cholinergic function and weight gain. Neuroreport 3:405–408

Lapchak PA, Araujo DM, Hilt DC, Sheng J, Jiao S (1997) Adenoviral vector-mediated GDNF gene therapy in a rodent lesion model of late stage Parkinson's disease. Brain Res 777:153–160

Ledoux S, Choquet M, Manfredi R (1993) Associated factors for self-reported binge eating among male and female adolescents. J Adolesc 16:75–91

Leinninger GM, Jo YH, Leshan RL, Louis GW, Yang H, Barrera JG, Wilson H, Opland DM, Faouzi MA, Gong Y, Jones JC, Rhodes CJ, Chua S Jr, Diano S, Horvath TL, Seeley RJ, Becker JB, Munzberg H, Myers MG Jr (2009) Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. Cell Metab 10:89–98

Lommatzsch M, Braun A, Mannsfeldt A, Botchkarev VA, Botchkareva NV, Paus R, Fischer A, Lewin GR, Renz H (1999) Abundant production of brain-derived neurotrophic factor by adult visceral epithelia. Implications for paracrine and target-derived neurotrophic functions. Am J Pathol 155:1183–1193

Luiten PG, Room P (1980) Interrelations between lateral, dorsomedial and ventromedial hypothalamic nuclei in the rat. An HRP study. Brain Res 190:321–332

Lyons WE, Mamounas LA, Ricaurte GA, Coppola V, Reid SW, Bora SH, Wihler C, Koliatsos VE, Tessarollo L (1999) Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. Proc Natl Acad Sci U S A 96:15239–15244

MacLennan AJ, Vinson EN, Marks L, McLaurin DL, Pfeifer M, Lee N (1996) Immunohistochemical localization of ciliary neurotrophic factor receptor alpha expression in the rat nervous system. J Neurosci 16:621–630

Manfredsson FP, Tumer N, Erdos B, Landa T, Broxson CS, Sullivan LF, Rising AC, Foust KD, Zhang Y, Muzyczka N, Gorbatyuk OS, Scarpace PJ, Mandel RJ (2009) Nigrostriatal

rAAV-mediated GDNF overexpression induces robust weight loss in a rat model of age-related obesity. Mol Ther 17:980–991
Martin-Iverson MT, Todd KG, Altar CA (1994) Brain-derived neurotrophic factor and neurotrophin-3 activate striatal dopamine and serotonin metabolism and related behaviors: interactions with amphetamine. J Neurosci 14:1262–1270
Matson CA, Wiater MF, Kuijper JL, Weigle DS (1997) Synergy between leptin and cholecystokinin (CCK) to control daily caloric intake. Peptides 18:1275–1278
McAllister AK, Katz LC, Lo DC (1997) Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. Neuron 18:767–778
Mercader JM, Saus E, Aguera Z, Bayes M, Boni C, Carreras A, Cellini E, de Cid R, Dierssen M, Escaramis G, Fernandez-Aranda F, Forcano L, Gallego X, Gonzalez JR, Gorwood P, Hebebrand J, Hinney A, Nacmias B, Puig A, Ribases M, Ricca V, Romo L, Sorbi S, Versini A, Gratacos M, Estivill X (2008) Association of NTRK3 and its interaction with NGF suggest an altered cross-regulation of the neurotrophin signaling pathway in eating disorders. Hum Mol Genet 17:1234–1244
Monteleone P, Zanardini R, Tortorella A, Gennarelli M, Castaldo E, Canestrelli B, Maj M (2006) The 196G/A (val66met) polymorphism of the BDNF gene is significantly associated with binge eating behavior in women with bulimia nervosa or binge eating disorder. Neurosci Lett 406:133–137
Moreno G, Piermaria J, Gaillard RC, Spinedi E (2011) In vitro functionality of isolated embryonic hypothalamic vasopressinergic and oxytocinergic neurons: modulatory effects of brain-derived neurotrophic factor and angiotensin II. Endocrine 39:83–88
Nakagawa T, Tsuchida A, Itakura Y, Nonomura T, Ono M, Hirota F, Inoue T, Nakayama C, Taiji M, Noguchi H (2000) Brain-derived neurotrophic factor regulates glucose metabolism by modulating energy balance in diabetic mice. Diabetes 49:436–444
Nicholson JR, Peter JC, Lecourt AC, Barde YA, Hofbauer KG (2007) Melanocortin-4 receptor activation stimulates hypothalamic brain-derived neurotrophic factor release to regulate food intake, body temperature and cardiovascular function. J Neuroendocrinol 19:974–982
Nonomura T, Tsuchida A, Ono-Kishino M, Nakagawa T, Taiji M, Noguchi H (2001) Brain-derived neurotrophic factor regulates energy expenditure through the central nervous system in obese diabetic mice. Int J Exp Diabetes Res 2:201–209
Numan S, Seroogy KB (1999) Expression of trkB and trkC mRNAs by adult midbrain dopamine neurons: a double-label in situ hybridization study. J Comp Neurol 403:295–308
Numan S, Lane-Ladd SB, Zhang L, Lundgren KH, Russell DS, Seroogy KB, Nestler EJ (1998) Differential regulation of neurotrophin and trk receptor mRNAs in catecholaminergic nuclei during chronic opiate treatment and withdrawal. J Neurosci 18:10700–10708
Okazawa H, Murata M, Watanabe M, Kamei M, Kanazawa I (1992) Dopaminergic stimulation up-regulates the in vivo expression of brain-derived neurotrophic factor (BDNF) in the striatum. FEBS Lett 313:138–142
Pan Y, Chau L, Liu S, Avshalumov MV, Rice ME, Carr KD (2011) A food restriction protocol that increases drug reward decreases tropomyosin receptor kinase B in the ventral tegmental area, with no effect on brain-derived neurotrophic factor or tropomyosin receptor kinase B protein levels in dopaminergic forebrain regions. Neuroscience 197:330–338
Patapoutian A, Reichardt LF (2001) Trk receptors: mediators of neurotrophin action. Curr Opin Neurobiol 11:272–280
Pelleymounter MA, Cullen MJ, Wellman CL (1995) Characteristics of BDNF-induced weight loss. Exp Neurol 131:229–238
Penicaud L, Larue-Achagiotis C, Le Magnen J (1983) Endocrine basis for weight gain after fasting or VMH lesion in rats. Am J Physiol 245:E246–E252
Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, Horvath TL (2004) Rapid rewiring of arcuate nucleus feeding circuits by leptin. Science 304:110–115

Pissios P, Frank L, Kennedy AR, Porter DR, Marino FE, Liu FF, Pothos EN, Maratos-Flier E (2008) Dysregulation of the mesolimbic dopamine system and reward in MCH-/- mice. Biol Psychiatry 64(3):184–191

Pothos EN, Creese I, Hoebel BG (1995a) Restricted eating with weight loss selectively decreases extracellular dopamine in the nucleus accumbens and alters dopamine response to amphetamine, morphine, and food intake. J Neurosci 15:6640–6650

Pothos EN, Hernandez L, Hoebel BG (1995b) Chronic food deprivation decreases extracellular dopamine in the nucleus accumbens: implications for a possible neurochemical link between weight loss and drug abuse. Obes Res 3(Suppl 4):525S–529S

Pu L, Liu QS, Poo MM (2006) BDNF-dependent synaptic sensitization in midbrain dopamine neurons after cocaine withdrawal. Nat Neurosci 9:605–607

Rada P, Avena NM, Hoebel BG (2005) Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience 134:737–744

Rauggi R, Scheggi S, Cassanelli A, De Montis MG, Tagliamonte A, Gambarana C (2005) The mesolimbic dopaminergic response to novel palatable food consumption increases dopamine-D1 receptor-mediated signalling with complex modifications of the DARPP-32 phosphorylation pattern. J Neurochem 92:867–877

Reichardt LF (2006) Neurotrophin-regulated signalling pathways. Philos Trans R Soc Lond B Biol Sci 361:1545–1564

Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, Lechan RM, Jaenisch R (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol Endocrinol 15:1748–1757

Saper CB, Swanson LW, Cowan WM (1979) An autoradiographic study of the efferent connections of the lateral hypothalamic area in the rat. J Comp Neurol 183:689–706

Schwartz GJ (2000) The role of gastrointestinal vagal afferents in the control of food intake: current prospects. Nutrition 16:866–873

Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG (1996) Identification of targets of leptin action in rat hypothalamus. J Clin Invest 98:1101–1106

Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P, Baskin DG (1997) Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 46:2119–2123

Sears RM, Liu RJ, Narayanan NS, Sharf R, Yeckel MF, Laubach M, Aghajanian GK, DiLeone RJ (2010) Regulation of nucleus accumbens activity by the hypothalamic neuropeptide melanin-concentrating hormone. J Neurosci 30:8263–8273

Shimizu E, Hashimoto K, Iyo M (2004) Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. Am J Med Genet B Neuropsychiatr Genet 126:122–123

Simpson KA, Martin NM, Bloom SR (2009) Hypothalamic regulation of food intake and clinical therapeutic applications. Arq Bras Endocrinol Metabol 53:120–128

Skledar M, Nikolac M, Dodig-Curkovic K, Curkovic M, Borovecki F, Pivac N (2012) Association between brain-derived neurotrophic factor Val66Met and obesity in children and adolescents. Prog Neuropsychopharmacol Biol Psychiatry 36(1):136–140

Sleeman MW, Garcia K, Liu R, Murray JD, Malinova L, Moncrieffe M, Yancopoulos GD, Wiegand SJ (2003) Ciliary neurotrophic factor improves diabetic parameters and hepatic steatosis and increases basal metabolic rate in db/db mice. Proc Natl Acad Sci U S A 100: 14297–14302

Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, Allen HL, Lindgren CM, Luan J, Magi R, Randall JC, Vedantam S, Winkler TW, Qi L, Workalemahu T, Heid IM, Steinthorsdottir V, Stringham HM, Weedon MN, Wheeler E, Wood AR, Ferreira T, Weyant RJ, Segre AV, Estrada K, Liang L, Nemesh J, Park JH, Gustafsson S, Kilpelainen TO, Yang J, Bouatia-Naji N, Esko T, Feitosa MF, Kutalik Z, Mangino M, Raychaudhuri S, Scherag A, Smith AV, Welch R, Zhao JH, Aben KK, Absher DM, Amin N, Dixon AL, Fisher E, Glazer NL, Goddard ME, Heard-Costa NL, Hoesel V, Hottenga JJ, Johansson A, Johnson T, Ketkar S, Lamina C, Li S,

Moffatt MF, Myers RH, Narisu N, Perry JR, Peters MJ, Preuss M, Ripatti S, Rivadeneira F, Sandholt C, Scott LJ, Timpson NJ, Tyrer JP, van Wingerden S, Watanabe RM, White CC, Wiklund F, Barlassina C, Chasman DI, Cooper MN, Jansson JO, Lawrence RW, Pellikka N, Prokopenko I, Shi J, Thiering E, Alavere H, Alibrandi MT, Almgren P, Arnold AM, Aspelund T, Atwood LD, Balkau B, Balmforth AJ, Bennett AJ, Ben-Shlomo Y, Bergman RN, Bergmann S, Biebermann H, Blakemore AI, Boes T, Bonnycastle LL, Bornstein SR, Brown MJ, Buchanan TA et al (2010) Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet 42(11):937–948

Sternson SM, Shepherd GM, Friedman JM (2005) Topographic mapping of VMH → arcuate nucleus microcircuits and their reorganization by fasting. Nat Neurosci 8:1356–1363

Stice E, Spoor S, Bohon C, Veldhuizen MG, Small DM (2008) Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study. J Abnorm Psychol 117:924–935

Sugiyama N, Kanba S, Arita J (2003) Temporal changes in the expression of brain-derived neurotrophic factor mRNA in the ventromedial nucleus of the hypothalamus of the developing rat brain. Brain Res Mol Brain Res 115:69–77

Teegarden SL, Nestler EJ, Bale TL (2008) Delta FosB-mediated alterations in dopamine signaling are normalized by a palatable high-fat diet. Biol Psychiatry 64:941–950

Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, Styrkarsdottir U, Gretarsdottir S, Thorlacius S, Jonsdottir I, Jonsdottir T, Olafsdottir EJ, Olafsdottir GH, Jonsson T, Jonsson F, Borch-Johnsen K, Hansen T, Andersen G, Jorgensen T, Lauritzen T, Aben KK, Verbeek AL, Roeleveld N, Kampman E, Yanek LR, Becker LC, Tryggvadottir L, Rafnar T, Becker DM, Gulcher J, Kiemeney LA, Pedersen O, Kong A, Thorsteinsdottir U, Stefansson K (2009) Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet 41:18–24

Toni R, Lechan RM (1993) Neuroendocrine regulation of thyrotropin-releasing hormone (TRH) in the tuberoinfundibular system. J Endocrinol Invest 16:715–753

Tonra JR, Ono M, Liu X, Garcia K, Jackson C, Yancopoulos GD, Wiegand SJ, Wong V (1999) Brain-derived neurotrophic factor improves blood glucose control and alleviates fasting hyperglycemia in C57BLKS-Lepr(db)/lepr(db) mice. Diabetes 48:588–594

Toriya M, Maekawa F, Maejima Y, Onaka T, Fujiwara K, Nakagawa T, Nakata M, Yada T (2010) Long-term infusion of brain-derived neurotrophic factor reduces food intake and body weight via a corticotrophin-releasing hormone pathway in the paraventricular nucleus of the hypothalamus. J Neuroendocrinol 22:987–995

Tran PV, Lee MB, Marin O, Xu B, Jones KR, Reichardt LF, Rubenstein JR, Ingraham HA (2003) Requirement of the orphan nuclear receptor SF-1 in terminal differentiation of ventromedial hypothalamic neurons. Mol Cell Neurosci 22:441–453

Tran PV, Akana SF, Malkovska I, Dallman MF, Parada LF, Ingraham HA (2006) Diminished hypothalamic bdnf expression and impaired VMH function are associated with reduced SF-1 gene dosage. J Comp Neurol 498:637–648

Tsao D, Thomsen HK, Chou J, Stratton J, Hagen M, Loo C, Garcia C, Sloane DL, Rosenthal A, Lin JC (2008) TrkB agonists ameliorate obesity and associated metabolic conditions in mice. Endocrinology 149:1038–1048

Tsuchida A, Nonomura T, Ono-Kishino M, Nakagawa T, Taiji M, Noguchi H (2001) Acute effects of brain-derived neurotrophic factor on energy expenditure in obese diabetic mice. Int J Obes Relat Metab Disord 25:1286–1293

Tsuchida A, Nonomura T, Nakagawa T, Itakura Y, Ono-Kishino M, Yamanaka M, Sugaru E, Taiji M, Noguchi H (2002) Brain-derived neurotrophic factor ameliorates lipid metabolism in diabetic mice. Diabetes Obes Metab 4:262–269

Tumer N, Scarpace PJ, Dogan MD, Broxson CS, Matheny M, Yurek DM, Peden CS, Burger C, Muzyczka N, Mandel RJ (2006) Hypothalamic rAAV-mediated GDNF gene delivery ameliorates age-related obesity. Neurobiol Aging 27:459–470

Unger TJ, Calderon GA, Bradley LC, Sena-Esteves M, Rios M (2007) Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. J Neurosci 27:14265–14274

Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguel P (2000) Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. J Clin Invest 106:253–262

Wang C, Bomberg E, Billington C, Levine A, Kotz CM (2007a) Brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus increases energy expenditure by elevating metabolic rate. Am J Physiol Regul Integr Comp Physiol 293(3):R992–R1002

Wang C, Bomberg E, Billington C, Levine A, Kotz CM (2007b) Brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus reduces energy intake. Am J Physiol Regul Integr Comp Physiol 293:R1003–R1012

Wang C, Bomberg E, Levine A, Billington C, Kotz CM (2007c) Brain-derived neurotrophic factor in the ventromedial nucleus of the hypothalamus reduces energy intake. Am J Physiol Regul Integr Comp Physiol 293:R1037–R1045

Wang C, Bomberg E, Billington CJ, Levine AS, Kotz CM (2010a) Brain-derived neurotrophic factor (BDNF) in the hypothalamic ventromedial nucleus increases energy expenditure. Brain Res 1336:66–77

Wang C, Godar RJ, Billington CJ, Kotz CM (2010b) Chronic administration of brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus reverses obesity induced by high-fat diet. Am J Physiol Regul Integr Comp Physiol 298:R1320–R1332

Wang J, Carnicella S, Ahmadiantehrani S, He DY, Barak S, Kharazia V, Ben Hamida S, Zapata A, Shippenberg TS, Ron D (2010c) Nucleus accumbens-derived glial cell line-derived neurotrophic factor is a retrograde enhancer of dopaminergic tone in the mesocorticolimbic system. J Neurosci 30:14502–14512

Watt MJ, Dzamko N, Thomas WG, Rose-John S, Ernst M, Carling D, Kemp BE, Febbraio MA, Steinberg GR (2006) CNTF reverses obesity-induced insulin resistance by activating skeletal muscle AMPK. Nat Med 12:541–548

Williams LR (1991) Hypophagia is induced by intracerebroventricular administration of nerve growth factor. Exp Neurol 113:31–37

Williams DL, Kaplan JM, Grill HJ (2000) The role of the dorsal vagal complex and the vagus nerve in feeding effects of melanocortin-3/4 receptor stimulation. Endocrinology 141: 1332–1337

Williams DL, Baskin DG, Schwartz MW (2009) Hindbrain leptin receptor stimulation enhances the anorexic response to cholecystokinin. Am J Physiol Regul Integr Comp Physiol 297: R1238–R1246

Xu B, Dube MG, Kalra PS, Farmerie WG, Kaibara A, Moldawer LL, Martin D, Kalra SP (1998) Anorectic effects of the cytokine, ciliary neurotropic factor, are mediated by hypothalamic neuropeptide Y: comparison with leptin. Endocrinology 139:466–473

Xu B, Goulding EH, Zang K, Cepoi D, Cone RD, Jones KR, Tecott LH, Reichardt LF (2003) Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 6:736–742

Xu AW, Ste-Marie L, Kaelin CB, Barsh GS (2007) Inactivation of signal transducer and activator of transcription 3 in proopiomelanocortin (Pomc) neurons causes decreased pomc expression, mild obesity, and defects in compensatory refeeding. Endocrinology 148:72–80

Yan Q, Radeke MJ, Matheson CR, Talvenheimo J, Welcher AA, Feinstein SC (1997) Immunocytochemical localization of TrkB in the central nervous system of the adult rat. J Comp Neurol 378:135–157

Yang F, Feng L, Zheng F, Johnson SW, Du J, Shen L, Wu CP, Lu B (2001) GDNF acutely modulates excitability and A-type K(+) channels in midbrain dopaminergic neurons. Nat Neurosci 4:1071–1078

Yeo GS, Connie Hung CC, Rochford J, Keogh J, Gray J, Sivaramakrishnan S, O'Rahilly S, Farooqi IS (2004) A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. Nat Neurosci 7:1187–1189

Zamir N, Skofitsch G, Jacobowitz DM (1986) Distribution of immunoreactive melanin-concentrating hormone in the central nervous system of the rat. Brain Res 373:240–245

Zheng H, Corkern M, Stoyanova I, Patterson LM, Tian R, Berthoud HR (2003) Peptides that regulate food intake: appetite-inducing accumbens manipulation activates hypothalamic orexin neurons and inhibits POMC neurons. Am J Physiol Regul Integr Comp Physiol 284: R1436–R1444

Zheng H, Patterson LM, Morrison C, Banfield BW, Randall JA, Browning KN, Travagli RA, Berthoud HR (2005) Melanin concentrating hormone innervation of caudal brainstem areas involved in gastrointestinal functions and energy balance. Neuroscience 135:611–625

The Biology of Neurotrophins: Cardiovascular Function

Costanza Emanueli, Marco Meloni, Wohaib Hasan, and Beth A. Habecker

Abstract

This chapter addresses the role of neurotrophins in the development of the heart, blood vessels, and neural circuits that control cardiovascular function, as well as the role of neurotrophins in the mature cardiovascular system. The cardiovascular system includes the heart and vasculature whose functions are tightly controlled by the nervous system. Neurons, cardiomyocytes, endothelial cells, vascular smooth muscle cells, and pericytes are all targets for neurotrophin action during development. Neurotrophin expression continues throughout life, and several common pathologies that impact cardiovascular function involve changes in the expression or activity of neurotrophins. These include atherosclerosis, hypertension, diabetes, acute myocardial infarction, and heart failure. In many of these conditions, altered expression of neurotrophins and/or neurotrophin receptors has direct effects on vascular endothelial and smooth muscle cells in addition to effects on nerves that modulate vascular resistance and cardiac function. This chapter summarizes the effects of neurotrophins in cardiovascular physiology and pathophysiology.

Keywords

NT-3 • TrkC • BDNF • TrkB • NGF • TrkA • Pericytes • Vascular endothelial growth factor-A (VEGF-A) • Cardiomyocytes • Angiogenesis • Diabetes • Myocardial ischemia • Hypertension • Sympathetic nervous system • Parasympathetic nervous system • p75NTR

C. Emanueli • M. Meloni
Regenerative Medicine Section, School of Clinical Sciences, Bristol Heart Institute, University of Bristol, Bristol, UK
e-mail: c.emanueli@yahoo.co.uk; marcomelni77@yahoo.it

W. Hasan • B.A. Habecker (✉)
Department of Physiology and Pharmacology, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, L334, Portland, OR 97239, USA
e-mail: hasan@ohsu.edu; habecker@ohsu.edu

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_12,

1 Introduction

The neurotrophins are a family of growth factors that exert diverse effects on the developing and mature cardiovascular system. Details about the structure and function of pro and mature nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) are examined elsewhere in this volume and are not a major focus here. Likewise, a detailed analysis of signaling through the Trk receptors or p75NTR and its various co-receptors is not the aim of this chapter. This chapter will focus instead on the role of neurotrophins and their receptors in cardiovascular development, function, and disease.

The heart and vasculature form the cardiovascular system, and their function is tightly controlled by the nervous system. Neurons, cardiomyocytes, endothelial cells, vascular smooth muscle cells (VSMCs), and pericytes are all targets for neurotrophin action during development and in the mature system. The first section of this chapter will examine the role of neurotrophins in development of the heart, blood vessels, and the neural circuits that control cardiovascular function. Later sections will examine the role of neurotrophins in the mature cardiovascular system, including disease states. Due to space limitations, we have not been able to include detailed information from, or cite, all of the relevant studies. For a more detailed review of direct neurotrophin actions on the heart and blood vessels, please see (Caporali and Emanueli 2009).

2 Neurotrophins and Cardiovascular Development

Vascular system development and maturation require highly coordinated and regulated complex processes including endothelial cell proliferation, migration and invasion, as well as support of peri-endothelial cells, including VSMCs and pericytes. The interaction among endothelial cells and peri-endothelial cells leads to the formation of a complex network of capillaries, arterioles, arteries, and veins. *Vasculogenesis* is the process of blood vessel formation by a de novo production of endothelial cells from vascular progenitor cells. Vasculogenesis is a crucial process for blood vessel formation during embryonic development and contributes to vessel growth in the adult (Risau and Flamme 1995). *Angiogenesis* is a general term for describing the growth and remodeling process that turns the primitive vascular network into a complex network, including the growth of endothelial sprouts from preexisting postcapillary venules (Carmeliet 2000).

Neurotrophins and their receptors are expressed by the developing heart and blood vessels (Scarisbrick et al. 1993). Studies using global knockout mouse models have identified specific roles for BDNF, NT-3, and their cognate Trk receptors in the formation of the heart and the myocardial vasculature (Donovan et al. 1996, 2000; Hiltunen et al. 1996; Huber et al. 1996; Tessarollo et al. 1997; Tessarollo 1998), but the cardiovascular phenotype of mice lacking NGF or TrkA has not been studied (Crowley et al. 1994; Smeyne et al. 1994). The lack of BDNF

reduces endothelial cell–cell contact in the embryonic heart, leading to intraventricular hemorrhage and reduction of cardiac contractility (Donovan et al. 2000). Similarly, TrkB−/− mice show a marked reduction of blood vessel density and increased number of apoptotic endothelial cells, especially in the subepicardial region of the developing heart (Wagner et al. 2005). Thus, BDNF activation of TrkB is required for survival of endothelial cells and development of the cardiac vasculature. NT3 activation of TrkC is required for the development of the atria, ventricles, and cardiac outflow tracts, so that genetic deletion of either NT-3 or TrkC results in impaired cardiac morphogenesis (Donovan et al. 1996; Tessarollo et al. 1997). The lack of NT3 leads to septal defects and tetralogy of Fallot, which resemble some of the most common congenital malformations in humans (Donovan et al. 1996). Overexpression of a dominant negative version of TrkC also leads to development of cardiovascular abnormalities, further highlighting its crucial role in proper development of the heart (Palko et al. 1999). Some of these developmental defects appear before the onset of cardiac innervation in mice (embryonic day 9.5), suggesting direct effects of neurotrophins on cardiovascular development (Tessarollo 1998).

The p75NTR is also present in the cardiovascular system during prenatal development. Immunohistochemistry for p75NTR together with the endothelial cell marker PECAM-1 and the VSMC marker α-smooth muscle actin (α-SMA) in wild-type murine embryos (at E11.5) showed the presence of p75NTR in both vascular cell types of large blood vessels (von Schack et al. 2001). Deletion of p75NTR by disrupting Exon IV in mice (*p75NTRExonIV*−/− mice) results in the development of a defective vascular system (von Schack et al. 2001). *p75NTRExonIV*−/− mice die at late gestational stages or around birth, with an aorta that has a thinner wall and increased lumen diameter, and many embryos show vascular ruptures and blood cell leakage (von Schack et al. 2001). More recent studies indicate that the *p75NTRExonIV*−/− mice produce a pro-apoptotic fragment of the p75NTR protein (Paul et al. 2004). This complicates interpretation of the phenotype, but nevertheless implicates neurotrophin signaling through p75NTR as critical for proper vascular development.

An alternative and useful approach to investigate the role of neurotrophins during differentiation is to test their activity on embryonic and fetal stem cells. Shmelkov et al investigated the effect of BDNF on CD133+ stem cells extracted from the human fetal liver (Shmelkov et al. 2005) and found that BDNF given alone or together with vascular endothelial growth factor-A (VEGF-A) stimulates CD133 + stem cells to differentiate toward the endothelial lineage as well as giving rise to beating cardiomyocytes. These cardiomyocytes, once transplanted into the mouse ear, are able to generate electrical action potentials. Human embryonic stem cells (hESC) express Trk receptors, and BDNF, NT-3, and NT-4/5 are produced by hESC to mediate their survival by an autocrine Trk-PI3K/Akt mechanism (Pyle et al. 2006). Addition of neurotrophins to hESC cultures induces greater clonal survival (Pyle et al. 2006), and culturing hESC on three-dimensional scaffolds in the presence of neurotrophins leads to formation of vascular structures (Levenberg et al. 2005).

These developmental studies revealed that neurotrophins have direct effects on cardiovascular cells and stem cells, which express neurotrophin receptors. However, the generation of new animal models lacking or overexpressing the genes for neurotrophin receptors in selected cardiovascular cells is required to fully elucidate the direct cardiovascular actions of neurotrophins during development and adulthood.

3 Neurotrophins and the Development of Neurons Involved in Cardiovascular Control

Neurotrophins play an important role in the development of the autonomic circuits that provide neural control to the cardiovascular system. Sympathetic and parasympathetic nerves provide the final common pathway for neural control of cardiovascular targets. Sympathetic nerves stimulate vasoconstriction, increase heart rate, and enhance cardiac contractility through the release of norepinephrine (NE), while parasympathetic nerves lower heart rate through the release of acetylcholine (ACh). Both sympathetic and parasympathetic neurons are controlled by preganglionic projections from the nucleus tractus solitarii (nTS) in the brainstem. The nTS receives sensory input from baroreceptor and chemoreceptor afferents as well as inputs from higher brain centers, and integrates this information to modulate sympathetic and parasympathetic transmission (Potts 2002; Boscan et al. 2002; Andresen et al. 2004). All aspects of this circuit—from sensory afferents to the brainstem to post-ganglionic sympathetic neurons—are impacted by neurotrophin actions.

Neurotrophins are required for the survival of neurons involved in cardiovascular homeostasis, so that the lack of specific neurotrophins or their cognate Trk receptors results in the loss of different types of neurons. For example, BDNF activation of TrkB is required for the survival of arterial baroreceptors during development (Brady et al. 1999) while NT-3, NT-4, and BDNF are all involved in the development of chemoafferent sensory neurons that innervate the carotid body (Conover et al. 1995; Erickson et al. 1996). The lack of NT-4 results in the loss of 20–30 % of preganglionic neurons projecting to the stellate ganglia (Roosen et al. 2001), which contains most of the sympathetic neurons that innervate the heart. NGF activation of TrkA is required for survival of the sympathetic (Crowley et al. 1994) and sensory neurons that innervate the heart (Ieda et al. 2006). In addition, multiple neurotrophins can play a role in the development of a single population of neurons. For example, postganglionic sympathetic neurons require NT-3 for their initial survival and axon outgrowth to targets and then become dependent on target-derived NGF (Glebova and Ginty 2005). Similarly, some sensory neurons from the nodose–petrosal ganglion require first NT-3 and NT-4, and then later in development BDNF, for survival (ElShamy and Ernfors 1997; Brady et al. 1999). Thus, one critical role of neurotrophins in cardiovascular function is in supporting the survival of nerves required to sense changes in arterial

pressure and oxygenation and then trigger compensatory changes in peripheral vascular resistance, heart rate, and stroke volume.

Neurotrophins can control other aspects of cardiovascular circuit development and function in addition to neuron survival. For example, BDNF is released from sensory afferents onto neurons in the nTS (Martin et al. 2009), where it can modulate cell excitability (Balkowiec et al. 2000) and contributes to development of normal autonomic control (Kline et al. 2010). The cardiac ventricles are innervated by TrkA-expressing sympathetic and sensory neurons whose production of neuropeptides can be regulated by NGF (Ieda et al. 2006; McMahon et al. 1995; Patel et al. 2000). These peptides can have direct effects on the heart and vasculature (Henning and Sawmiller 2001; Li and Peng 2002) in addition to modulating neurotransmitter release in the atria (Smith-White et al. 2003; Herring et al. 2008). NGF activation of TrkA also stimulates extension of sympathetic axons into the heart (Kohn et al. 1999; Glebova and Ginty 2004; Kuruvilla et al. 2004), synapse formation between pre- and post-ganglionic sympathetic neurons (Sharma et al. 2010), expression of tyrosine hydroxylase (TH), and NE synthesis (Max et al. 1978; Thoenen 1972). NGF activation of receptor complexes containing p75NTR during development modulates the density and distribution of sympathetic fibers in the atria (Habecker et al. 2008) and left ventricle (Lorentz et al. 2010). Thus, neurotrophin signaling is critical for the development and maintenance of several different aspects of neural control of cardiovascular function.

4 Neurotrophins in Adult Cardiovascular Physiology and Pathophysiology

Neurotrophin expression continues throughout life, and several common pathologies that impact cardiovascular function involve changes in the expression or activity of neurotrophins. These include atherosclerosis, hypertension, diabetes, acute myocardial infarction, and heart failure. In many of these conditions altered expression of neurotrophins and/or neurotrophin receptors has direct effects on vascular endothelial and smooth muscle cells in addition to effects on nerves that modulate vascular resistance and cardiac function. In addition, changes in vascular and cardiac neurotrophin production can alter neurotransmitter and peptide synthesis and release, which in turn can impact expression of neurotrophins in the target cell. For ease of understanding we will first discuss neurotrophin control of angiogenesis under normal conditions and in several types of pathology. We will then discuss the effects of neurotrophins on the innervation of the heart and vasculature and highlight reciprocal interactions between neurotransmission and neurotrophin expression.

5 Neurotrophin Regulation of Angiogenesis

Postnatal angiogenesis occurs physiologically in the cycling ovary and the placenta and is reactivated during wound healing, tissue repair, and under several pathological conditions (Carmeliet 2005). The driving force of angiogenesis is hypoxia in the surrounding tissue. Thus, ischemia provides a potent stimulus to angiogenesis and the subsequent development of collateral vasculature that in part maintains and/or revitalizes the ischemic tissue (Ejiri et al. 1990; Kodama et al. 1996) Sprouting of capillaries leads to an increase of their density improving blood perfusion of hypoxic tissue which is necessary to maintain or restore local oxygen and nutrition supply (Heil et al. 2006). Arteriogenesis is the maturation of capillaries or arterioles and the formation of arterial collaterals (Luttun et al. 2002; Skoff and Adler 2006) that is important for post-ischemic blood flow recovery. Several neurotrophins play a role in adult neovascularization.

NGF was the first neurotrophin found to be involved in postnatal angiogenesis (Santos et al. 1991). NGF is produced by endothelial cells that also express TrkA and p75NTR (Cantarella et al. 2002; Rahbek et al. 2005). NGF activation of TrkA promotes survival, proliferation, and migration/invasion of endothelial cells, while selective activation of p75NTR induces endothelial cell death (Kim et al. 2004; Caporali et al. 2008a; Skoff and Adler 2006). TrkA-dependent survival and proliferation of endothelial cells is at least in part due to increased production of VEGF-A (Emanueli et al. 2002; Graiani et al. 2004; Salis et al. 2004) and may be mediated by activation of ERK 1/2 (Cantarella et al. 2002). NGF-induced migration of endothelial cells is mediated by the simultaneous activation of the PI3K/Akt and ERK 1/2 signaling pathways (Rahbek et al. 2005; Dolle et al. 2005). The end result of NGF-TrkA-stimulated survival, proliferation, and migration of endothelial cells is increased angiogenesis, as seen during the healing of cutaneous wounds (Graiani et al. 2004).

5.1 Hindlimb Ischemia

NGF and TrkA are upregulated following the insurgence of ischemia in the leg or heart (Emanueli et al. 2002; Hiltunen et al. 2001; Meloni et al. 2010), and NGF participates in reparative capillarization triggered by the ischemic insult (Emanueli et al. 2002; Meloni et al. 2010). Indeed, in a mouse model of hindlimb ischemia, blockade of endogenous NGF by a neutralizing antibody disrupts the angiogenic response to muscular ischemia, while exogenous NGF supplementation to ischemic muscles enhances the spontaneous formation of capillaries and arterioles in the target tissue and accelerates blood flow recovery (Emanueli et al. 2002; Salis et al. 2004). The pro-angiogenic effect of NGF in the ischemic limb muscle seems to be mediated by increasing expression of VEGF-A (Emanueli et al. 2002) and possibly VEGF receptors (Hansen-Algenstaedt et al. 2006) followed by activation of Akt, nitric oxide production, and upregulation of matrix metalloproteinase-2 (MMP2) expression (Park et al. 2007; Rahbek et al. 2005).

NGF-induced angiogenesis in ischemic limb muscles is prevented by a neutralizing antibody for VEGF-A, as well as by suppressing nitric oxide production by using a nitric oxide synthase inhibitor (L-NAME) or by gene transfer with a dominant negative mutant form of Akt (Emanueli et al. 2002).

BDNF can stimulate angiogenesis in tissues where a subpopulation of vascular endothelial cells expresses TrkB (Kermani et al. 2005). BDNF expression is increased in response to hypoxia (Kim et al. 2004; Wang et al. 2006), and exogenous BDNF stimulates in vitro angiogenesis via TrkB and activation of the PI3K/Akt pathway (Kim et al. 2004). BDNF expression is upregulated in ischemic limb muscles, and BDNF gene therapy in mice with limb ischemia accelerates post-ischemic blood flow recovery and increases capillary density in the ischemic muscle. Importantly, these effects appear to be mediated by TrkB, as the effects of exogenous BDNF are attenuated in haplodeficient animals ($trkB^{+/-}$) (Kermani et al. 2005). In addition, adenovirus-mediated BDNF overexpression induces the mobilization of Sca-1^{pos}/CD11b^{pos} hematopoietic progenitor cells from the bone marrow into the circulation during mouse limb ischemia, which may play a role in BDNF-stimulated angiogenesis (Kermani et al. 2005).

The NT-3 receptor TrkC is expressed in human veins and mouse skeletal muscle endothelial cells, and recent studies indicate that NT-3 can stimulate angiogenesis (Cristofaro et al. 2010). NT-3 stimulates endothelial cell proliferation, survival, migration, and network formation on Matrigel in vitro (Cristofaro et al. 2010), while in vivo overexpression of NT-3 induces neovascularization in a rat mesenteric angiogenesis assay and a mouse model of hindlimb ischemia (Cristofaro et al. 2010). In the rat mesentery, newly formed vessels show an enhanced branch point density and diameter compared with the control group, and they also display increased coverage by mural cells. Adenovirus-mediated NT-3 gene transfer to murine ischemic hindlimbs stimulates the proliferation of capillary endothelial cells, thus increasing capillary density and promoting blood flow recovery to the ischemic foot. Activation of the PI3K/Akt/eNOS pathway is critical for NT-3-induced angiogenesis both in vitro and in vivo (Cristofaro et al. 2010), and stimulation of rat brain endothelial cells with NT-3 also increases eNOS levels and nitric oxide production (Takeo et al. 2003).

5.2 Diabetes

Type I diabetes mellitus downregulates the content of NGF and TrkA in ischemic skeletal muscles and concomitantly induces p75NTR expression in capillary endothelial cells (Caporali et al. 2008a, b). In contrast to Trk actions, activation of p75NTR induces endothelial cell death (Kim et al. 2004). Transduction of human umbilical vein endothelial cells (HUVEC) with p75NTR impairs their pro-angiogenic capacity (Caporali et al. 2008b), and p75NTR is responsible for diabetes-induced impairment in neovascularization of ischemic limb muscles (Caporali et al. 2008a). In diabetic mice treated with an adenovirus carrying a dominant negative p75NTR in their ischemic limbs, post-ischemic angiogenesis

and blood perfusion recovery were normalized to levels observed in normoglycemic mice (Caporali et al. 2008a). Interestingly, NGF supplementation, rather than initiating apoptosis of diabetic endothelial cells *via* p75NTR, downregulates p75NTR expression by a mechanism that has not yet been clarified and promotes endothelial cell survival and vascular regeneration (Graiani et al. 2004; Salis et al. 2004).

5.3 Myocardial Ischemia

More recently, NGF has been shown to exert its pro-angiogenic effects also in the setting of myocardial infarction (Meloni et al. 2010). Both NGF and TrkA are increased in the peri-infarct area of human and mouse heart after myocardial infarction, where NGF participates in the spontaneous angiogenic response. Blockade of endogenous NGF by a neutralizing antibody abrogates the spontaneous capillary growth and reduces the density of small arterioles in the mouse peri-infarct zone. By contrast, cardiac NGF overexpression improves angiogenesis and cardiac perfusion, leading to improved cardiac performance and reduced mortality in mice (Meloni et al. 2010). In contrast to limb muscles, NGF does not act through VEGF-A to activate reparative angiogenesis in the infarcted heart. In the post-infarcted heart, NGF promotes reparative neovascularization acting on the pro-angiogenic and pro-survival Akt-Foxo-3A pathway (Meloni et al. 2010; Potente et al. 2005). Moreover, local NGF gene therapy expands the number of Lineage negative c-kit positive (lin^{-neg} $c\text{-}kit^{-pos}$) cells with cardiogenic and vasculogenic capacities in the infarcted heart by increasing the expression of the c-kit ligand stem cell factor (SCF) (Beltrami et al. 2003; Meloni et al. 2010). lin^{-neg} $c\text{-}kit^{-pos}$ cells are involved in myocardial repair and regeneration after myocardial infarction (Beltrami et al. 2003; Cimini et al. 2007), and SCF induces neovascularization in the adult myocardium (Xiang et al. 2009). Thus, NGF-stimulated expansion of cardiac $c\text{-}kit^{pos}$ progenitor cells and SCF expression represent a new therapeutic possibility for improving cardiac regeneration.

Interesting new data indicate that proNGF is also elevated in the human heart after myocardial infarction (Siao et al. 2012). Similarly, upregulation of proNGF was observed in the mouse heart soon after ischemia–reperfusion injury, accompanied by increased expression of p75NTR by microvascular pericytes. Further studies in the mouse revealed that proNGF expression in the heart decreased pericyte process length and increased vascular permeability, resulting in microvascular damage and expansion of the cardiac scar (Siao et al. 2012). Thus, degradation or blockade of proNGF in the heart might provide a therapeutic target to limit cardiac damage after myocardial infarction.

5.4 Heart Failure

The role of NGF and its possible cardiac protective effects on the failing heart are still under investigation. NGF is protective in a cardiotoxic model of heart failure in zebrafish (Abstract 17596, AHA Scientific Session 2010). Heart failure was induced by exposure of zebrafish embryos to aristolochic acid which reportedly generates heart failure via inflammation (Huang et al. 2007). In the zebrafish model of cardiac injury, NGF reduces the incidence of heart failure and mortality. The effect of NGF was mediated *via* a regenerative response rather than by a reduction in apoptosis, and this response was accompanied by upregulation of the LIM-homeodomain protein Islet-1, which is expressed by cardiovascular progenitor cells (Barzelay et al. 2010; Genead et al. 2010). In a mouse model of heart failure associated with diabetic cardiomyopathy, NGF gene therapy by adeno-associated vectors showed promise (Meloni et al. 2012). Diabetes-induced deterioration of cardiac function was prevented by NGF overexpression. Moreover, increased NGF cardiac expression prevented the enlargement of left ventricular chamber volume and maintained the left ventricular internal diameter. NGF overexpression also prevented the diabetes-induced microvascular rarefaction in the left ventricle. These data suggest that NGF can be a relevant factor in promoting cardiac regeneration and angiogenesis in the failing heart.

5.5 Atherosclerosis

Atherosclerosis is a complex chronic inflammatory process of the arterial wall that involves endothelial cell activation by inflammatory cytokines, followed by increased adhesion of circulating monocytes to the endothelium, and by the migration of VSMCs into the developing neointima. Lipid accumulation and modulation of vascular cell phenotypes by extracellular matrix proteins (especially metalloproteases) stimulate the development of an atherosclerotic plaque, which progressively obstructs the vascular lumen, thus reducing blood flow and increasing arterial pressure. Human and rat VSMCs express all the neurotrophins as well as p75NTR and Trk receptors both in vivo and in vitro (Donovan et al. 1995; Kraemer et al. 1999). The expression of NGF, BDNF, TrkA, and TrkB are dramatically upregulated by arterial balloon injury in rats and increased levels persist during neointima formation (Donovan et al. 1995). BDNF, NT-3, NT-4/5, TrkB, TrkC, and p75NTR are also present in VSMCs in human atherosclerotic lesions (Donovan et al. 1995), thus suggesting that neurotrophins may regulate responses of VSMCs to vascular injury. Additional studies indicate that activation of TrkA stimulates VSMC migration (Donovan et al. 1995; Kraemer et al. 1999), while p75NTR activation induces VSMC apoptosis during remodeling of the established vascular lesion (Wang et al. 2000). Thus, neurotrophins may contribute to the development and remodeling of atherosclerotic plaques.

6 Neurotrophin Regulation of Nerves Involved in Cardiovascular Function

Neurotrophins have widespread effects on the mature nervous system just as they continue to stimulate angiogenesis in the adult. Neurotrophins are no longer required for neuron survival, but they can regulate neurotransmission at many levels including synapses formation (Lockhart et al. 2000; Sharma et al. 2010), neural excitability (Luther and Birren 2009), production of neurotransmitters (Max et al. 1978; Thoenen 1972), and neuropeptide synthesis (Shadiack et al. 2001; Skoff and Adler 2006). Mature fully processed forms of the neurotrophins are a minor species in most peripheral tissues including the heart and vasculature (Bierl et al. 2005), and recent studies identified proteases in sympathetic neurons that can cleave proNGF to the mature form (Saygili et al. 2011). Pro-neurotrophins preferentially bind a receptor complex containing p75NTR rather than Trk receptors, generating a distinct set of responses including cell death and axon degeneration (Lee et al. 2001; Al Shawi et al. 2007; Nykjaer et al. 2004). Thus, changes in the expression or processing of neurotrophins in the heart, vasculature, or innervation can impact the nerves controlling cardiovascular function, and in some instances contribute to the development of pathology.

6.1 Myocardial Ischemia and Congestive Heart Failure

Neurotrophin expression and actions have been studied in the context of acute myocardial infarction, heart failure, and hypertrophy. Nerve Growth Factor (NGF) mRNA is elevated in the infarct following ischemia–reperfusion (Hiltunen et al. 2001), while BDNF mRNA is transiently expressed in myocytes at the border of the infarct and intact tissue, and NT-3 mRNA changes little (Hiltunen et al. 2001). Endogenous NGF protein is increased in the infarcted left ventricle after both ischemia–reperfusion (Abe et al. 1997; Zhou et al. 2004) and chronic ischemia (Hasan et al. 2006; Meloni et al. 2010; Oh et al. 2006), although the methods used to quantify cardiac neurotrophin expression did not distinguish between pro and mature forms. New data using a proNGF-selective antibody indicate that proNGF is also elevated in mouse and human heart after ischemia–reperfusion (Siao et al. 2012). The myocardium is an important source of NGF, but several other sources have been identified in the heart including neural-crest stem cells, inflammatory cells, as well as sympathetic and parasympathetic neurons (Drapeau et al. 2005; Hasan et al. 2003, 2006; Hasan and Smith 2009; Saygili et al. 2011). Mechanical stretch of sympathetic neurons, as may occur with myocardial hypertrophy, can also increase neural synthesis of NGF (Hasan et al. 2003; Rana et al. 2010). The extent to which these localized sources of NGF affect nerve growth in cardiac pathology remains to be determined.

Increased production of NGF soon after myocardial infarction leads to development of focal sympathetic hyperinnervation. In human studies increased NGF is observed in the peri-infarct area of postmortem tissue (Meloni et al. 2010), and

regions of peri-infarct sympathetic hyperinnervation correlate with a clinical history of ventricular arrhythmias (Chen et al. 2001). In canine studies, hyperinnervation from sympathetic nerves contributes to ventricular arrhythmia generation (Chen et al. 2001; Zhou et al. 2004). The peak of ventricular NGF expression in rodent models corresponds to the peak in tyrosine hydroxylase (TH) levels in the first week after chronic cardiac ischemia, followed by decreases in both NGF and TH (Hasan et al. 2006; Kimura et al. 2010). A major source of NGF in the infarct is inflammatory cells, specifically myofibroblasts and macrophages, which are spatially and temporally associated with changes in innervation density (Hasan et al. 2006). Neurite outgrowth from peri-infarct tissue in explant culture can be blocked by addition of function-blocking NGF antibodies (Hasan et al. 2006), and anti-inflammatory therapies reduce both NGF expression within the failing heart and sympathetic hyperinnervation (Wernli et al. 2009). Thus, early increases in NGF stimulate sympathetic axon growth and increased NE production in the cardiac sympathetic innervation following myocardial infarction.

Sympathetic nerves innervating the heart have been a major focus of investigation because their dysfunction contributes to human pathology (Esler et al. 1997; Rubart and Zipes 2005), but the heart is also innervated by parasympathetic and sensory nerves. Postganglionic parasympathetic fibers project from the cardiac ganglia to the atria where they control the activity of pacemaker cells and modulate NE release from sympathetic fibers (Levy 1990). Cardiac parasympathetic neurons express the TrkC and p75NTR receptors (Hiltunen et al. 1996), but they are not altered by the lack of p75NTR (Habecker et al. 2008), and their response to neurotrophins after myocardial infarction has not been examined. Cardiac parasympathetic neurons synthesize mature NGF, and this protein may help maintain axo-axonal connections with sympathetic neurons in cardiac pacemaker regions (Hasan and Smith 2009). Epicardial and ventricular myocardium is richly innervated by NGF-dependent calcitonin gene-related peptide (CGRP)-expressing sensory nerves (Ieda et al. 2006; Park et al. 2010). Although comprehensive studies are lacking on sensory nerves after myocardial infarction, few CGRP-immunoreactive nerve fibers were observed in post-infarct myocardium despite sympathetic hyperinnervation (Hasan et al. 2006). Since hyperinnervation after infarction has only been observed for sympathetic nerves, it is possible that sympathetics compete more effectively for local NGF than sensory nerves in that context. Indeed, after sympathectomy in spontaneously hypertensive rats (SHRs), ventricular NGF levels increase accompanied by increased utilization of NGF by sensory nerves (Supowit et al. 2005).

In contrast to the increased cardiac NGF observed soon after myocardial infarction, the progression of myocardial damage to congestive heart failure (CHF) is associated with decreased production of NGF (Kaye et al. 2000; Kimura et al. 2010; Qin et al. 2002). The transition to CHF is promoted by an overactive sympathetic nervous system (Esler et al. 1997; Thomas and Marks 1978). In addition to increased sympathetic drive in CHF, NE handling is disrupted so that the normal balance between NE storage/release/reuptake is replaced by increased NE release and decreased reuptake (Eisenhofer et al. 1996). The loss of NE reuptake, increased

release, and corresponding increase in extracellular NE causes hypertrophy in the human heart (Corea et al. 1983, 1984) and in animal models (Laycock et al. 1996; Tsoporis et al. 1998). Eventually, the chronic increase in extracellular NE is pathological (Lai et al. 1996; Bacaner et al. 2004) and may contribute to the decrease in cardiac NGF expression (Qin et al. 2002; Kimura et al. 2010). Low NGF in turn promotes decreased reuptake, as the loss of NE uptake is preceded by a decrease in cardiac NGF and NT-3 (Kreusser et al. 2008), and injection of NGF into stellate ganglia can restore NE uptake in nerve terminals within the failing heart (Kreusser et al. 2006). Neuron–target interactions, including altered production of neurotrophins, play an important role in the development of heart failure.

6.2 Diabetes

Cardiac autonomic neuropathy is a common complication of type I and type II diabetes (Pop-Busui 2010), leading to complex changes in the cardiac sympathetic innervation. The causes of diabetic autonomic neuropathy have been characterized in animal models using streptozotocin to induce type I diabetes. Studies in rats revealed increased cardiac NGF several weeks after streptozotocin injection (Hellweg and Hartung 1990) that was followed by decreased cardiac NGF content several months after the induction of diabetes (Hellweg and Hartung 1990; Schmid et al. 1999). At the 6-month time point, the cardiac sympathetic innervation exhibited significant heterogeneity, with distal denervation that closely tracked with a gradient of cardiac NGF content that was highest in the proximal ventricle and lowest in the distal ventricle (Schmid et al. 1999). The decreased production of NGF, and proximal to distal gradient of NGF content in the diabetic rat heart, is especially interesting because it may explain the proximal to distal gradient of innervation density observed in patients with type 1 diabetes (Stevens et al. 1998). Heterogeneity in the distribution and density of sympathetic nerves in the heart in diabetes or other conditions increases the risk of sudden cardiac death (Rubart and Zipes 2005; Stevens et al. 1998; Ieda and Fukuda 2009). Decreased cardiac NGF in diabetes may contribute to the loss of uptake through the NE transporter that is a component of diabetic autonomic neuropathy (Langer et al. 1995), since a similar loss of NGF contributes to decreased NE uptake in heart failure (Kreusser et al. 2006).

The heart is also innervated by NGF-responsive sensory nerves from dorsal root ganglia (Ieda et al. 2006) that sense cardiac ischemia via acid-sensitive channels (Yagi et al. 2006). The loss of pain perception during myocardial ischemia is a complication of diabetes that increases a patient's risk for complications or even death since they do not sense the cardiac ischemia and seek treatment. The loss of pain sensation in the heart has long been associated with diabetic autonomic neuropathy (Faerman et al. 1977), suggesting that both sensory and autonomic neurons in the heart are affected in diabetic neuropathy. Recent studies using streptozotocin to produce diabetes in mice (Ieda et al. 2006) found decreased NGF expression in the heart 4 months after streptozotocin injection accompanied

by fewer CGRP + sensory nerve fibers. Ieda and colleagues then tested whether the loss of NGF caused the loss of sensory nerve fibers and CGRP production by inducing diabetes in mice whose cardiac myocytes were overexpressing NGF. Increased cardiac NGF completely rescued the sensory innervation, preventing deficits in nerve function despite the presence of hyperglycemia (Ieda et al. 2006). To confirm that simply preventing the diabetes-induced loss of NGF was sufficient to prevent sensory neuropathy, they then used viral overexpression to add back NGF in diabetic rat hearts and blocked the degeneration of sensory fibers (Ieda et al. 2006). These studies show that decreased cardiac NGF in diabetes is a major contributor to cardiac diabetic neuropathy.

6.3 Hypertension

Neurotrophins play a role in the development of atherosclerosis-induced hypertension through their effects on plaque formation as described above, but they can contribute to the development of hypertension through other means as well. The SHR is a genetic model of essential hypertension that arose from Wistar-Kyoto rats. Analysis of SHR rats compared to Wistar-Kyoto controls revealed that the underlying cause of hypertension was sympathetic hyperinnervation of vascular smooth muscle (Head 1989). Further studies implicated increased expression of NGF in the vasculature (Falckh et al. 1992; Zettler and Rush 1993) as causing the development of sympathetic hyperinnervation and then hypertension. Injection of function blocking anti-NGF antibodies at 3 weeks of age normalized arterial pressure in adult rats (Brock et al. 1996), confirming that elevated NGF was the ultimate source of hyperinnervation and hypertension. More recent studies indicate that NT-3 is also elevated in SHR rats compared to Wistar-Kyoto control rats (Zhang and Rush 2001), but it is not clear if NT-3 contributes to the hyperinnervation of mesenteric arteries. Increased sympathetic transmission in the heart and vasculature plays an important role in a large fraction of human hypertension cases (Grassi et al. 2010), but the underlying cause of hyperinnervation and increased nerve activity is not known. Elevated NGF production in these targets may be an underlying cause, but further studies in humans are required to determine if that is indeed the case.

7 Summary

Neurotrophins have widespread affects on the development of the heart and vasculature as well as the neural circuits that control their function. Neurotrophins continue to exert an effect on the cardiovascular system in the adult, acting directly on vessels and cardiac myocytes in addition to actions in the nervous system. Increased expression of neurotrophins in response to injury or other pathological conditions can play an important role in stimulating angiogenesis and other reparative processes. However, changes in neurotrophin expression can also contribute to

the development of atherosclerosis, hypertension, diabetic sensory neuropathy, and pathological heterogeneity in the cardiac sympathetic innervation.

References

Abe T, Morgan DA, Gutterman DD (1997) Protective role of nerve growth factor against postischemic dysfunction of sympathetic coronary innervation. Circulation 95:213–220

Al Shawi R, Hafner A, Chun S, Raza S, Crutcher K, Thrasivoulou C, Simons P, Cowen T (2007) ProNGF, sortilin, and age-related neurodegeneration. Ann N Y Acad Sci 1119:208–215

Andresen MC, Doyle MW, Bailey TW, Jin YH (2004) Differentiation of autonomic reflex control begins with cellular mechanisms at the first synapse within the nucleus tractus solitarius. Braz J Med Biol Res 37:549–558

Bacaner M, Brietenbucher J, LaBree J (2004) Prevention of ventricular fibrillation, acute myocardial infarction (myocardial necrosis), heart failure, and mortality by bretylium: is ischemic heart disease primarily adrenergic cardiovascular disease? Am J Ther 11:366–411

Balkowiec A, Kunze DL, Katz DM (2000) Brain-derived neurotrophic factor acutely inhibits AMPA-mediated currents in developing sensory relay neurons. J Neurosci 20:1904–1911

Barzelay A, Ben-Shoshan J, Entin-Meer M, Maysel-Auslender S, Afek A, Barshack I, Keren G, George J (2010) A potential role for islet-1 in post-natal angiogenesis and vasculogenesis. Thromb Haemost 103:188–197

Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P (2003) Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 114:763–776

Bierl MA, Jones EE, Crutcher KA, Isaacson LG (2005) 'Mature' nerve growth factor is a minor species in most peripheral tissues. Neurosci Lett 380:133–137

Boscan P, Pickering AE, Paton JF (2002) The nucleus of the solitary tract: an integrating station for nociceptive and cardiorespiratory afferents. Exp Physiol 87:259–266

Brady R, Zaidi SI, Mayer C, Katz DM (1999) BDNF is a target-derived survival factor for arterial baroreceptor and chemoafferent primary sensory neurons. J Neurosci 19:2131–2142

Brock JA, Van Helden DF, Dosen P, Rush RA (1996) Prevention of high blood pressure by reducing sympathetic innervation in the spontaneously hypertensive rat. J Auton Nerv Syst 61:97–102

Cantarella G, Lempereur L, Presta M, Ribatti D, Lombardo G, Lazarovici P, Zappala G, Pafumi C, Bernardini R (2002) Nerve growth factor-endothelial cell interaction leads to angiogenesis in vitro and in vivo. FASEB J 16:1307–1309

Caporali A, Emanueli C (2009) Cardiovascular actions of neurotrophins. Physiol Rev 89:279–308

Caporali A, Pani E, Horrevoets AJ, Kraenkel N, Oikawa A, Sala-Newby GB, Meloni M, Cristofaro B, Graiani G, Leroyer AS, Boulanger CM, Spinetti G, Yoon SO, Madeddu P, Emanueli C (2008a) Neurotrophin p75 receptor (p75NTR) promotes endothelial cell apoptosis and inhibits angiogenesis: implications for diabetes-induced impaired neovascularization in ischemic limb muscles. Circ Res 103:e15–e26

Caporali A, Sala-Newby GB, Meloni M, Graiani G, Pani E, Cristofaro B, Newby AC, Madeddu P, Emanueli C (2008b) Identification of the prosurvival activity of nerve growth factor on cardiac myocytes. Cell Death Differ 15:299–311

Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis. Nat Med 6:389–395

Carmeliet P (2005) Angiogenesis in life, disease and medicine. Nature 438:932–936

Chen PS, Chen LS, Cao JM, Sharifi B, Karagueuzian HS, Fishbein MC (2001) Sympathetic nerve sprouting, electrical remodeling and the mechanisms of sudden cardiac death. Cardiovasc Res 50:409–416

Cimini M, Fazel S, Zhuo S, Xaymardan M, Fujii H, Weisel RD, Li RK (2007) c-kit dysfunction impairs myocardial healing after infarction. Circulation 116:I77–I82

Conover JC, Erickson JT, Katz DM, Bianchi LM, Poueymirou WT, McClain J, Pan L, Helgren M, Ip NY, Boland P (1995) Neuronal deficits, not involving motor neurons, in mice lacking BDNF and/or NT4. Nature 375:235–238

Corea L, Bentivoglio M, Verdecchia P (1983) Echocardiographic left ventricular hypertrophy as related to arterial pressure and plasma norepinephrine concentration in arterial hypertension. Reversal by atenolol treatment. Hypertension 5:837–843

Corea L, Bentivoglio M, Verdecchia P, Motolese M (1984) Plasma norepinephrine and left ventricular hypertrophy in systemic hypertension. Am J Cardiol 53:1299–1303

Cristofaro B, Stone OA, Caporali A, Dawbarn D, Ieronimakis N, Reyes M, Madeddu P, Bates DO, Emanueli C (2010) Neurotrophin-3 is a novel angiogenic factor capable of therapeutic neovascularization in a mouse model of limb ischemia. Arterioscler Thromb Vasc Biol 30:1143–1150

Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S, Armaninl MP, Ling LH, McMahon SB, Shelton DL, Levinson AD, Phillips HS (1994) Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 76:1001–1011

Dolle JP, Rezvan A, Allen FD, Lazarovici P, Lelkes PI (2005) Nerve growth factor-induced migration of endothelial cells. J Pharmacol Exp Ther 315:1220–1227

Donovan MJ, Miranda RC, Kraemer R, McCaffrey TA, Tessarollo L, Mahadeo D, Sharif S, Kaplan DR, Tsoulfas P, Parada L (1995) Neurotrophin and neurotrophin receptors in vascular smooth muscle cells. Regulation of expression in response to injury. Am J Pathol 147:309–324

Donovan MJ, Hahn R, Tessarollo L, Hempstead BL (1996) Identification of an essential nonneuronal function of neurotrophin 3 in mammalian cardiac development. Nat Genet 14:210–213

Donovan MJ, Lin MI, Wiegn P, Ringstedt T, Kraemer R, Hahn R, Wang S, Ibanez CF, Rafii S, Hempstead BL (2000) Brain derived neurotrophic factor is an endothelial cell survival factor required for intramyocardial vessel stabilization. Development 127:4531–4540

Drapeau J, El-Helou V, Clement R, Bel-Hadj S, Gosselin H, Trudeau LE, Villeneuve L, Calderone A (2005) Nestin-expressing neural stem cells identified in the scar following myocardial infarction. J Cell Physiol 204:51–62

Eisenhofer G, Friberg P, Rundqvist B, Quyyumi AA, Lambert G, Kaye DM, Kopin IJ, Goldstein DS, Esler MD (1996) Cardiac sympathetic nerve function in congestive heart failure. Circulation 93:1667

Ejiri M, Fujita M, Sakai O, Miwa K, Asanoi H, Sasayama S (1990) Development of collateral circulation after acute myocardial infarction: its role in preserving left ventricular function. J Cardiol 20:31–37

ElShamy WM, Ernfors P (1997) Brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4 complement and cooperate with each other sequentially during visceral neuron development. J Neurosci 17:8667–8675

Emanueli C, Salis MB, Pinna A, Graiani G, Manni L, Madeddu P (2002) Nerve growth factor promotes angiogenesis and arteriogenesis in ischemic hindlimbs. Circulation 106:2257–2262

Erickson JT, Conover JC, Borday V, Champagnat J, Barbacid M, Yancopoulos G, Katz DM (1996) Mice lacking brain-derived neurotrophic factor exhibit visceral sensory neuron losses distinct from mice lacking NT4 and display a severe developmental deficit in control of breathing. J Neurosci 16:5361–5371

Esler M, Kaye D, Lambert G, Esler D, Jennings G (1997) Adrenergic nervous system in heart failure. Am J Cardiol 80:7L–14L

Faerman I, Faccio E, Milei J, Nunez R, Jadzinsky M, Fox D, Rapaport M (1977) Autonomic neuropathy and painless myocardial infarction in diabetic patients. Histologic evidence of their relationship. Diabetes 26:1147–1158

Falckh PH, Harkin LA, Head RJ (1992) Nerve growth factor mRNA content parallels altered sympathetic innervation in the spontaneously hypertensive rat. Clin Exp Pharmacol Physiol 19:541–545

Genead R, Danielsson C, Andersson AB, Corbascio M, Franco-Cereceda A, Sylven C, Grinnemo KH (2010) Islet-1 cells are cardiac progenitors present during the entire lifespan: from the embryonic stage to adulthood. Stem Cells Dev 19:1601–1615

Glebova NO, Ginty DD (2004) Heterogeneous requirement of NGF for sympathetic target innervation in vivo. J Neurosci 24:743–751

Glebova NO, Ginty DD (2005) Growth and survival signals controlling sympathetic nervous system development. Annu Rev Neurosci 28:191–222

Graiani G, Emanueli C, Desortes E, Van Linthout S, Pinna A, Figueroa CD, Manni L, Madeddu P (2004) Nerve growth factor promotes reparative angiogenesis and inhibits endothelial apoptosis in cutaneous wounds of Type 1 diabetic mice. Diabetologia 47:1047–1054

Grassi G, Seravalle G, Quarti-Trevano F (2010) The 'neuroadrenergic hypothesis' in hypertension: current evidence. Exp Physiol 95:581–586

Habecker BA, Bilimoria P, Linick C, Gritman K, Lorentz CU, Woodward W, Birren SJ (2008) Regulation of cardiac innervation and function via the p75 neurotrophin receptor. Auton Neurosci 140:40–48

Hansen-Algenstaedt N, Algenstaedt P, Schaefer C, Hamann A, Wolfram L, Cingoz G, Kilic N, Schwarzloh B, Schroeder M, Joscheck C, Wiesner L, Ruther W, Ergun S (2006) Neural driven angiogenesis by overexpression of nerve growth factor. Histochem Cell Biol 125:637–649

Hasan W, Smith PG (2009) Modulation of rat parasympathetic cardiac ganglion phenotype and NGF synthesis by adrenergic nerves. Auton Neurosci 145:17–26

Hasan W, Pedchenko T, Krizsan-Agbas D, Baum L, Smith PG (2003) Sympathetic neurons synthesize and secrete pro-nerve growth factor protein. J Neurobiol 57:38–53

Hasan W, Jama A, Donohue T, Wernli G, Onyszchuk G, Al Hafez B, Bilgen M, Smith PG (2006) Sympathetic hyperinnervation and inflammatory cell NGF synthesis following myocardial infarction in rats. Brain Res 1124:142–154

Head RJ (1989) Hypernoradrenergic innervation: its relationship to functional and hyperplastic changes in the vasculature of the spontaneously hypertensive rat. Blood Vessels 26:1–20

Heil M, Eitenmuller I, Schmitz-Rixen T, Schaper W (2006) Arteriogenesis versus angiogenesis: similarities and differences. J Cell Mol Med 10:45–55

Hellweg R, Hartung HD (1990) Endogenous levels of nerve growth factor (NGF) are altered in experimental diabetes mellitus: a possible role for NGF in the pathogenesis of diabetic neuropathy. J Neurosci Res 26:258–267

Henning RJ, Sawmiller DR (2001) Vasoactive intestinal peptide: cardiovascular effects. Cardiovasc Res 49:27–37

Herring N, Lokale MN, Danson EJ, Heaton DA, Paterson DJ (2008) Neuropeptide Y reduces acetylcholine release and vagal bradycardia via a Y2 receptor-mediated, protein kinase C-dependent pathway. J Mol Cell Cardiol 44:477–485

Hiltunen JO, Arumae U, Moshnyakov M, Saarma M (1996) Expression of mRNAs for neurotrophins and their receptors in developing rat heart. Circ Res 79:930–939

Hiltunen JO, Laurikainen A, Vakeva A, Meri S, Saarma M (2001) Nerve growth factor and brain-derived neurotrophic factor mRNAs are regulated in distinct cell populations of rat heart after ischaemia and reperfusion. J Pathol 194:247–253

Huang CC, Chen PC, Huang CW, Yu J (2007) Aristolochic acid induces heart failure in zebrafish embryos that is mediated by inflammation. Toxicol Sci 100:486–494

Huber LJ, Hempstead B, Donovan MJ (1996) Neurotrophin and neurotrophin receptors in human fetal kidney. Dev Biol 179:369–381

Ieda M, Fukuda K (2009) Cardiac innervation and sudden cardiac death. Curr Cardiol Rev 5:289–295

Ieda M, Kanazawa H, Ieda Y, Kimura K, Matsumura K, Tomita Y, Yagi T, Onizuka T, Shimoji K, Ogawa S, Makino S, Sano M, Fukuda K (2006) Nerve growth factor is critical for cardiac sensory innervation and rescues neuropathy in diabetic hearts. Circulation 114:2351–2363

Kaye DM, Vaddadi G, Gruskin SL, Du XJ, Esler MD (2000) Reduced myocardial nerve growth factor expression in human and experimental heart failure. Circ Res 86:E80–E84

Kermani P, Rafii D, Jin DK, Whitlock P, Schaffer W, Chiang A, Vincent L, Friedrich M, Shido K, Hackett NR, Crystal RG, Rafii S, Hempstead BL (2005) Neurotrophins promote revascularization by local recruitment of TrkB + endothelial cells and systemic mobilization of hematopoietic progenitors. J Clin Invest 115:653–663

Kim H, Li Q, Hempstead BL, Madri JA (2004) Paracrine and autocrine functions of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in brain-derived endothelial cells. J Biol Chem 279:33538–33546

Kimura K, Kanazawa H, Ieda M, Kawaguchi-Manabe H, Miyake Y, Yagi T, Arai T, Sano M, Fukuda K (2010) Norepinephrine-induced nerve growth factor depletion causes cardiac sympathetic denervation in severe heart failure. Auton Neurosci 156:27–35

Kline DD, Ogier M, Kunze DL, Katz DM (2010) Exogenous brain-derived neurotrophic factor rescues synaptic dysfunction in Mecp2-null mice. J Neurosci 30:5303–5310

Kodama K, Kusuoka H, Sakai A, Adachi T, Hasegawa S, Ueda Y, Mishima M, Hori M, Kamada T, Inoue M, Hirayama A (1996) Collateral channels that develop after an acute myocardial infarction prevent subsequent left ventricular dilation. J Am Coll Cardiol 27:1133–1139

Kohn J, Aloyz RS, Toma JG, Haak-Frendscho M, Miller FD (1999) Functionally antagonistic interactions between the TrkA and p75 neurotrophin receptors regulate sympathetic neuron growth and target innervation. J Neurosci 19:5393–5408

Kraemer R, Nguyen H, March KL, Hempstead B (1999) NGF activates similar intracellular signaling pathways in vascular smooth muscle cells as PDGF-BB but elicits different biological responses. Arterioscler Thromb Vasc Biol 19:1041–1050

Kreusser MM, Haass M, Buss SJ, Hardt SE, Gerber SH, Kinscherf R, Katus HA, Backs J (2006) Injection of nerve growth factor into stellate ganglia improves norepinephrine reuptake into failing hearts. Hypertension 47:209–215

Kreusser MM, Buss SJ, Krebs J, Kinscherf R, Metz J, Katus HA, Haass M, Backs J (2008) Differential expression of cardiac neurotrophic factors and sympathetic nerve ending abnormalities within the failing heart. J Mol Cell Cardiol 44:380–387

Kuruvilla R, Zweifel LS, Glebova NO, Lonze BE, Valdez G, Ye H, Ginty DD (2004) A neurotrophin signaling cascade coordinates sympathetic neuron development through differential control of TrkA trafficking and retrograde signaling. Cell 118:243–255

Lai LP, Fan TH, Delehanty JM, Yatani A, Liang CS (1996) Elevated myocardial interstitial norepinephrine concentration contributes to the regulation of Na+, K(+)-ATPase in heart failure. Eur J Pharmacol 309:235–241

Langer A, Freeman MR, Josse RG, Armstrong PW (1995) Metaiodobenzylguanidine imaging in diabetes mellitus: assessment of cardiac sympathetic denervation and its relation to autonomic dysfunction and silent myocardial ischemia. J Am Coll Cardiol 25:610–618

Laycock SK, Kane KA, McMurray J, PARRATT JR (1996) Captopril and norepinephrine-induced hypertrophy and haemodynamics in rats. J Cardiovasc Pharmacol 27:667–672

Lee R, Kermani P, Teng KK, Hempstead BL (2001) Regulation of cell survival by secreted proneurotrophins. Science 294:1945–1948

Levenberg S, Burdick JA, Kraehenbuehl T, Langer R (2005) Neurotrophin-induced differentiation of human embryonic stem cells on three-dimensional polymeric scaffolds. Tissue Eng 11:506–512

Levy MN (1990) Autonomic interactions in cardiac control. Ann N Y Acad Sci 601:209–221

Li YJ, Peng J (2002) The cardioprotection of calcitonin gene-related peptide-mediated preconditioning. Eur J Pharmacol 442:173–177

Lockhart ST, Mead JN, Pisano JM, Slonimsky JD, Birren SJ (2000) Nerve growth factor collaborates with myocyte-derived factors to promote development of presynaptic sites in cultured sympathetic neurons. J Neurobiol 42:460–476

Lorentz CU, Alston EN, Belcik JT, Lindner JR, Giraud GD, Habecker BA (2010) Heterogeneous ventricular sympathetic innervation, altered beta adrenergic receptor expression, and rhythm instability in mice lacking p75 neurotrophin receptor. Am J Physiol Heart Circ Physiol 298: H1652–H1660

Luther JA, Birren SJ (2009) p75 and TrkA signaling regulates sympathetic neuronal firing patterns via differential modulation of voltage-gated currents. J Neurosci 29:5411–5424
Luttun A et al (2002) Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. Nat Med 8:831–840
Martin JL, Jenkins VK, Hsieh HY, Balkowiec A (2009) Brain-derived neurotrophic factor in arterial baroreceptor pathways: implications for activity-dependent plasticity at baroafferent synapses. J Neurochem 108:450–464
Max SR, Rohrer H, Otten U, Thoenen H (1978) Nerve growth factor-mediated induction of tyrosine hydroxylase in rat superior cervical ganglia in vitro. J Biol Chem 253:8013–8015
McMahon SB, Bennett DL, Priestley JV, Shelton DL (1995) The biological effects of endogenous nerve growth factor on adult sensory neurons revealed by a trkA-IgG fusion molecule. Nat Med 1:774–780
Meloni M, Caporali A, Graiani G, Lagrasta C, Katare R, Van Linthout S, Spillmann F, Campesi I, Madeddu P, Quaini F, Emanueli C (2010) Nerve growth factor promotes cardiac repair following myocardial infarction. Circ Res 106:1275–1284
Meloni M, Descamps B, Caporali A, Zentilin L, Floris I, Giacca M, Emanueli C (2012) Nerve growth factor gene therapy using adeno-associated viral vectors prevents cardiomyopathy in type 1 diabetic mice. Diabetes 61:229–240
Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, Jacobsen C, Kliemannel M, Schwarz E, Willnow TE, Hempstead BL, Petersen CM (2004) Sortilin is essential for proNGF-induced neuronal cell death. Nature 427:843–848
Oh YS, Jong AY, Kim DT, Li H, Wang C, Zemljic-Harpf A, Ross RS, Fishbein MC, Chen PS, Chen LS (2006) Spatial distribution of nerve sprouting after myocardial infarction in mice. Heart Rhythm 3:728–736
Palko ME, Coppola V, Tessarollo L (1999) Evidence for a role of truncated trkC receptor isoforms in mouse development. J Neurosci 19:775–782
Park MJ, Kwak HJ, Lee HC, Yoo DH, Park IC, Kim MS, Lee SH, Rhee CH, Hong SI (2007) Nerve growth factor induces endothelial cell invasion and cord formation by promoting matrix metalloproteinase-2 expression through the phosphatidylinositol 3-kinase/Akt signaling pathway and AP-2 transcription factor. J Biol Chem 282:30485–30496
Park KA, Fehrenbacher JC, Thompson EL, Duarte DB, Hingtgen CM, Vasko MR (2010) Signaling pathways that mediate nerve growth factor-induced increase in expression and release of calcitonin gene-related peptide from sensory neurons. Neuroscience 171:910–923
Patel TD, Jackman A, Rice FL, Kucera J, Snider WD (2000) Development of sensory neurons in the absence of NGF/TrkA signaling in vivo. Neuron 25:345–357
Paul CE, Vereker E, Dickson KM, Barker PA (2004) A pro-apoptotic fragment of the p75 neurotrophin receptor is expressed in p75NTRExonIV null mice. J Neurosci 24:1917–1923
Pop-Busui R (2010) Cardiac autonomic neuropathy in diabetes: a clinical perspective. Diabetes Care 33:434–441
Potente M, Urbich C, Sasaki K, Hofmann WK, Heeschen C, Aicher A, Kollipara R, DePinho RA, Zeiher AM, Dimmeler S (2005) Involvement of Foxo transcription factors in angiogenesis and postnatal neovascularization. J Clin Invest 115:2382–2392
Potts JT (2002) Neural circuits controlling cardiorespiratory responses: baroreceptor and somatic afferents in the nucleus tractus solitarius. Clin Exp Pharmacol Physiol 29:103–111
Pyle AD, Lock LF, Donovan PJ (2006) Neurotrophins mediate human embryonic stem cell survival. Nat Biotechnol 24:344–350
Qin F, Vulapalli RS, Stevens SY, Liang CS (2002) Loss of cardiac sympathetic neurotransmitters in heart failure and NE infusion is associated with reduced NGF. Am J Physiol Heart Circ Physiol 282:H363–H371
Rahbek UL, Dissing S, Thomassen C, Hansen AJ, Tritsaris K (2005) Nerve growth factor activates aorta endothelial cells causing PI3K/Akt- and ERK-dependent migration. Pflugers Arch 450:355–361

Rana OR, Schauerte P, Hommes D, Schwinger RH, Schroder JW, Hoffmann R, Saygili E (2010) Mechanical stretch induces nerve sprouting in rat sympathetic neurocytes. Auton Neurosci 155:25–32

Risau W, Flamme I (1995) Vasculogenesis. Annu Rev Cell Dev Biol 11:73–91

Roosen A, Schober A, Strelau J, Bottner M, Faulhaber J, Bendner G, McIlwrath SL, Seller H, Ehmke H, Lewin GR, Unsicker K (2001) Lack of neurotrophin-4 causes selective structural and chemical deficits in sympathetic ganglia and their preganglionic innervation. J Neurosci 21:3073–3084

Rubart M, Zipes DP (2005) Mechanisms of sudden cardiac death. J Clin Invest 115:2305–2315

Salis MB, Graiani G, Desortes E, Caldwell RB, Madeddu P, Emanueli C (2004) Nerve growth factor supplementation reverses the impairment, induced by Type 1 diabetes, of hindlimb post-ischaemic recovery in mice. Diabetologia 47:1055–1063

Santos PM, Winterowd JG, Allen GG, Bothwell MA, Rubel EW (1991) Nerve growth factor: increased angiogenesis without improved nerve regeneration. Otolaryngol Head Neck Surg 105:12–25

Saygili E, Schauerte P, Pekassa M, Saygili E, Rackauskas G, Schwinger RH, Weis J, Weber C, Marx N, Rana OR (2011) Sympathetic neurons express and secrete MMP-2 and MT1-MMP to control nerve sprouting via Pro-NGF conversion. Cell Mol Neurobiol 31:17–25

Scarisbrick IA, Jones EG, Isackson PJ (1993) Coexpression of mRNAs for NGF, BDNF, and NT-3 in the cardiovascular system of the pre- and postnatal rat. J Neurosci 13:875–893

Schmid H, Forman LA, Cao X, Sherman PS, Stevens MJ (1999) Heterogeneous cardiac sympathetic denervation and decreased myocardial nerve growth factor in streptozotocin-induced diabetic rats: implications for cardiac sympathetic dysinnervation complicating diabetes. Diabetes 48:603–608

Shadiack AM, Sun Y, Zigmond RE (2001) Nerve growth factor antiserum induces axotomy-like changes in neuropeptide expression in intact sympathetic and sensory neurons. J Neurosci 21:363–371

Sharma N, Deppmann CD, Harrington AW, St Hillaire C, Chen ZY, Lee FS, Ginty DD (2010) Long-distance control of synapse assembly by target-derived NGF. Neuron 67:422–434

Shmelkov SV, Meeus S, Moussazadeh N, Kermani P, Rashbaum WK, Rabbany SY, Hanson MA, Lane WJ, St Clair R, Walsh KA, Dias S, Jacobson JT, Hempstead BL, Edelberg JM, Rafii S (2005) Cytokine preconditioning promotes codifferentiation of human fetal liver CD133+ stem cells into angiomyogenic tissue. Circulation 111:1175–1183

Siao CJ, Lorentz CU, Kermani P, Marinic T, Carter J, McGrath K, Padow VA, Mark W, Falcone DJ, Cohen-Gould L, Parrish DC, Habecker BA, Nykjaer A, Ellenson LH, Tessarollo L, Hempstead BL (2012) ProNGF, a cytokine induced after myocardial infarction in humans, targets pericytes to promote microvascular damage and activation. J Exp Med 209 (12):2291–2305

Skoff AM, Adler JE (2006) Nerve growth factor regulates substance P in adult sensory neurons through both TrkA and p75 receptors. Exp Neurol 197:430–436

Smeyne RJ, Klein R, Schnapp A, Long LK, Bryant S, Lewin A, Lira SA, Barbacid M (1994) Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature 368:246–249

Smith-White MA, Iismaa TP, Potter EK (2003) Galanin and neuropeptide Y reduce cholinergic transmission in the heart of the anaesthetised mouse. Br J Pharmacol 140:170–178

Stevens MJ, Raffel DM, Allman KC, Dayanikli F, Ficaro E, Sandford T, Wieland DM, Pfeifer MA, Schwaiger M (1998) Cardiac sympathetic dysinnervation in diabetes: implications for enhanced cardiovascular risk. Circulation 98:961–968

Supowit SC, Ethridge RT, Zhao H, Katki KA, Dipette DJ (2005) Calcitonin gene-related peptide and substance P contribute to reduced blood pressure in sympathectomized rats. Am J Physiol Heart Circ Physiol 289:H1169–H1175

Takeo C, Nakamura S, Tanaka T, Uchida D, Noguchi Y, Nagao T, Saito Y, Tatsuno I (2003) Rat cerebral endothelial cells express trk C and are regulated by neurotrophin-3. Biochem Biophys Res Commun 305:400–406

Tessarollo L (1998) Pleiotropic functions of neurotrophins in development. Cytokine Growth Factor Rev 9:125–137

Tessarollo L, Tsoulfas P, Donovan MJ, Palko ME, Blair-Flynn J, Hempstead BL, Parada LF (1997) Targeted deletion of all isoforms of the trkC gene suggests the use of alternate receptors by its ligand neurotrophin-3 in neuronal development and implicates trkC in normal cardiogenesis. Proc Natl Acad Sci USA 94:14776–14781

Thoenen H (1972) Comparison between the effect of neuronal activity and nerve growth factor on the enzymes involved in the synthesis of norepinephrine. Pharmacol Rev 24:255–267

Thomas JA, Marks BH (1978) Plasma norepinephrine in congestive heart failure. Am J Cardiol 41:233–243

Tsoporis JN, Marks A, Kahn HJ, Butany JW, Liu PP, O'Hanlon D, Parker TG (1998) Inhibition of norepinephrine-induced cardiac hypertrophy in s100beta transgenic mice. J Clin Invest 102:1609–1616

von Schack D, Casademunt E, Schweigreiter R, Meyer M, Bibel M, Dechant G (2001) Complete ablation of the neurotrophin receptor p75NTR causes defects both in the nervous and the vascular system. Nat Neurosci 4:977–978

Wagner N, Wagner KD, Theres H, Englert C, Schedl A, Scholz H (2005) Coronary vessel development requires activation of the TrkB neurotrophin receptor by the Wilms' tumor transcription factor Wt1. Genes Dev 19:2631–2642

Wang S, Bray P, McCaffrey T, March K, Hempstead BL, Kraemer R (2000) p75(NTR) mediates neurotrophin-induced apoptosis of vascular smooth muscle cells. Am J Pathol 157:1247–1258

Wang H, Ward N, Boswell M, Katz DM (2006) Secretion of brain-derived neurotrophic factor from brain microvascular endothelial cells. Eur J Neurosci 23:1665–1670

Wernli G, Hasan W, Bhattacherjee A, van Rooijen N, Smith PG (2009) Macrophage depletion suppresses sympathetic hyperinnervation following myocardial infarction. Basic Res Cardiol 104:681–693

Xiang FL, Lu X, Hammoud L, Zhu P, Chidiac P, Robbins J, Feng Q (2009) Cardiomyocyte-specific overexpression of human stem cell factor improves cardiac function and survival after myocardial infarction in mice. Circulation 120:1065–1074

Yagi J, Wenk HN, Naves LA, McCleskey EW (2006) Sustained currents through ASIC3 ion channels at the modest pH changes that occur during myocardial ischemia. Circ Res 99:501–509

Zettler C, Rush RA (1993) Elevated concentrations of nerve growth factor in heart and mesenteric arteries of spontaneously hypertensive rats. Brain Res 614:15–20

Zhang SH, Rush RA (2001) Neurotrophin 3 is increased in the spontaneously hypertensive rat. J Hypertens 19:2251–2256

Zhou S, Chen LS, Miyauchi Y, Miyauchi M, Kar S, Kangavari S, Fishbein MC, Sharifi B, Chen PS (2004) Mechanisms of cardiac nerve sprouting after myocardial infarction in dogs. Circ Res 95:76–83

Neurotrophin Signalling and Transcription Programmes Interactions in the Development of Somatosensory Neurons

F. Marmigère and P. Carroll

Abstract

Somatosensory neurons of the dorsal root ganglia are generated from multipotent neural crest cells by a process of progressive specification and differentiation. Intrinsic transcription programmes active in somatosensory neuron progenitors and early post-mitotic neurons drive the cell-type expression of neurotrophin receptors. In turn, signalling by members of the neurotrophin family controls expression of transcription factors that regulate neuronal sub-type specification. This chapter explores the mechanisms by which this crosstalk between neurotrophin signalling and transcription programmes generates the diverse functional sub-types of somatosensory neurons found in the mature animal.

Keywords

Neurotrophins • Somatosensory neuron functional diversity • Transcription factors • Specification of neuronal identity

1 Introduction

In this chapter, we will concentrate on the roles of neurotrophins in the differentiation and cell fate specification of somatosensory neurons of the peripheral nervous system (PNS). This system, because of its relative simplicity and accessibility, has been immensely instructive for our understanding of the key roles of neurotrophin signalling in shaping nervous system development. We will review current ideas about how complex interactions between neurotrophin signalling pathways and intrinsic transcriptional codes together (1) generate the diverse array of functionally

F. Marmigère • P. Carroll (✉)
INSERM U1051, Institut des Neurosciences de Montpellier (INM), 80, rue Augustin Fliche, 34091, Montpellier, France
e-mail: carroll@univ-montp2.fr

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_13,

distinct sensory neurons of the PNS (2) enable the formation of appropriate central and peripheral projections of these neurons.

The PNS comprises several ganglia or plexi with different functions and specificities disseminated in the whole body. Body sensations are detected by sensory neurons located in dorsal root ganglia (DRG) along the spinal cord and in the trigeminal ganglia located at the base of the brain. Sensory neurons are characterised by the type of primary sensory stimulus they transmit to the CNS: mechanoreceptors that respond to mechanical stimuli, proprioceptors that respond to limb and muscle movement, thermoreceptors that respond to temperature, nociceptors that respond to painful or pruritic (itch) stimuli (for reviews see Delmas et al. 2011; Han and Simon 2011; Woolf and Ma 2007). Classical physiological studies showed that these different types of sensory neurons innervate specific target end-organs in the skin, muscle, tendons and organs of the body, form highly stereotypic connections with central target neurons in the spinal cord, generating a topographic map of the surface of the body and of the positions of joints and muscles. Information from somatosensory neurons is integrated into spinal circuits that control reflexes and coordinated movements.

A characteristic of peripheral neurons is their great diversity, although they come from the same progenitor cells: diversity regarding their size, their biochemistry, the targets they contact and their physiological functioning. In recent years, the functional diversity within different classes of sensory neurons is being revealed by the identification of specific molecular markers and genetic labelling techniques combined with physiological analyses. Several types of functionally distinct nociceptors can be distinguished by expression of ion channels and receptors (Liu and Ma 2011). Some of these are also distinguishable by the Mas-related G protein coupled receptor (Mrgpr) expression (Dong et al. 2001), and specific sub-types of Mgrprs have been shown to be necessary for itch sensation (Liu et al. 2009). Another remarkable example is the diversity of sensory neurons responding to temperature. The characterisation of the expression of the different Trp class of channels in various thermoreceptors differentially activated by a wide range of temperature allowed a better understanding of how sensory neuron discriminate painful cold or heat from pleasant warm or cool stimuli (Caterina et al. 1997; Peier et al. 2002; Jordt et al. 2003; Patapoutian et al. 2003; McKemy 2005). Similarly, the calcium binding protein parvalbumin is a marker for proprioceptive neurons (Carr and Nagy 1993). Myelinated Aβ sub-types of low threshold mechanoreceptors have been identified by transcription factor gene and Ret receptor expression (Bourane et al. 2009; Luo et al. 2009; Wende et al. 2012). Genetic labelling techniques in mice are now being used to identify and trace the fine anatomy of the projections of different types of somatosensory neurons innervating the skin (Liu et al. 2007; Badea et al. 2012; Li et al. 2012).

During embryonic development, the vast majority of the PNS derives from a transient population of multipotent stem cells: the neural crest cells (NCCs). This is the case of the cells composing the DRG, the autonomic nervous system including the enteric nervous plexus, the parasympathetic ganglia and the sympathetic chain. The DRGs are formed by NCCs that migrate ventrally between the somite and the

neural tube. Other transient embryonic structures, the neurogenic placodes, participate in the formation of the cranial ganglia, together or not with NCCs. Thus, the vestibulo-acoustic (VIII), facial (VII) and the glossopharyngeal (IX), petrosal and nodose cranial ganglia exclusively derives from neurogenic placodes whereas the trigeminal ganglion is a mixed population arising from both neurogenic placodes and neural crest origins in which nociceptors arise from NCCs. The jugular ganglion is exclusively of NCC origin. Beside their common embryonic origins and their function in maintaining the communication between the external environment and the internal milieu, neurons composing the PNS share other similarities. They all contact a peripheral usually non-neuronal target located in the different organs of the body and, for afferent neurons, central target neurons located in the central nervous system. Peripheral neurons thus cross long distance in the body to find, recognise and establish contacts with their appropriate peripheral and central targets.

Cellular diversity is created according to a classical principle of developmental biology: the specification or cell lineage segregation. Multipotent stem cells proliferate in a tightly controlled manner to self-renew and give birth to progenitors with restricted potential regarding proliferative rates and phenotypic fate. These progenitor cells migrate long distances and undergo several transitions and phenotypic transformations during their journey, changing shape, polarity, size, biochemical markers and their responses to the local environment. As differentiation progresses, the fate potential of multipotent progenitor cells is progressively restricted in a "step-by-step" manner. This process – called lineage segregation – is achieved by two main mechanisms: epigenetic signals between cells and cell autonomous genetic programmes acting within each cell.

Thus, cell fate commitment and phenotypic differentiation result from the integration of distinct transcriptional programmes that are regulated by different signalling pathways. In this concept, under the influence of extrinsic signals activating intrinsic cell-autonomous programmes, the progression of a multipotent progenitor towards a more differentiated state is accompanied by a loss of competence. External cues are mainly ligands secreted by local organising centres acting on specific membrane-bound receptors. Cell-autonomous signals are mainly transcription factor networks acting within the cell nucleus to regulate specific transcriptional programmes. During development of the nervous system, morphogens such as sonic hedgehog, retinoic acid, growth factors and members of the wingless (Wnts) and bone morphogenetic proteins (BMPs) families are produced by local organising centres and diffuse from their source in the surrounding embryonic tissues, combining at variable distances depending on their diffusion properties. In early embryogenesis, these molecules pattern the different tissues in a highly organised manner to create the embryo.

Over the last several years, some of the transcription factors involved in the progressive lineage restriction of multipotent neural crest stem cells into the diverse array of fully differentiated somatosensory neurons have been identified (for reviews see Pavan and Raible 2012; Lallemend and Ernfors 2012; Marmigere and Ernfors 2007). During delamination from the dorsal neural tube and their ventral

migration, NCCs express the sex-determining region of Y chromosome (SRY)-related high mobility group (HMG)-box transcription factor Sox10 (Kim et al. 2003) and Pax3 (Goulding et al. 1991; Nakazaki et al. 2008). These two factors are important for NCCs specification and appear to be necessary to maintain the multipotent state, since gene inactivation leads to premature neural differentiation (George et al. 2010; Nakazaki et al 2008), an effect that may due in part to repression of proneural gene expression such as Ngn2 (Nakazaki et al. 2008). Sox10 maintains NCCs in the multipotent state (Kim et al 2003) and Pax3 seems to be necessary for NCC migration (Conway et al. 1997; Serbedzija and McMahon 1997). The future neurons of the ganglia are derived from neuronal precursors that are produced in three successive and overlapping waves of neurogenesis between E9.5 and E11 in the mouse embryo (Marmigere and Ernfors 2007). The first wave produces mostly precursors of future myelinated afferents of the proprioceptor and mechanoreceptor functional sub-classes; the second wave produces future non-myelinated nociceptive neurons and C-fibre mechanoreceptors sub-classes. A minor population of nociceptive neurons arises from the later migration of precursors from a transient neural crest-derived structure called the boundary cap, located at the dorsal root entry zone and representing a reservoir of multipotent sensory precursors (Maro et al. 2004; Hjerling-Leffler et al. 2005).

Under the influence of morphogens such as Wnt1 (Lee et al. 2004), BMPs (Raible and Ragland 2005), fibroblast growth factors (FGFs) (Murphy et al. 1994; Barembaum and Bronner-Fraser 2005; Ota and Ito 2006; Stuhlmiller and Garcia-Castro 2012) and Notch signalling (Hu et al. 2011; Mead and Yutzey 2012), NCCs that will form the future sensory neurons begin to express proneural basic helix-loop-helix (bHLH) transcription factors neurogenin 2 (Ngn2) and neurogenin 1 (Ngn1) between E9.5 and E10.5. Ngn1 and Ngn2 are the earliest lineage markers of sensory precursors and are essential for the development of DRG neurons since double Ngn1/2 mutants display agenesis of the DRG (Ma et al. 1999). In particular, high levels of Wnt signalling through its intracellular target β-catenin plays an instructive role in driving sensory neuron specification. Mutation of β-catenin in neural crest results in a loss of Ngn2 expression and reduced sensory neurogenesis (Hari et al. 2002). Inversely, maintaining a sustained β-catenin activity in NCCs results in a premature and ectopic Ngn2 expression with mislocated and overabundant sensory neurons at the expense of other neural crest derivatives (Lee et al. 2004). These transient expressions of Ngn2 and 1 in two temporally successive but overlapping timeframes identify the two first waves of neurogenesis mentioned earlier. Ngn2 expression appears in the first wave of progenitors that gives rise to most of the future myelinated afferents in the DRG, including neurons that contribute to the proprioceptive, mechanoceptive and to the lightly myelinated Aδ nociceptive populations, whereas the Ngn1 population gives rise to some myelinated afferents and most unmyelinated nociceptive neurons at later stages (Ma et al. 1999; Bachy et al. 2011). However, in the absence of Ngn2, Ngn1-expressing precursors are capable of generating most functional types of sensory neurons, indicating a developmental plasticity in the generation of these neurons. Nevertheless, many of the details of the factors governing the numbers of each

progenitor type produced and the exact roles of the Ngns in these cells have still to be worked out. Are Ngn1 and Ngn2 functionally equivalent generic proneural factors differentially regulated in time or do they have specific roles associated with their expression in the early and late progenitor populations?

The transition from neuronal precursors to post-mitotic sensory neurons requires the expression of the transcription factors Islet1 and Brn3a. A series of elegant studies by the Turner group using single and double mutant mice lines has shown that these factors appear to act together to suppress the progenitor state and drive sensory differentiation to the exclusion of other neuronal fates (Fedtsova and Turner 1995; Eng et al. 2007; Sun et al. 2008; Lanier et al. 2009; Dykes et al. 2011). In the absence of these molecules, sensory progenitors express the general neuronal marker βIII-tubulin but fail to up-regulate a series of genes characteristic of sensory neurons. The FoxS1 transcription factor is also induced in sensory precursors and post-mitotic neurons during this transition (Heglind et al. 2005; Montelius et al. 2007). However, its role in this process is not known since no sensory phenotype has been described in mouse mutants for this gene. Having consolidated a generic sensory phenotype, early post-mitotic sensory neurons begin to unfold the transcriptional programmes that will specify them into the different functional sub-classes of neurons in the adult organism.

As somatosensory neurons extend processes during embryonic growth, retrograde signals pattern their growth and pathfinding in the peripheral tissues. These retrograde signals include neurotrophins and other neurotrophic factors, secreted from target tissues, as well as repulsive and attractive guidance molecules that together cooperate with intrinsic transcriptional programmes to coordinate the appropriate specification, differentiation, survival and neurite growth of PNS neurons. The importance of neurotrophins in the harmonious development of this system is exemplified by the deleterious effects of loss, overexpression or mutation of neurotrophins or their receptors.

2 Neurotrophin-Trk Signalling and Functional Sub-classes of Somatosensory Neurons (Fig. 1)

The neurotrophins (NGF, BDNF, NT3 and NT4) and their receptors (TrkA, TrkB, TrkC and p75) belong to the superfamily of growth factors/receptors and their appearance during development coincides with neurogenesis. From the moment of their birth, it appears that all somatosensory and autonomic sensory neurons express at least one neurotrophin receptor. Neurotrophins are secreted peptides that diffuse poorly and act as autocrine/paracrine factors regulating neural precursor selection and early neurogenesis. Later in nervous system development, they serve as long distance factors regulating axonal growth, cell survival, specification and phenotypic stabilisation. For these late functions, neurotrophin receptors are synthesised by the neurons whereas their ligands are secreted by neuronal targets thus establishing an elaborate communication system to establish

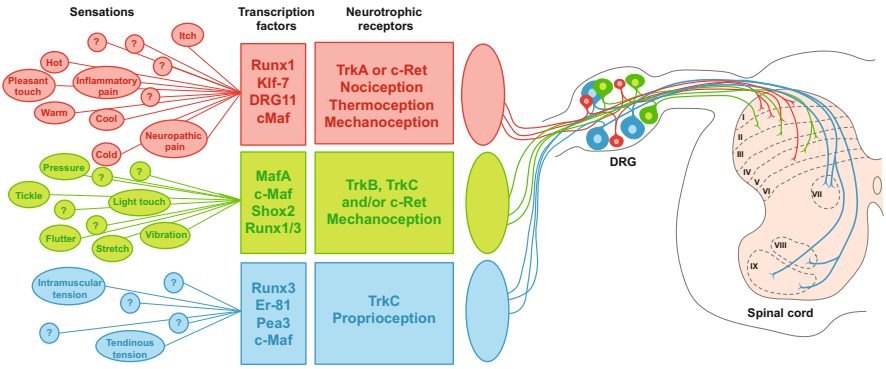

Fig. 1 Molecular characteristics of somatosensory neuron functional sub-types. The major classes of somatosensory neurons (thermo- and nociceptors, C-fibre low-threshold mechanoreceptors; *red*), myelinated low-threshold mechanoreceptors (*green*) and muscle spindle and Golgi tendon organ afferents (*blue*) and the sensory modalities that they transduce are shown. Different functional sub-types project to different regions of the spinal cord. Different functional sub-types express different neurotrophic factor receptors (Trks and Ret) in adult stages. Transcription factors associated with the development of different functional sub-types are shown

appropriate recognition between a neuron and its specific target. In this scenario, neurotrophins signalling first promotes long range signalling bringing the axonal projections to the vicinity of the source of neurotrophins. According to the neurotrophic hypothesis, neurotrophins are secreted by the target in limited amounts sufficient to promote neuronal survival of a limited number of neurons. Thus, whereas neurons are initially produced in excess, the competition for limiting amounts of retrograde neurotrophin signalling leads to the elimination of superfluous neurons and neurons not presenting the appropriate high affinity neurotrophins receptor (for recent review on neurotrophins and neuronal survival see Ichim et al. 2012).

Studies on Trk receptors expression and analyses of mice carrying mutations at neurotrophin and *trk* receptor loci have initially led to the general idea that different neurotrophin-Trk receptor combinations are necessary for the development of the three main classes of somatosensory neurons: NGF-TrkA for nociceptors, BDNF/NT4-TrkB for mechanoreceptors and NT-3-TrkC for proprioceptors. However, further biochemical characterisation of sensory neurons allowing a better discrimination of the numerous sub-classes has revealed a more complex reality. In fact, the expression of Trk receptors in sensory neurons is highly dynamic during development. Trk receptors display overlapping and sequential expression patterns in sensory neurons as they progressively mature towards their ultimate differentiated phenotype in the adult (for review see Ernsberger 2009). Accordingly, the neuronal loss found in mutant mice lacking the different neurotrophins and their receptors is much greater than the numbers of Trk-expressing neurons at a given stage. Many newly born DRG neurons express multiple Trk receptors and probably respond to short range autocrine/paracrine cues.

TrkC is the first neurotrophin receptor to be expressed and is found in a majority of DRG neurons at early stages in all studied species. Genetic lineage tracing studies using a TrkC-Cre mouse line crossed with a Cre reporter line showed that most sensory neurons in the trigeminal ganglion express TrkC at some stage of their development (Funfschilling et al. 2004). TrkC expression is rapidly followed by TrkB and TrkA is the last member of the family to be expressed (Ernsberger 2009). The dynamic nature of Trks expression is illustrated by the changes in co-expression of TrkB and TrkC during development. TrkB is expressed in a subset of post-mitotic neurons at E11 (Farinas et al. 1998) and co-localises with TrkC in 75 % of the neurons. The co-incidence of TrkB and TrkC drops to 40 % at E12 and to 10 % by E12.5, finally falling to zero at E14 (Kramer et al. 2006). However, in adult mice 20 % of the DRG neurons co-express TrkB and TrkC (McMahon et al. 1994; Karchewski et al. 1999). In neonatal mice, 40 % of neurons express TrkC (Liebl et al. 1997, 2000). Similarly in adult rats, 20 % of the neurons express TrkC (Wetmore and Olson 1995).

The onset of TrkA expression in mouse is E10.5 (Wright and Snider 1995; Phillips and Armanini 1996), starts in few neurons and increases dramatically by E11.5 to 20 % of the neurons and by E13 and E15 to 80 %, falling to 30 % in the adult (Wright and Snider 1995; Molliver et al. 1997; Molliver and Snider 1997; Farinas et al. 1998; Luo et al. 2007). In late gestation, the decrease in TrkA-expressing neurons has been shown to be due to a switch in expression from TrkA to c-Ret (Molliver et al. 1997), and this postnatal loss of TrkA depends on Ret signalling (Luo et al. 2007). Nevertheless, whereas TrkA exhibits a more and more restricted expression in subpopulations of thermo- and nociceptors during development, its signalling is necessary for the development of all nociceptors since TrkA or NGF mice mutants lack all types of nociceptors by birth (Crowley et al. 1994; Smeyne et al. 1994). In the adult mouse, the TrkA-positive population represents the peptidergic nociceptors and lightly myelinated Aδ nociceptors, and the Ret-positive population includes the IB4-lectin binding non-peptidergic nociceptors. TrkC-NT3 signalling is necessary for the survival of proprioceptive neurons (Ernfors et al. 1994; Klein et al. 1994), and mouse mutants at these loci display uncoordinated movements. TrkC is also expressed in some cutaneous myelinated afferents. Single unit recordings on isolated nerve-skin preparations and chronic application of NT3 in chick embryos showed that cutaneous slowly adapting mechanoreceptors depend on NT3-TrkC for their survival during development (Airaksinen et al. 1996; Oakley et al. 2000). However, their firing properties in the adult are determined by BDNF-TrkB signalling (Carroll et al. 1998). TrkB-NT4 signalling is necessary for the survival of adult D-hair mechanoreceptors (Stucky et al. 2002), but 40 % of these afferents are also lost during early development in NT3-deficient mice. The above examples demonstrate that different aspects of sensory neuron development (survival, axon growth, physiological functions) can be under the influence of different neurotrophins-Trk receptor signalling at different times during their development. In addition, a series of experiments in which null-mutant strains of mice for neurotrophins or their receptors were crossed into a Bax-null mutant background preventing apoptosis

in DRG demonstrated at least for NGF/TrkA and NT3/TrkC signalling a function beyond neuronal survival in the full phenotypic maturation of nociceptive and proprioceptive neurons including the expression of specific ion channels and neuropeptides and the establishment of stereotyped projection pattern characteristic of their physiological functions (Patel et al. 2000, 2003). Such an instructive role of neurotrophins signalling for sensory neurons specification was further confirmed by knock-in experiments in which the expression of TrkC receptor from the *TrkA* locus was able to switch the fate of a small subset of nociceptive neurons in proprioceptive neurons (Moqrich et al. 2004). The demonstration of this late essential role of neurotrophins signalling thus placed these molecules at the centre of studies related to sensory neuron specification and diversification.

3 Early Effects of Neurotrophins

Differentiation/fate differentiation

The process of neuronal specification is the acquisition of definitive phenotypic characteristics for a given sub-class of neurons during embryonic development. This acquisition can be divided into several interdependent and sequential phases, from the time point when progenitor cells exit the cell cycle towards the newly formed and the perfectly differentiated neuron gains its definitive physiological function. These steps include phases of specific biochemical characteristics acquisition, axonal growth, survival, set-up and maintenance of connectivity with the appropriate targets. Any disturbance of one of these steps compromises the following one and leads to the elimination of the cell mainly through apoptosis.

3.1 Proliferation and Cell Cycle Exit

The early expression of Trk receptors in sensory neuron precursors before they establish synaptic contact with their peripheral target led to the general idea that early in development, local (non-target-derived) neurotrophins act on their receptor to influence proliferation, survival and differentiation of neural progenitors in the PNS (Schecterson and Bothwell 1992; Buchman and Davies 1993; Elkabes et al. 1994; Farinas et al. 1996; White et al. 1996; Rifkin et al. 2000). Accordingly, several studies in chick, quail and mice have further demonstrated a role for NT3 in proliferating sensory neuron precursors. PNS stem cells in vivo respond to NT3 during gangliogenesis which can arrest their proliferation. In vitro, NT3 is able to stimulate the proliferation of chick and quail NCCs as well as early rat DRG cells (Kalcheim et al. 1992; Pinco et al. 1993; Memberg and Hall 1995). NT3 also promotes neurogenesis for a subset of NCCs in vitro (Henion et al. 1995) as well as the maturation of early isolated chick DRG neurons in vitro (Wright et al. 1992). Addition of anti-NT3 antibodies in chick at the time of gangliogenesis induces a dramatic loss of sensory neurons both in DRG and nodose ganglion (Wright et al. 1992; Lefcort et al. 1996), and the loss occurs in precursor cells (Lefcort

et al. 1996). Thus, at early stages, in vivo functions of NT3 include regulation of neuronal precursor proliferation or induction of differentiation of precursors into TrkC-expressing neurons that will become dependent on NT3 for survival. These effects are specific to NT3 and could not be mediated via NGF signalling (Gaese et al. 1994). These effects of NT-3 on NCCs proliferation seems to be mediated by activation of full-length TrkC receptor (Hapner et al. 1998). All these effects exist before the target-dependent neurotrophic period and are due to locally produced NT3. Surprisingly, addition of NT3 in the same experiment, i.e. directly to the chick embryo before gangliogenesis when neuroblasts are still proliferating, also reduced the numbers of sensory neurons in DRG and nodose ganglia as well as a reduction of proliferating neuroblasts (Ockel et al. 1996). If NT3 is applied later, the number of neurons is increased, consistent with the role of NT3 in promoting survival of mature neurons (Ockel et al. 1996). In mice mutants for NT3 at E12, the numbers of neurons were normal, but there were fewer precursor cells (Farinas et al. 1996). This was interpreted as a depletion of the progenitor pool by premature neurogenesis in the absence of NT3. However, since no expression of Trk receptors has been detected in proliferating sensory precursors in mice (Farinas et al. 1998), these effects of NT3 could be indirect (Farinas et al. 2002).

Since the DRG neuronal loss in TrkC mutant is much less pronounced than in NT3 mutants at early stages (Ernfors et al. 1994; Klein et al. 1994; Minichiello et al. 1995), it is believed that early locally produced NT3 acts via TrkA activation (Davies et al. 1995; Huang et al. 1999). However, whereas TrkA and NGF mutants showed a synchronous cell death at E13.5, TrkC and NT3 null mutants exhibit cell death at E11.5. In the trigeminal ganglion, similar results were observed (ElShamy and Ernfors 1996; Pinon et al. 1996; Wilkinson et al. 1996). Additional early role in neural crest commitment/differentiation to the sensory lineage have been attributed to BDNF in vitro (Sieber-Blum et al. 1993). However, such a function has never been demonstrated in vivo.

3.2 From Dividing Neuronal Progenitors to Post-mitotic Sensory Neurons

The transition from a dividing neuronal progenitor to a post-mitotic sensory neuron involves the down-regulation of the proneural genes *neurogenin-1* and *-2* and the induction of transcription programmes characteristic of the post-mitotic state. The transcription factors Islet1 and Brn3a play an essential role in this transition in sensory neurons, since in mice lacking both of these factors, Ngn expression is maintained and all studied early sensory neuron markers, including Trk receptors, are absent (Dykes et al. 2011). However, the general neural marker βIII-tubulin was expressed almost normally, showing that neurogenesis was not affected, whereas the process of sub-type specification was completely compromised. Brn3a expression is correlated with the onset of expression of Trk receptors in post-mitotic neurons, and mice lacking Brn3a fail to express TrkA in sensory neurons and subsequently die presumably due to lack of trophic support from neurotrophins

(McEvilly et al. 1996). NT-3 acting on progenitors may play a role in this process, since it has been shown at least in trigeminal progenitors in culture that NT3 can induce Brn3a expression (Wyatt et al. 1998).

4 Transcription Programmes in the Development of Somatosensory Neurons (Fig. 2)

Depending on the cellular context and the developmental stage, the further development of sensory neurons is characterised by crosstalk between transcriptional programmes and neurotrophins-Trk receptors and several other neurotrophic factor signalling pathways. As described above, Trk receptor expression in sensory neurons is highly dynamic throughout embryonic development. Because of the restricted neurotrophin receptor expression profiles in sub-types of specified sensory neurons, one strategy used by developmental neurobiologists to identify such transcriptional programmes has been to study the transcriptional regulation of the neurotrophins receptors TrkA, TrkB and TrkC (for review see Lei and Parada 2007).

Regulation of the TrkA promoter by transcription factor binding

The first and best-characterised enhancer of a neurotrophin receptor gene is the promoter of the *trkA* gene (Sacristan et al. 1999; Ma et al. 2000, 2003). In this promoter, several binding sites for the transcription factor Brn3a, conserved across species, have been characterised (Ma et al. 2003; Valderrama and Misra 2008). However, loss of Brn3a results in a loss of TrkA expression and neurons in the sensory trigeminal ganglion but not in the DRG (McEvilly et al. 1996; Xiang et al. 1996; Huang et al. 1999; Eng et al. 2001). The Kruppel-like zinc-finger transcription factor Klf-7 has been found to be expressed in TrkA-positive neurons in the developing sensory ganglia (Lei et al. 2001) and cooperates with Brn3a to activate the *trkA* enhancer (Lei et al. 2006). Mutation of the *Klf7* gene led to a loss of TrkA expression and subsequent neuronal death by apoptosis of nociceptive sub-classes (Lei et al. 2005). Similarly, the homeodomain interacting protein kinase 2 (HIPK2) interacts with Brn3a to promote its binding to DNA but suppresses its activation of TrkA transcription. Mutant mice displayed increased TrkA expression and neuronal numbers in the trigeminal ganglia (Wiggins et al. 2004). However, no effect of HIPK2 inactivation was reported in DRG neurons.

Other DNA binding protein such as Zhangfei/Crebzf (Valderrama et al. 2008), the deltaNp73 isoform of the *p73* gene (Zhang and Chen 2007), have been shown to bind the *trkA* promoter and to modulate its transcription. However, although they might be expressed in developing DRG, their roles in the specification of the nociceptive sub-class of sensory neurons remain to be demonstrated.

Transcription factors and the regulation of neurotrophin receptor genes expression

As mentioned above, Brn3a and Islet have been shown to be critically important for the development of sensory neurons (Dykes et al. 2011). In DRGs of Brn3a/

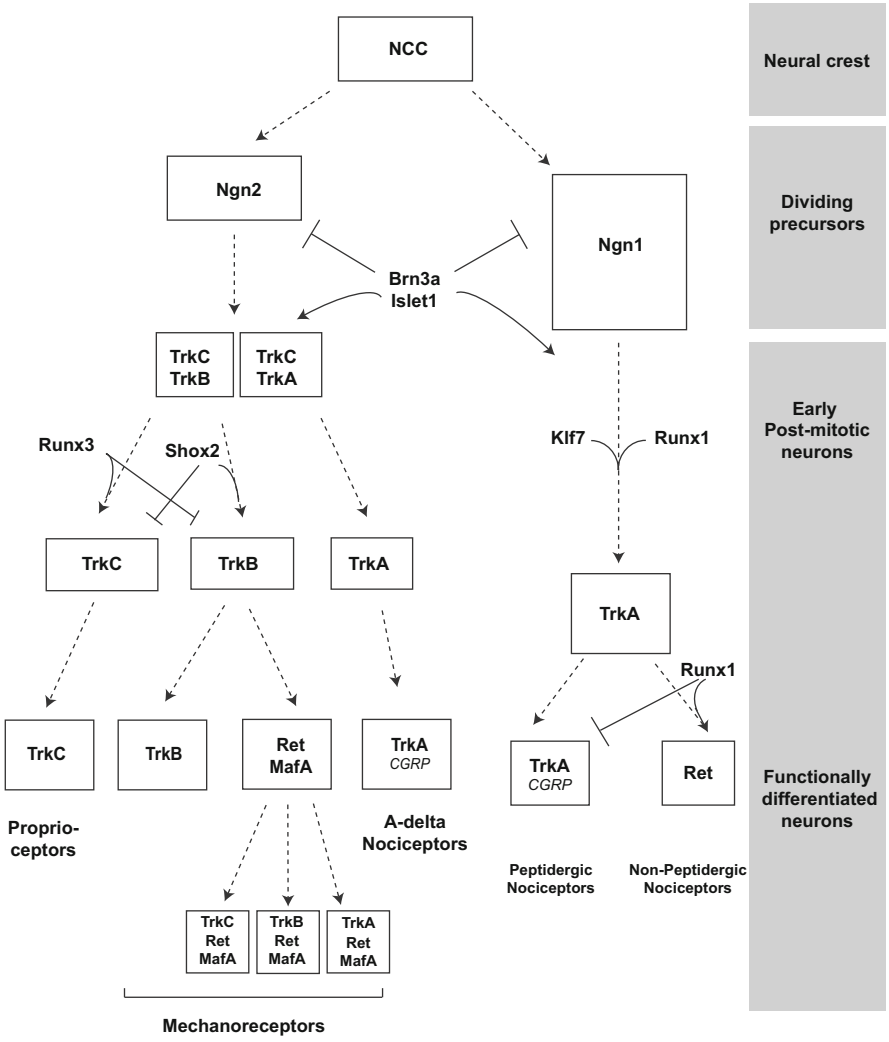

Fig. 2 Differentiation of somatosensory neurons from multipotent neural crest cells. Neural crest cells (NCC) give rise to two major sub-classes of sensory neuron precursors expressing the proneural genes *ngn1* and *ngn2*. Ngn2 precursors generate most of the myelinated afferent sensory neurons, whereas umyelinated nociceptors, thermoceptors and low-threshold C-fibres are generated from the Ngn1 precursor population. Brn3a and Islet1 suppress Ngn expression and drive neuronal differentiation and neurotrophin survival dependence, in part through the regulation of Trk receptor expression. By cross-inhibitory mechanisms, Runx3 and Shox2 transcription factors further differentiate neurons towards the proprioceptor and mechanoreceptor lineages. Maf transcription factors play roles in the generation of specific sub-types of mechanoreceptor neurons. In the Ngn1-dependent lineage, Klf7 and Runx1 drive TrkA expression at early stages and Runx1, via regulation of TrkA expression, plays an essential role in the generation of diversity of thermo- and nociceptors later in development

Islet1 double mutant mice at E13, there was an absence of expression of TrkA, -B and -C as well as several other transcription factors (Runx3, Runx1, ER81) and signalling molecules (Ret) necessary for the generation of sensory neuron sub-types. Transcription factors of the Runx family play important roles in the specification of the proprioceptor and nociceptor functional classes of sensory neurons. In the proprioceptive lineage Runx3 is required for the expression of TrkC as well as parvalbumin and represses that of TrkB (Inoue et al. 2002, 2007; Levanon et al. 2002; Kramer et al. 2006). At later stages of development Runx3 is also expressed in skin innervating sensory neurons that have not yet been functionally identified and may also regulate the expression of TrkA and CGRP in these neurons (Nakamura et al. 2008). A potential mechanism for the direct regulation of *trkB* gene transcription by Runx3 was suggested by the identification of a negative regulatory sequence in intron 7 of the *trkB* gene that contains Runx binding sites and is responsive to TrkC signalling (Inoue et al. 2007). The level of Runx3 expression in proprioceptive and cutaneous afferents neurons also controls the projection termination region of these afferents along the dorso-ventral axis of the spinal cord (Chen et al. 2006a) although it has been suggested that this could be a secondary effect of inefficient peripheral target innervation in Runx3 mutants that results in loss of access to retrograde signals that control the expression of ER81 (Lallemend et al. 2012). A recent study showed that Runx3 expression levels also influence the rate of axon growth of proprioceptive neurons in the periphery and thus could be a mechanism for adapting axon growth rate to the proximo-distal position of target muscles (Lallemend et al. 2012).

The cross-inhibitory mechanisms by which transcription factors promote a particular cellular fate while simultaneously suppressing alternative fates are well illustrated by the inhibition of the mechanoreceptive neuron factor Shox2 by Runx3 (Abdo et al. 2011; Scott et al. 2011). Shox2 is necessary for the development of TrkB-positive neurons. Runx3 suppresses Shox2 and TrkB expression in TrkC proprioceptive neurons. Conversely, in the putative mechanoreceptor neuron population, Shox2 promotes TrkB expression and inhibits TrkC expression.

Small cell-body diameter unmyelinated neuron sub-classes include all nociceptors, thermoreceptors, pruriceptors and low-threshold C-fibre mechanosensory neurons. These neurons all arise from the Ngn1-expressing precursor population (Ma et al. 1999; Kramer et al. 2006). As they exit the cell cycle, they express Runx1 and are characterised by the early expression of TrkA. As is the case for Runx3 expression in the proprioceptive lineage, early Runx1 expression depends on Brn3a and Islet1 (Dykes et al. 2011). Later in development (E15 to postnatal stages), Runx1 plays an essential role in the diversification of nociceptors into two major classes: the TrkA-positive peptidergic nociceptors expressing CGRP and substance P and the Ret-positive non-peptidergic IB4 lectin binding nociceptors (Chen et al. 2006b; Kramer et al. 2006; Yoshikawa et al. 2007). Thus, NGF signalling through TrkA causes down-regulation of Runx1 in peptidergic nociceptors. Reciprocally, Runx1 is essential for the down-regulation of TrkA and the up-regulation of Ret in the non-peptidergic nociceptor population (Luo et al. 2007). Most of the functions of Runx1 including its role in the

diversification into peptidergic and non-peptidergic sub-classes require a genetic interaction with the homeodomain transcription factor Tlx3, which is broadly expressed in developing DRG neurons (Lopes et al. 2012). Although absence of Runx1 does not appear to affect the initiation of TrkA expression in the mouse in some studies (Yoshikawa et al. 2007), others have reported an early loss of TrkA-positive neurons in another Runx1-deficient mouse model (Kobayashi et al. 2012). Nevertheless, gain or loss of function experiments in the chick and in vitro analysis of TrkA promoter activity, together with the late function of Runx1 in the perinatal diversification of the two main sub-classes of nociceptors, suggest that Runx1 can directly modulate *trkA* transcription (Mulloy et al. 2005; Marmigere et al. 2006; Chen et al. 2006b; Kramer et al. 2006; Yoshikawa et al. 2007). As demonstrated for Runx3 and proprioceptive neurons (Lallemend et al. 2012), the levels of Runx1 seem to be important in determining the axonal rate growth of nociceptive neurons (Chen et al. 2006b; Marmigere et al. 2006; Marmigere and Ernfors 2007). Runx1 is necessary for the correct central projections of IB4-positive non-peptidergic neurons in the dorsal horn, and Runx1 mutant mice display altered inflammatory and neuropathic pain behaviours (Chen et al. 2006b). Further detailed analyses revealed that Runx1 characterises two populations of nociceptors, distinguished by persistent or transient Runx1 expression and that these sub-classes of nociceptors play roles in inflammatory or neuropathic pain, respectively (Abdel Samad et al. 2010).

How are the Runx3 and Runx1 lineages established? Analysis of single Brn3a and Islet1 knockout mice suggest that these factors have a partially selective role in sub-type specification, in that Brn3a and Islet1 are the principal regulators of Runx3 and Runx1 expression, respectively, but loss of both factors is required to completely extinguish expression of these sub-type markers (Dykes et al. 2011). In line with this, mice lacking Islet1 alone lose most cutaneous innervation whereas muscle proprioceptors are normal (Sun et al. 2008). Nevertheless, it is not yet known if Brn3a and Islet1 directly regulate *runx* genes, or if they create a cellular environment that allows other signalling pathways to induce gene expression.

DRG11/Prrxl1 is a paired homeodomain protein that plays a role in the correct spatio-temporal projections of primary nociceptive neurons to the superficial laminae of the spinal cord (Chen et al. 2001). Expression of DRG11 appears at E12 in mouse DRG neurons (Chen et al. 2001), and two different isoforms are expressed in the same sensory neuron sub-classes (Rebelo et al. 2009). Genetic ablation of DRG11 leads to loss of central projections of nociceptive afferents during embryonic stages and a reduction in peptidergic and non-peptidergic nociceptive neuron numbers post-natally accompanied by behavioural deficits in nociception without affecting large DRG neurons. However, analysis of the expression of series of molecular markers of nociceptive neurons including TrkA showed no differences between wild-type and mutant mice DRGs throughout embryonic development (Chen et al. 2001; Rebelo et al. 2006), contrasting with the effect of Runx1 inactivation on the expression of the same set of genes. Interestingly, DRG11 is also expressed in second-order interneurons in the superficial dorsal horn, and the expression of PKC gamma, a marker of a subset of spinal interneurons

involved in pain processing, was lost in mutant mice. C-fibre innervation of peripheral tissues was also reduced at postnatal stages in mutant mice (Rebelo et al. 2006). Although expressed in the same neurons at the same time, it appears that DRG11 and Runx1 act on different sets of genes to exert their functions. Runx1 would appear to specify nociceptor sub-type identity whereas DRG11, by as yet unknown mechanisms, is important for spatiotemporal control of nociceptor projections. The postnatal growth of peptidergic and non-peptidergic nociceptive neurons strongly depends on TrkA and Ret signalling, respectively. Whether DRG11 transcriptionally regulates or is activated downstream of these signalling pathways has not been explored in detail.

The homeobox gene *hoxD1* is expressed in the TrkA-expressing nociceptive population beginning at E12 in mouse development (Guo et al. 2012). Interestingly, HoxD1 expression in nociceptors is particular to mammals and seems to be important for differences in nociceptive circuitry between mice and chick (Guo et al. 2012). In HoxD1 mutant mice, several classes of skin innervating nociceptors display abnormal termination patterns. In addition, central projections of nociceptors in the spinal cord were aberrant and resembled those of the chick. It was therefore suggested that the *hoxD1* gene was co-opted during mammalian evolution to play a role in determining the mammal-specific characteristic features of nociceptive circuits.

Until recently, much less was known about the transcriptional programmes that drive the specification and differentiation of low-threshold mechanoreceptor neurons responsible for touch sensation. Progress was hampered by the very low representation of these neurons, their high diversity and the lack of specific markers to identify them. Mafs are members of the leucine zipper transcription factor superfamily. Two members of the Maf family have now been shown to be important for the development of highly specific sub-types of myelinated low-threshold mechanoreceptors, namely the rapidly adapting afferents (RA-LTMs) that innervate hair follicles, Pacinian corpuscles and Meissner corpuscles of the glabrous skin. cMaf and MafA are both expressed in these neuronal sub-types (Bourane et al. 2009; Wende et al. 2012). These neurons specifically express the Ret tyrosine kinase receptor from early stages and loss of Ret causes defects in their peripheral and central target innervation (Luo et al. 2009). cMaf is essential for the development of these neurons. Mutant mice display defective innervation of hair follicles and reduced and/or atrophied Pacinian and Meissner corpuscles and altered electrophysiological response of RA-LTMs. Human patients carrying point mutations in the cMaf gene present aberrant sensitivity to vibration stimuli, in accordance with the observed defect in the Pacinian corpuscle innervating afferents in the mutant mice (Wende et al. 2012). Interestingly, cMaf is also necessary for the expression of the voltage-gated potassium channel KCNQ4. KCNQ4 is found at the peripheral nerve endings of Aβ-hair follicle afferents and Meissner corpuscles and is required for the proper velocity coding and frequency tuning of these receptors in both mice and human (Heidenreich et al. 2012). The role of MafA in these neurons is less clear, and loss of MafA in mutant mice could potentially be compensated by cMaf. The expression of MafA is dependent on cMaf expression, but MafA has been

shown to regulate the proportions of TrkB and Ret myelinated afferents (Bourane et al. 2009). In line with a putative redundancy between these two factors, cMaf/MafA double mutants have a more severe Pacinian corpuscle phenotype than cMaf mutant alone (Wende et al. 2012). cMaf is necessary for the maintenance but not the initiation of Ret expression in these neurons, and Ret expression is progressively lost in RA-LTMs in cMaf mutants between E13 and P0. Thus, some of the defects seen in cMaf mutants are most likely due to loss of Ret signalling. Indeed, cMaf and Ret mutants display several similar phenotypes such as aberrant peripheral and central projections of RA-LTMs (Bourane et al. 2009; Luo et al. 2009; Wende et al. 2012).

The homeobox protein Shox2 was shown to be widely expressed in early DRG neurons, later becoming progressively restricted to the medium diameter cell body neurons that are TrkB-positive putative mechanoreceptor neurons (Abdo et al. 2011; Scott et al. 2011). Conditional ablation of Shox2 in neural crest derivatives caused a 60 % reduction in the numbers of TrkB-positive neurons by P0 (Scott et al. 2011). However, no evidence of cell death was found suggesting a failure of differentiation of TrkB neurons. Analysis of cutaneous innervation in Shox2 mutant skin showed deficits in the innervation of Merkel cells, hair follicles and Meissner corpuscles whereas Pacinian corpuscles were unaffected (Abdo et al. 2011). Loss of a single *shox2* allele caused an intermediate reduction in the numbers of TrkB-positive neurons, and heterozygous Shox2 mutant mice displayed a reduced sensitivity to mechanical stimulation of the paw (Abdo et al. 2011). Whether and how *Shox2* and *Maf* genes interact genetically in the development of mechanoreceptors has not been addressed so far.

The homeodomain transcription factor Cux2 is expressed in subsets of post-mitotic neurons in the DRG from early stages of development (Bachy et al. 2011). It was shown that a part of this neuronal population that expresses TrkA is derived from the Ngn2-positive first wave of DRG sensory neurogenesis. These early-born TrkA-positive neurons are thought to become Aδ nociceptors that have lightly myelinated fibres and convey sharp pain in response to mechanical insults. Mouse mutants at the *cux2* locus did not show any changes in neuronal numbers or in the numbers of cells expressing sub-type-specific markers during development. However, adult Cux2 mutant mice were hypersensitive to mechanical stimuli (Bachy et al. 2011).

The three members of the Brn3 family of transcription factors (Brn3a, -b and -c) are expressed in subsets of DRG neurons. Genetic labelling in mice using Brn3-Cre alleles crossed with reporter mice allowed the visualisation of afferent arbours in the skin and spinal cord of adult animals (Badea et al. 2012). It was shown that Brn3a is expressed in a wide variety of DRG neurons whereas Brn3b and Brn3c were restricted to hair follicle innervating mechanoreceptors and peptidergic nociceptors, respectively. No somatosensory neuron phenotype was detected in Brn3b and Brn3c mutant mice (Badea et al. 2012).

In conclusion, there is a growing body of information about the transcriptional programmes regulating the different stages of neurogenesis, specification and differentiation of different functional sub-classes of sensory neurons. These

programmes could have direct effects on the transcriptional regulation of neurotrophin receptor genes and/or be regulated by neurotrophins signalling as evidenced by studies on the Runx and Maf families of transcription factors. As was the case for the initial thinking that one Trk receptor was expressed by one functional sub-class of neurons, recent data on Runx1 and Runx3 expression in sensory neurons during development suggest that the dichotomy of Runx1/nociceptors and Runx3/proprioceptors is an over-simplification (Yoshikawa et al. 2013). Indeed, Runx transcription factors expression appears to be also highly dynamic throughout development, overlapping with TrkB, c-Ret and TrkC in some mechanoreceptive neurons (Yoshikawa et al. 2013). Similarly, the c-Maf transcription factor is also found expressed in some TrkA/CGRP and TrkC/parvalbumin neurons (Wende et al. 2012). Thus, it is tempting to speculate that if all these factors are involved in the transcriptional regulation of *trk* genes, their levels of expression together with their appropriate combinations will be the key to crack the code specifying each sensory sub-type. To fully resolve this issue, many questions remain to be answered, such as how these factors are induced? What are the hierarchical relationships between many of the transcription factors expressed in the same neuronal lineage? What are the downstream transcriptional targets responsible for the observed phenotypes in the respective mutants? When different factors are expressed in the same neuronal sub-type, do they cooperate in their actions or function in parallel to drive specific aspects of neuronal differentiation? Technical advances such as ChIP will help to further progress in this area. Besides, the role of epigenetic factors such as chromatin modifications and histone acetylation in DRG neuron specification has only been touched on (Eng et al. 2007) but remains to be explored in detail.

4.1 Retrograde Control of Transcription Factor Expression by Neurotrophin Signalling

Whereas early steps such as neurogenesis and process outgrowth depend on intrinsic transcriptional programmes, target dependent signals are an essential part of the mechanisms by which neurons are integrated into neuronal circuits (Hippenmeyer et al. 2004). Limb ablation experiments in chick embryo were instrumental in the discovery of target-dependent survival and the identification of NGF. The importance of target-derived neurotrophins in the survival of different functional sub-types of somatosensory neurons has been well documented. With the identification of transcription factors specific to functional sub-types of sensory neurons, it became possible to study the role of signals from target tissues for the correct development of neurons that innervate them. After early specification by Runx3 has occurred, expression of two members of the ETS family of transcription factors (ER81 and Pea3) is turned on in proprioceptive neurons, as well as in specific pools of motoneurons in the spinal cord (Lin et al. 1998; Arber et al. 2000; Patel et al. 2000). In mouse and chick, ER81 was shown to regulate late steps in the differentiation of these neurons (Arber et al. 2000; Patel et al. 2003; Lee

et al. 2012). ER81 functions to control appropriate projections of proprioceptive neurons since mutation of the *er81* gene results in failure of these afferents to invade the ventral spinal cord and form synapses on motoneurons (Arber et al. 2000), in spite of the fact that peripheral projections to muscle are normal. Thus, in ER81 mutant mice, Ia proprioceptive afferents terminate prematurely in the intermediate spinal cord and fail to make monosynaptic connections with α-motoneurons. Limb ablation in the chick showed that limb-derived signals were necessary for the expression of these factors in sensory neurons during a period preceding programmed cell death (Lin et al. 1998). Subsequently, it was shown, by analyzing NT3/Bax double mutant mice in which proprioceptive Ia afferents survive in the absence of NT3, that NT3 produced in muscle is necessary for the expression of ER81. Accordingly, forced expression of NT3 in muscle of NT3 knockout mice restored ER81 expression in Ia proprioceptive neurons (Patel et al. 2003).

NGF retrograde signalling is also necessary for definitive phenotypic differentiation of nociceptive neurons. In TrkA/Bax double mutant, nociceptive neurons also survive in the absence of neurotrophins retrograde signalling (Patel et al. 2000). Using this mutant, it was possible to demonstrate that the expression of CGRP and Substance P, two neuropeptides expressed by TrkA-positive peptidergic nociceptors, as well as the expression of c-Ret by non-peptidergic nociceptors depend on TrkA/NGF signalling. Furthermore, this model was used to show that NGF/TrkA signalling is responsible for the acquisition of mechanosensitivity in nociceptors (Lechner et al 2009). In the case of Runx1, this factor is essential for the early induction of TrkA expression in the nociceptive neuron lineage but is itself subsequently down-regulated by TrkA signalling during the transition of the nociceptive class of neurons into peptidergic TrkA-positive and non-peptidergic Ret-positive sub-classes. Runx1 expression in the non-peptidergic neuron inhibits TrkA expression. This "reiterative" transcription factor/Trk receptor interaction has been proposed to be a hallmark of sensory neuron development (Lallemend and Ernfors 2012).

Recently, it was shown that NGF/TrkA signalling is necessary of the induction of *HoxD1* gene in mouse nociceptors both in vitro and in vivo (Guo et al. 2012). HoxD1, through the regulation of as-yet-unknown effector genes, subsequently plays an important role in controlling the correct peripheral and central target innervation by nociceptors. In an interesting evolutionary twist, Guo et al demonstrated that this NGF/TrkA/HoxD1 signalling pathway is specific to mammals and is not present in lower vertebrates.

Conclusions and Future Questions

As sensory neuron progenitors exit the cell cycle and begin to differentiate, they rapidly turn on the expression of transcription factors that drive their diversification into broad functional sub-classes. Under the influence of Brn3a and Islet1, newly born DRG neurons express multiple Trk receptors and probably respond to short range autocrine/paracrine cues. Neuronal specification involves refinement of Trk receptor expression by Runx transcription factors for the

proprioceptive and nociceptive lineages whereas Shox2 is important for TrkB expression in mechanoreceptor neurons. Members of the Maf family of transcription factors are induced very early in mechanoreceptor neurons sub-types by as-yet unknown mechanisms and are essential for their further development. Certain of these transcription factors (Runx3, cMaf, MafA and Runx1) are expressed from very early stages and are, to an extent, predictive for the final functional phenotype of these neurons in the adult animal, suggesting that the broad functional classes, i.e. proprioceptive, mechanoceptive and nociceptive are specified just after neuronal birth, before the neurons have extended projections to their target tissues. Other neurotrophic factor signalling pathways, notably those acting through Ret and Met receptors (Gascon et al. 2012), function in a complex interplay with the Trk receptors to further refine and sub-divide the different functional sub-types of sensory neurons. As sensory neurons innervate their target tissues, they receive trophic and differentiation signals, probably summing biochemical and electrophysiological information that control the transcriptional networks necessary for the consolidation of neuronal identity and to form appropriate projections to central target regions.

Since the discovery of neurotrophins and their receptors, somatosensory neurons of the DRG, because they express all Trk receptors, have been a model of choice to decipher the functions of neurotrophins signalling. Therefore, the effects we discussed here on survival, axonal growth, early and late neuronal specification are among the plethora of neurotrophins functions that were initially unravelled in sensory neurons. In the future, novel functions will certainly be attributed to neurotrophins signalling and the peripheral nervous system will definitively remain a precious model for such discovery. For instance, in the sympathetic nervous system, a novel function of NGF involving retrograde transport from the peripheral target in the control of synapse assembly with the central target has been demonstrated (Sharma et al. 2010). Besides, emerging data on pro-neurotrophins and new Trk receptor interacting molecules are revealing unexpected functions and even antagonistic effects to the well-established role of Trk receptors, as shown by the collapsing effect of proBDNF on DRG neurite outgrowth (Sun et al. 2012) or the enhancing effect of sortilin on retrograde transport of neurotrophins by interacting with TrkA, B and C (Vaegter et al. 2011).

References

Abdel Samad O, Liu Y, Yang FC, Kramer I, Arber S, Ma Q (2010) Characterization of two Runx1-dependent nociceptor differentiation programs necessary for inflammatory versus neuropathic pain. Mol Pain 6:45

Abdo H, Li L, Lallemend F, Bachy I, Xu XJ, Rice FL, Ernfors P (2011) Dependence on the transcription factor Shox2 for specification of sensory neurons conveying discriminative touch. Eur J Neurosci 34:1529–1541

Airaksinen MS, Koltzenburg M, Lewin GR, Masu Y, Helbig C, Wolf E, Brem G, Toyka KV, Thoenen H, Meyer M (1996) Specific subtypes of cutaneous mechanoreceptors require neurotrophin-3 following peripheral target innervation. Neuron 16:287–295

Arber S, Ladle DR, Lin JH, Frank E, Jessell TM (2000) ETS gene Er81 controls the formation of functional connections between group Ia sensory afferents and motor neurons. Cell 101:485–498

Bachy I, Franck MC, Li L, Abdo H, Pattyn A, Ernfors P (2011) The transcription factor Cux2 marks development of an A-delta sublineage of TrkA sensory neurons. Dev Biol 360:77–86

Badea TC, Williams J, Smallwood P, Shi M, Motajo O, Nathans J (2012) Combinatorial expression of Brn3 transcription factors in somatosensory neurons: genetic and morphologic analysis. J Neurosci 32:995–1007

Barembaum M, Bronner-Fraser M (2005) Early steps in neural crest specification. Semin Cell Dev Biol 16:642–646

Bourane S, Garces A, Venteo S, Pattyn A, Hubert T, Fichard A, Puech S, Boukhaddaoui H, Baudet C, Takahashi S, Valmier J, Carroll P (2009) Low-threshold mechanoreceptor subtypes selectively express MafA and are specified by Ret signaling. Neuron 64:857–870

Buchman VL, Davies AM (1993) Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. Development 118:989–1001

Carr PA, Nagy JI (1993) Emerging relationships between cytochemical properties and sensory modality transmission in primary sensory neurons. Brain Res Bull 30:209–219

Carroll P, Lewin GR, Koltzenburg M, Toyka KV, Thoenen H (1998) A role for BDNF in mechanosensation. Nat Neurosci 1:42–46

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824

Chen ZF, Rebelo S, White F, Malmberg AB, Baba H, Lima D, Woolf CJ, Basbaum AI, Anderson DJ (2001) The paired homeodomain protein DRG11 is required for the projection of cutaneous sensory afferent fibers to the dorsal spinal cord. Neuron 31:59–73

Chen AI, de Nooij JC, Jessell TM (2006a) Graded activity of transcription factor Runx3 specifies the laminar termination pattern of sensory axons in the developing spinal cord. Neuron 49:395–408

Chen CL, Broom DC, Liu Y, de Nooij JC, Li Z, Cen C, Samad OA, Jessell TM, Woolf CJ, Ma Q (2006b) Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. Neuron 49:365–377

Conway SJ, Henderson DJ, Copp AJ (1997) Pax3 is required for cardiac neural crest migration in the mouse: evidence from the splotch (Sp2H) mutant. Development 124:505–514

Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S, Armanini MP, Ling LH, McMahon SB, Shelton DL, Levinson AD et al (1994) Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 76:1001–1011

Davies AM, Minichiello L, Klein R (1995) Developmental changes in NT3 signalling via TrkA and TrkB in embryonic neurons. EMBO J 14:4482–4489

Delmas P, Hao J, Rodat-Despoix L (2011) Molecular mechanisms of mechanotransduction in mammalian sensory neurons. Nat Rev Neurosci 12:139–153

Dong X, Han S, Zylka MJ, Simon MI, Anderson DJ (2001) A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. Cell 106:619–632

Dykes IM, Tempest L, Lee SI, Turner EE (2011) Brn3a and Islet1 act epistatically to regulate the gene expression program of sensory differentiation. J Neurosci 31:9789–9799

Elkabes S, Dreyfus CF, Schaar DG, Black IB (1994) Embryonic sensory development: local expression of neurotrophin-3 and target expression of nerve growth factor. J Comp Neurol 341:204–213

ElShamy WM, Ernfors P (1996) A local action of neurotrophin-3 prevents the death of proliferating sensory neuron precursor cells. Neuron 16:963–972

Eng SR, Gratwick K, Rhee JM, Fedtsova N, Gan L, Turner EE (2001) Defects in sensory axon growth precede neuronal death in Brn3a-deficient mice. J Neurosci 21:541–549

Eng SR, Dykes IM, Lanier J, Fedtsova N, Turner EE (2007) POU-domain factor Brn3a regulates both distinct and common programs of gene expression in the spinal and trigeminal sensory ganglia. Neural Dev 2:3

Ernfors P, Lee KF, Kucera J, Jaenisch R (1994) Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. Cell 77:503–512

Ernsberger U (2009) Role of neurotrophin signalling in the differentiation of neurons from dorsal root ganglia and sympathetic ganglia. Cell Tissue Res 336:349–384

Farinas I, Yoshida CK, Backus C, Reichardt LF (1996) Lack of neurotrophin-3 results in death of spinal sensory neurons and premature differentiation of their precursors. Neuron 17:1065–1078

Farinas I, Wilkinson GA, Backus C, Reichardt LF, Patapoutian A (1998) Characterization of neurotrophin and Trk receptor functions in developing sensory ganglia: direct NT-3 activation of TrkB neurons in vivo. Neuron 21:325–334

Farinas I, Cano-Jaimez M, Bellmunt E, Soriano M (2002) Regulation of neurogenesis by neurotrophins in developing spinal sensory ganglia. Brain Res Bull 57:809–816

Fedtsova NG, Turner EE (1995) Brn-3.0 expression identifies early post-mitotic CNS neurons and sensory neural precursors. Mech Dev 53:291–304

Funfschilling U, Ng YG, Zang K, Miyazaki J, Reichardt LF, Rice FL (2004) TrkC kinase expression in distinct subsets of cutaneous trigeminal innervation and nonneuronal cells. J Comp Neurol 480:392–414

Gaese F, Kolbeck R, Barde YA (1994) Sensory ganglia require neurotrophin-3 early in development. Development 120:1613–1619

Gascon E, Gaillard S, Malapert P, Liu Y, Rodat-Despoix L, Samokhvalov IM, Delmas P, Helmbacher F, Maina F, Moqrich A (2012) Hepatocyte growth factor-Met signaling is required for Runx1 extinction and peptidergic differentiation in primary nociceptive neurons. J Neurosci 30:12414–12423

George L, Kasemeier-Kulesa J, Nelson BR, Koyano-Nakagawa N, Lefcort F (2010) Patterned assembly and neurogenesis in the chick dorsal root ganglion. J Comp Neurol 518:405–422

Goulding MD, Chalepakis G, Deutsch U, Erselius JR, Gruss P (1991) Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. EMBO J 10:1135–1147

Guo T, Mandai K, Condie BG, Wickramasinghe SR, Capecchi MR, Ginty DD (2012) An evolving NGF-Hoxd1 signaling pathway mediates development of divergent neural circuits in vertebrates. Nat Neurosci 14:31–36

Han SK, Simon MI (2011) Intracellular signaling and the origins of the sensations of itch and pain. Sci Signal 4:pe38

Hapner SJ, Boeshore KL, Large TH, Lefcort F (1998) Neural differentiation promoted by truncated trkC receptors in collaboration with p75(NTR). Dev Biol 201:90–100

Hari L, Brault V, Kleber M, Lee HY, Ille F, Leimeroth R, Paratore C, Suter U, Kemler R, Sommer L (2002) Lineage-specific requirements of beta-catenin in neural crest development. J Cell Biol 159:867–880

Heglind M, Cederberg A, Aquino J, Lucas G, Ernfors P, Enerback S (2005) Lack of the central nervous system- and neural crest-expressed forkhead gene Foxs1 affects motor function and body weight. Mol Cell Biol 25:5616–5625

Heidenreich M, Lechner SG, Vardanyan V, Wetzel C, Cremers CW, De Leenheer EM, Aránguez G, Moreno-Pelayo MÁ, Jentsch TJ, Lewin GR (2012) KCNQ4 K(+) channels tune mechanoreceptors for normal touch sensation in mouse and man. Nat Neurosci 15:138–145

Henion PD, Garner AS, Large TH, Weston JA (1995) trkC-mediated NT-3 signaling is required for the early development of a subpopulation of neurogenic neural crest cells. Dev Biol 172:602–613

Hippenmeyer S, Kramer I, Arber S (2004) Control of neuronal phenotype: what targets tell the cell bodies. Trends Neurosci 27:482–488

Hjerling-Leffler J, Marmigere F, Heglind M, Cederberg A, Koltzenburg M, Enerback S, Ernfors P (2005) The boundary cap: a source of neural crest stem cells that generate multiple sensory neuron subtypes. Development 132:2623–2632

Hu ZL, Shi M, Huang Y, Zheng MH, Pei Z, Chen JY, Han H, Ding YQ (2011) The role of the transcription factor Rbpj in the development of dorsal root ganglia. Neural Dev 6:14

Huang EJ, Wilkinson GA, Farinas I, Backus C, Zang K, Wong SL, Reichardt LF (1999) Expression of Trk receptors in the developing mouse trigeminal ganglion: in vivo evidence for NT-3 activation of TrkA and TrkB in addition to TrkC. Development 126:2191–2203

Ichim G, Tauszig-Delamasure S, Mehlen P (2012) Neurotrophins and cell death. Exp Cell Res 318:1221–1228

Inoue K, Ozaki S, Shiga T, Ito K, Masuda T, Okado N, Iseda T, Kawaguchi S, Ogawa M, Bae SC, Yamashita N, Itohara S, Kudo N, Ito Y (2002) Runx3 controls the axonal projection of proprioceptive dorsal root ganglion neurons. Nat Neurosci 5:946–954

Inoue K, Ito K, Osato M, Lee B, Bae SC, Ito Y (2007) The transcription factor Runx3 represses the neurotrophin receptor TrkB during lineage commitment of dorsal root ganglion neurons. J Biol Chem 282:24175–24184

Jordt SE, McKemy DD, Julius D (2003) Lessons from peppers and peppermint: the molecular logic of thermosensation. Curr Opin Neurobiol 13:487–492

Kalcheim C, Carmeli C, Rosenthal A (1992) Neurotrophin 3 is a mitogen for cultured neural crest cells. Proc Natl Acad Sci U S A 89:1661–1665

Karchewski LA, Kim FA, Johnston J, McKnight RM, Verge VM (1999) Anatomical evidence supporting the potential for modulation by multiple neurotrophins in the majority of adult lumbar sensory neurons. J Comp Neurol 413(2):327–341

Kim J, Lo L, Dormand E, Anderson DJ (2003) SOX10 maintains multipotency and inhibits neuronal differentiation of neural crest stem cells. Neuron 38:17–31

Klein R, Silos-Santiago I, Smeyne RJ, Lira SA, Brambilla R, Bryant S, Zhang L, Snider WD, Barbacid M (1994) Disruption of the neurotrophin-3 receptor gene trkC eliminates la muscle afferents and results in abnormal movements. Nature 368:249–251

Kobayashi A, Senzaki K, Ozaki S, Yoshikawa M, Shiga T (2012) Runx1 promotes neuronal differentiation in dorsal root ganglion. Mol Cell Neurosci 49:23–31

Kramer I, Sigrist M, de Nooij JC, Taniuchi I, Jessell TM, Arber S (2006) A role for Runx transcription factor signaling in dorsal root ganglion sensory neuron diversification. Neuron 49:379–393

Lallemend F, Ernfors P (2012) Molecular interactions underlying the specification of sensory neurons. Trends Neurosci 35:373–381

Lallemend F, Sterzenbach U, Hadjab-Lallemend S, Aquino JB, Castelo-Branco G, Sinha I, Villaescusa JC, Levanon D, Wang Y, Franck MC, Kharchenko O, Adameyko I, Linnarsson S, Groner Y, Turner E, Ernfors P (2012) Positional differences of axon growth rates between sensory neurons encoded by Runx3. EMBO J 31:3718–3729

Lanier J, Dykes IM, Nissen S, Eng SR, Turner EE (2009) Brn3a regulates the transition from neurogenesis to terminal differentiation and represses non-neural gene expression in the trigeminal ganglion. Dev Dyn 238:3065–3079

Lechner SG, Frenzel H, Wang R, Lewin GR (2009) Developmental waves of mechanosensitivity acquisition in sensory neuron subtypes during embryonic development. EMBO J 28:1479–1491

Lee HY, Kleber M, Hari L, Brault V, Suter U, Taketo MM, Kemler R, Sommer L (2004) Instructive role of Wnt/beta-catenin in sensory fate specification in neural crest stem cells. Science 303:1020–1023

Lee J, Friese A, Mielich M, Sigrist M, Arber S (2012) Scaling proprioceptor gene transcription by retrograde NT3 signaling. PLoS One 7:e45551

Lefcort F, Clary DO, Rusoff AC, Reichardt LF (1996) Inhibition of the NT-3 receptor TrkC, early in chick embryogenesis, results in severe reductions in multiple neuronal subpopulations in the dorsal root ganglia. J Neurosci 16:3704–3713

Lei L, Parada LF (2007) Transcriptional regulation of Trk family neurotrophin receptors. Cell Mol Life Sci 64:522–532

Lei L, Ma L, Nef S, Thai T, Parada LF (2001) mKlf7, a potential transcriptional regulator of TrkA nerve growth factor receptor expression in sensory and sympathetic neurons. Development 128:1147–1158

Lei L, Laub F, Lush M, Romero M, Zhou J, Luikart B, Klesse L, Ramirez F, Parada LF (2005) The zinc finger transcription factor Klf7 is required for TrkA gene expression and development of nociceptive sensory neurons. Genes Dev 19:1354–1364

Lei L, Zhou J, Lin L, Parada LF (2006) Brn3a and Klf7 cooperate to control TrkA expression in sensory neurons. Dev Biol 300:758–769

Levanon D, Bettoun D, Harris-Cerruti C, Woolf E, Negreanu V, Eilam R, Bernstein Y, Goldenberg D, Xiao C, Fliegauf M, Kremer E, Otto F, Brenner O, Lev-Tov A, Groner Y (2002) The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. EMBO J 21:3454–3463

Li L, Rutlin M, Abraira VE, Cassidy C, Kus L, Gong S, Jankowski MP, Luo W, Heintz N, Koerber HR, Woodbury CJ, Ginty DD (2012) The functional organization of cutaneous low-threshold mechanosensory neurons. Cell 147:1615–1627

Liebl DJ, Tessarollo L, Palko ME, Parada LF (1997) Absence of sensory neurons before target innervation in brain-derived neurotrophic factor-, neurotrophin 3-, and TrkC-deficient embryonic mice. J Neurosci 17:9113–9121

Liebl DJ, Klesse LJ, Tessarollo L, Wohlman T, Parada LF (2000) Loss of brain-derived neurotrophic factor-dependent neural crest-derived sensory neurons in neurotrophin-4 mutant mice. Proc Natl Acad Sci U S A 97:2297–2302

Lin JH, Saito T, Anderson DJ, Lance-Jones C, Jessell TM, Arber S (1998) Functionally related motor neuron pool and muscle sensory afferent subtypes defined by coordinate ETS gene expression. Cell 95:393–407

Liu Y, Ma Q (2011) Generation of somatic sensory neuron diversity and implications on sensory coding. Curr Opin Neurobiol 21:52–60

Liu Q, Vrontou S, Rice FL, Zylka MJ, Dong X, Anderson DJ (2007) Molecular genetic visualization of a rare subset of unmyelinated sensory neurons that may detect gentle touch. Nat Neurosci 10:946–948

Liu Q, Tang Z, Surdenikova L, Kim S, Patel KN, Kim A, Ru F, Guan Y, Weng HJ, Geng Y, Undem BJ, Kollarik M, Chen ZF, Anderson DJ, Dong X (2009) Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. Cell 139:1353–1365

Lopes C, Liu Z, Xu Y, Ma Q (2012) Tlx3 and Runx1 act in combination to coordinate the development of a cohort of nociceptors, thermoceptors, and pruriceptors. J Neurosci 32:9706–9715

Luo W, Wickramasinghe SR, Savitt JM, Griffin JW, Dawson TM, Ginty DD (2007) A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpeptidergic DRG neurons. Neuron 54:739–754

Luo W, Enomoto H, Rice FL, Milbrandt J, Ginty DD (2009) Molecular identification of rapidly adapting mechanoreceptors and their developmental dependence on ret signaling. Neuron 64:841–856

Ma Q, Fode C, Guillemot F, Anderson DJ (1999) Neurogenin1 and neurogenin2 control two distinct waves of neurogenesis in developing dorsal root ganglia. Genes Dev 13:1717–1728

Ma L, Merenmies J, Parada LF (2000) Molecular characterization of the TrkA/NGF receptor minimal enhancer reveals regulation by multiple cis elements to drive embryonic neuron expression. Development 127:3777–3788

Ma L, Lei L, Eng SR, Turner E, Parada LF (2003) Brn3a regulation of TrkA/NGF receptor expression in developing sensory neurons. Development 130:3525–3534

Marmigere F, Ernfors P (2007) Specification and connectivity of neuronal subtypes in the sensory lineage. Nat Rev Neurosci 8:114–127

Marmigere F, Montelius A, Wegner M, Groner Y, Reichardt LF, Ernfors P (2006) The Runx1/AML1 transcription factor selectively regulates development and survival of TrkA nociceptive sensory neurons. Nat Neurosci 9:180–187

Maro GS, Vermeren M, Voiculescu O, Melton L, Cohen J, Charnay P, Topilko P (2004) Neural crest boundary cap cells constitute a source of neuronal and glial cells of the PNS. Nat Neurosci 7:930–938

McEvilly RJ, Erkman L, Luo L, Sawchenko PE, Ryan AF, Rosenfeld MG (1996) Requirement for Brn-3.0 in differentiation and survival of sensory and motor neurons. Nature 384:574–577

McKemy DD (2005) How cold is it? TRPM8 and TRPA1 in the molecular logic of cold sensation. Mol Pain 1:16

McMahon SB, Armanini MP, Ling LH, Phillips HS (1994) Expression and coexpression of Trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets. Neuron 12:1161–1171

Mead TJ, Yutzey KE (2012) Notch pathway regulation of neural crest cell development in vivo. Dev Dyn 241:376–389

Memberg SP, Hall AK (1995) Proliferation, differentiation, and survival of rat sensory neuron precursors in vitro require specific trophic factors. Mol Cell Neurosci 6:323–335

Minichiello L, Piehl F, Vazquez E, Schimmang T, Hokfelt T, Represa J, Klein R (1995) Differential effects of combined trk receptor mutations on dorsal root ganglion and inner ear sensory neurons. Development 121:4067–4075

Molliver DC, Snider WD (1997) Nerve growth factor receptor TrkA is down-regulated during postnatal development by a subset of dorsal root ganglion neurons. J Comp Neurol 381:428–438

Molliver DC, Wright DE, Leitner ML, Parsadanian AS, Doster K, Wen D, Yan Q, Snider WD (1997) IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. Neuron 19:849–861

Montelius A, Marmigere F, Baudet C, Aquino JB, Enerback S, Ernfors P (2007) Emergence of the sensory nervous system as defined by Foxs1 expression. Differentiation 75:404–417

Moqrich A, Earley TJ, Watson J, Andahazy M, Backus C, Martin-Zanca D, Wright DE, Reichardt LF, Patapoutian A (2004) Expressing TrkC from the TrkA locus causes a subset of dorsal root ganglia neurons to switch fate. Nat Neurosci 7:812–818

Mulloy JC, Jankovic V, Wunderlich M, Delwel R, Cammenga J, Krejci O, Zhao H, Valk PJ, Lowenberg B, Nimer SD (2005) AML1-ETO fusion protein up-regulates TRKA mRNA expression in human CD34+ cells, allowing nerve growth factor-induced expansion. Proc Natl Acad Sci U S A 102:4016–4021

Murphy M, Reid K, Ford M, Furness JB, Bartlett PF (1994) FGF2 regulates proliferation of neural crest cells, with subsequent neuronal differentiation regulated by LIF or related factors. Development 120:3519–3528

Nakamura S, Senzaki K, Yoshikawa M, Nishimura M, Inoue K, Ito Y, Ozaki S, Shiga T (2008) Dynamic regulation of the expression of neurotrophin receptors by Runx3. Development 135:1703–1711

Nakazaki H, Reddy AC, Mania-Farnell BL, Shen YW, Ichi S, McCabe C, George D, McLone DG, Tomita T, Mayanil CS (2008) Key basic helix-loop-helix transcription factor genes Hes1 and Ngn2 are regulated by Pax3 during mouse embryonic development. Dev Biol 316:510–523

Oakley RA, Lefcort FB, Plouffe P, Ritter A, Frank E (2000) Neurotrophin-3 promotes the survival of a limited subpopulation of cutaneous sensory neurons. Dev Biol 224:415–427

Ockel M, Lewin GR, Barde YA (1996) In vivo effects of neurotrophin-3 during sensory neurogenesis. Development 122:301–307

Ota M, Ito K (2006) BMP and FGF-2 regulate neurogenin-2 expression and the differentiation of sensory neurons and glia. Dev Dyn 235:646–655

Patapoutian A, Peier AM, Story GM, Viswanath V (2003) ThermoTRP channels and beyond: mechanisms of temperature sensation. Nat Rev Neurosci 4:529–539

Patel TD, Jackman A, Rice FL, Kucera J, Snider WD (2000) Development of sensory neurons in the absence of NGF/TrkA signaling in vivo. Neuron 25:345–357

Patel TD, Kramer I, Kucera J, Niederkofler V, Jessell TM, Arber S, Snider WD (2003) Peripheral NT3 signaling is required for ETS protein expression and central patterning of proprioceptive sensory afferents. Neuron 38:403–416

Pavan WJ, Raible DW (2012) Specification of neural crest into sensory neuron and melanocyte lineages. Dev Biol 366:55–63

Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A (2002) A TRP channel that senses cold stimuli and menthol. Cell 108:705–715

Phillips HS, Armanini MP (1996) Expression of the trk family of neurotrophin receptors in developing and adult dorsal root ganglion neurons. Philos Trans R Soc Lond B Biol Sci 351:413–416

Pinco O, Carmeli C, Rosenthal A, Kalcheim C (1993) Neurotrophin-3 affects proliferation and differentiation of distinct neural crest cells and is present in the early neural tube of avian embryos. J Neurobiol 24:1626–1641

Pinon LG, Minichiello L, Klein R, Davies AM (1996) Timing of neuronal death in trkA, trkB and trkC mutant embryos reveals developmental changes in sensory neuron dependence on Trk signalling. Development 122:3255–3261

Raible DW, Ragland JW (2005) Reiterated Wnt and BMP signals in neural crest development. Semin Cell Dev Biol 16:673–682

Rebelo S, Chen ZF, Anderson DJ, Lima D (2006) Involvement of DRG11 in the development of the primary afferent nociceptive system. Mol Cell Neurosci 33:236–246

Rebelo S, Lopes C, Lima D, Reguenga C (2009) Expression of a Prrxl1 alternative splice variant during the development of the mouse nociceptive system. Int J Dev Biol 53:1089–1095

Rifkin JT, Todd VJ, Anderson LW, Lefcort F (2000) Dynamic expression of neurotrophin receptors during sensory neuron genesis and differentiation. Dev Biol 227:465–480

Sacristan MP, de Diego JG, Bonilla M, Martin-Zanca D (1999) Molecular cloning and characterization of the 5′ region of the mouse trkA proto-oncogene. Oncogene 18:5836–5842

Schecterson LC, Bothwell M (1992) Novel roles for neurotrophins are suggested by BDNF and NT-3 mRNA expression in developing neurons. Neuron 9:449–463

Scott A, Hasegawa H, Sakurai K, Yaron A, Cobb J, Wang F (2011) Transcription factor short stature homeobox 2 is required for proper development of tropomyosin-related kinase B-expressing mechanosensory neurons. J Neurosci 31:6741–6749

Serbedzija GN, McMahon AP (1997) Analysis of neural crest cell migration in Splotch mice using a neural crest-specific LacZ reporter. Dev Biol 185:139–147

Sharma N, Deppmann CD, Harrington AW, St Hillaire C, Chen ZY, Lee FS, Ginty DD (2010) Long-distance control of synapse assembly by target-derived NGF. Neuron 67:422–434

Sieber-Blum M, Ito K, Richardson MK, Langtimm CJ, Duff RS (1993) Distribution of pluripotent neural crest cells in the embryo and the role of brain-derived neurotrophic factor in the commitment to the primary sensory neuron lineage. J Neurobiol 24(2):173–184

Smeyne RJ, Klein R, Schnapp A, Long LK, Bryant S, Lewin A, Lira SA, Barbacid M (1994) Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature 368:246–249

Stucky CL, Shin JB, Lewin GR (2002) Neurotrophin-4: a survival factor for adult sensory neurons. Curr Biol 12:1401–1404

Stuhlmiller TJ, Garcia-Castro MI (2012) Current perspectives of the signaling pathways directing neural crest induction. Cell Mol Life Sci 69:3715–3737

Sun Y, Dykes IM, Liang X, Eng SR, Evans SM, Turner EE (2008) A central role for Islet1 in sensory neuron development linking sensory and spinal gene regulatory programs. Nat Neurosci 11:1283–1293

Sun Y, Lim Y, Li F, Liu S, Lu JJ, Haberberger R, Zhong JH, Zhou XF (2012) ProBDNF collapses neurite outgrowth of primary neurons by activating RhoA. PLoS One 7:e35883

Vaegter CB, Jansen P, Fjorback AW, Glerup S, Skeldal S, Kjolby M, Richner M, Erdmann B, Nyengaard JR, Tessarollo L, Lewin GR, Willnow TE, Chao MV, Nykjaer A (2011) Sortilin associates with Trk receptors to enhance anterograde transport and neurotrophin signaling. Nat Neurosci 14:54–61

Valderrama X, Misra V (2008) Novel Brn3a cis-acting sequences mediate transcription of human trkA in neurons. J Neurochem 105:425–435

Valderrama X, Rapin N, Misra V (2008) Zhangfei, a novel regulator of the human nerve growth factor receptor, trkA. J Neurovirol 14:425–436

Wende H, Lechner SG, Cheret C, Bourane S, Kolanczyk ME, Pattyn A, Reuter K, Munier FL, Carroll P, Lewin GR, Birchmeier C (2012) The transcription factor c-Maf controls touch receptor development and function. Science 335:1373–1376

Wetmore C, Olson L (1995) Neuronal and nonneuronal expression of neurotrophins and their receptors in sensory and sympathetic ganglia suggest new intercellular trophic interactions. J Comp Neurol 353:143–159

White FA, Silos-Santiago I, Molliver DC, Nishimura M, Phillips H, Barbacid M, Snider WD (1996) Synchronous onset of NGF and TrkA survival dependence in developing dorsal root ganglia. J Neurosci 16:4662–4672

Wiggins AK, Wei G, Doxakis E, Wong C, Tang AA, Zang K, Luo EJ, Neve RL, Reichardt LF, Huang EJ (2004) Interaction of Brn3a and HIPK2 mediates transcriptional repression of sensory neuron survival. J Cell Biol 167:257–267

Wilkinson GA, Farinas I, Backus C, Yoshida CK, Reichardt LF (1996) Neurotrophin-3 is a survival factor in vivo for early mouse trigeminal neurons. J Neurosci 16:7661–7669

Woolf CJ, Ma Q (2007) Nociceptors – noxious stimulus detectors. Neuron 55:353–364

Wright DE, Snider WD (1995) Neurotrophin receptor mRNA expression defines distinct populations of neurons in rat dorsal root ganglia. J Comp Neurol 351:329–338

Wright EM, Vogel KS, Davies AM (1992) Neurotrophic factors promote the maturation of developing sensory neurons before they become dependent on these factors for survival. Neuron 9:139–150

Wyatt S, Ensor L, Begbie J, Ernfors P, Reichardt LF, Latchman DS (1998) NT-3 regulates expression of Brn3a but not Brn3b in developing mouse trigeminal sensory neurons. Brain Res Mol Brain Res 55:254–264

Xiang M, Gan L, Zhou L, Klein WH, Nathans J (1996) Targeted deletion of the mouse POU domain gene Brn-3a causes selective loss of neurons in the brainstem and trigeminal ganglion, uncoordinated limb movement, and impaired suckling. Proc Natl Acad Sci U S A 93:11950–11955

Yoshikawa M, Senzaki K, Yokomizo T, Takahashi S, Ozaki S, Shiga T (2007) Runx1 selectively regulates cell fate specification and axonal projections of dorsal root ganglion neurons. Dev Biol 303(2):663–674

Yoshikawa M, Murakami Y, Senzaki K, Masuda T, Ozaki S, Ito Y, Shiga T (2013) Coexpression of Runx1 and Runx3 in mechanoreceptive dorsal root ganglion neurons. Dev Neurobiol 73 (6):469–479

Zhang J, Chen X (2007) DeltaNp73 modulates nerve growth factor-mediated neuronal differentiation through repression of TrkA. Mol Cell Biol 27:3868–3880

Part IV

Neurotrophins in Pathological Conditions

Huntington's Disease

Chiara Zuccato and Elena Cattaneo

Abstract

Changes in the level and activity of brain-derived neurotrophic factor (BDNF) have been described in a number of neurodegenerative disorders since early 1990s. However, only in Huntington disease (HD) gain- and loss-of-function experiments have mechanistically linked these abnormalities with the genetic defect.

In this chapter we will describe how huntingtin protein, whose mutation causes HD, is involved in the physiological control of BDNF synthesis and transport in neurons and how both processes are simultaneously disrupted in HD. We will describe the underlying molecular mechanisms and discuss pre-clinical data concerning the impact of the experimental manipulation of BDNF levels on HD progression. These studies have revealed that a major loss of BDNF protein in the brain of HD patients may contribute to the clinical manifestations of the disease. The experimental strategies under investigation to increase brain BDNF levels in animal models of HD will also be described, with a view to ultimately improving the clinical treatment of this condition.

Keywords

Huntingtin • BDNF • BDNF polymorphism • RE-1 silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) • BDNF promotor • BDNF transport • Neurodegeneration • Post-mortem brain • Neurotrophin • Neurodegeneration

C. Zuccato (✉) • E. Cattaneo (✉)
Department of Biosciences and Centre for Stem cell Research, Università degli Studi di Milano, Via Viotti 3/5, 20133 Milan, Italy
e-mail: chiara.zuccato@unimi.it; elena.cattaneo@unimi.it

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_14,

Abbreviations

3NP	3-Nitropropionic acid
AAV	Adeno-associated viral vector
ALS	Amyotrophic lateral sclerosis
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BAC	Bacterial-derived artificial chromosome
ARNT2	Aryl hydrocarbon receptor nuclear translocator 2
BDNF	Brain-derived neurotrophic factor
CaM kinase II	α-Subunit of Ca^{2+}/calmodulin-dependent kinase II
cAMP	Cyclic adenosyne 3′ 5′ monophosphate
CaRE1/2/3	Ca^{2+} Responsive element 1, 2 and 3
CaRF	Calcium responsive transcription factor
CBP	CREB Binding protein
C/EBP/beta	CCAAT/Enhancer binding protein beta
ChIP	Chromatin immunoprecipitation
CNS	Central Nervous System
coREST	REST Co-repressor 1
CRE	cAMP/Ca^{2+} Responsive element
CREB	CRE Binding protein
DARPP-32	Dopamine- and cyclic AMP-regulated phosphoprotein 32 kDa
DR	Dietary restriction
ES	Embryonic stem
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and drug administration
GDNF	Glial cell line-derived neurothrophic factor
Emx	Empty spiracles homolog
eGFP	Enhanced green fluorescent protein
GSK-3β	Glycogen synthase kinase 3-beta
HAP1	Huntingtin-associated protein 1
HDAC	Histone deacetylase
HD	Huntington's disease
Hdh	Huntington disease gene homolog
hsp70	Heat shock protein cognate 70 kDa
HSJ1B	Heat shock protein DNAJ-containing protein 1b
muHTT	Mutant huntingtin
wtHTT	Wild-type huntingtin
IT15	Interesting transcript 15
LiCl	Lithium chloride
L-VDCC	L-Type voltage-dependent Ca^{2+} channel
MEF2	Myocyte enhancer factor-2
MeCP2	Methyl-CpG binding protein 2
MEKK	Mitogen-activated protein kinase kinase
MLK	Mixed lineage kinase

MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSNs	Medium sized spiny neurons
mTOR	Mammalian target of rapamycin
NGF	Nerve growth factor
NMDA	*N*-Methyl-D-aspartic acid
NPAS4	Neuronal PAS domain protein 4
$p75^{NTR}$	p75 Neurotrophin receptor
PCR	Polymerase chain reaction
$p150^{Glued}$	150 kDa Dynein-associated polypeptide
PasRE	Basic helix-loop-helix (bHLH)-PAS transcription factor response element
PGC-1alpha	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PKA	Protein kinase A
Pro-BDNF	BDNF Precursor
RE1/NRSE	Repressor element 1/neuron-restrictive silencer element
REST/NRSF	RE-1 Silencing transcription factor/neuron-restrictive silencer factor
RILP	REST/NRSF-Interacting LIM domain protein
Sin3a	Switch independent homologue 3a
SOX 11	SRY (sex determining region Y)-box 11
Sp1	Specificity protein 1
SSRIs	Selective serotonin reuptake inhibitors
SVZ	Subventricular zone
TAFII-130	TATA box binding protein (TBP)-associated factor 130 kDa
TGases	Transglutaminases
TrkB	Tyrosine receptor kinase B
USF	Upstream stimulatory factor
Val66Met	Valine-to-methionine substitution at position 66
YAC	Yeast-derived artificial chromosome

1 Introduction

Huntington's disease (HD) is a dominant inherited neurodegenerative disorder that is caused by an unstable expansion of a CAG repeat within the coding region of the *interesting transcript 15* (*IT15*) gene (HDCRG 1993). The gene encodes for a protein called huntingtin whose mutation results in an elongated stretch of glutamine in the N-terminal of the protein (HDCRG 1993). Prevalence of the mutation is about 7–8 cases per 100,000 in populations of Western European descent, with many more at risk of having inherited the mutant gene. Neuropathological and neuroimaging studies revealed that the consequence of carrying the HD mutation is a widespread brain neurodegeneration characterised by the prevalent loss of efferent medium spiny neurons (MSNs) in caudate nucleus and putamen of the basal

ganglia (Reiner et al. 1988; Rosas et al. 2008). The typical HD symptoms include personality changes, cognitive declines and generalised motor dysfunction. The disease is with no effective therapies and progresses inexorably for 10–15 years from the onset.

The expansion of the CAG tract in huntingtin is the triggering event that endows the protein with new toxic functions deleterious for brain cells. Since the discovery of the HD gene in 1993, most of the research has focussed on elucidating the toxic activities of mutant huntingtin (Zuccato et al. 2010). In addition, we now know that the HD mutation also impairs the ability of normal huntingtin to exert activities that are fundamental for the survival and functioning of neurons (Cattaneo et al. 2001, 2005). As we proposed in 2001 (Cattaneo et al. 2001), this *loss of function hypothesis* in HD originates from the evidence that an expanded polyQ tract is present also in other proteins that cause at least eight different neurodegenerative diseases characterised by the loss of different types of neurons. Accordingly, we put forward the idea that "*whereas the CAG domain always evokes cell death, the different proteins in whose backbone the CAG is expressed identify the neurons that will die. If such proteins have crucial functions for the neurons that die in the disease, the resulting selective neuronal death might be directly attributable to the loss of those functions*" (Cattaneo et al. 2001). A number of findings now indicate that the ubiquitously expressed huntingtin protein has physiological function(s) that are particularly important for the brain, both during development and in adulthood (Zuccato et al. 2010; Cattaneo et al. 2005). *It is in the context of these studies that brain-derived neurotrophic factor (BDNF) has been mechanistically linked, through gain and loss of function experiments, to normal and mutant huntingtin for the first time*.

Most of the striatal BDNF is produced in the cerebral cortex and anterogradely delivered via the cortico-striatal afferents to the *corpus striatum* where it controls the activity of the cortico-striatal synapse while promoting the survival and maturation of the medium spiny neurons that are affected in HD (Altar et al. 1997; Baquet et al. 2004; Rauskolb et al. 2010). A 50 % reduction in BDNF levels at this synaptic site may thus contribute to striatal and cortical vulnerability. The hypothesis of a link between huntingtin and BDNF is supported also by the fact that they are co-localised in 99 % of the pyramidal neurons of motor cortex (Fusco et al. 1999, 2003)

The first proof in favour of this hypothesis was obtained in 2001. We reported that a crucial function of wild-type huntingtin is to contribute to the pool of BDNF protein produced in the cerebral cortex and that a loss or reduction in wild-type huntingtin as well as the presence of the CAG expansion in huntingtin diminishes BDNF cortical production and its striatal level (Zuccato et al. 2001). We also showed that huntingtin's ability to control cortical BDNF production occurs at a transcriptional level. Two years later huntingtin's target on the BDNF promoter was identified (Zuccato et al. 2003). In 2004, a new piece of data was added by the group led by Frederic Saudou in Paris who showed that wild-type huntingtin, in addition to controlling BDNF production, also controls its transport, at least in cells in vitro. Huntingtin is part of the molecular machinery that drives BDNF vesicles along

microtubules, and reduced BDNF transport was found in cultured HD cells (Gauthier et al. 2004). In light of the evidence indicating reduced levels of BDNF in HD, a number of studies involving HD mice have tested the impact of reducing or augmenting the level of this neurotrophin on disease onset and progression. The general conclusion is that "*the BDNF loss*" contributes to clinical manifestations in mice. This has generated considerable excitement about the idea of establishing a "*BDNF therapy*" for HD.

In this chapter we will describe the relevant data indicating that the production and transport of BDNF are under the stimulatory control of wild-type huntingtin, and that the mutation in the huntingtin gene as it occurs in HD causes the loss of this stimulatory activity, leading to a reduced BDNF protein level in cortex and striatum. We will emphasise the experiments performed on HD animal models and on tissue from patients with HD, as these have revealed defects in BDNF transcription, intracellular transport and postsynaptic targeting, as well as alterations in downstream signalling pathways. We will also present the available evidence highlighting the effect of reduced BDNF in HD, along with data showing that increased levels of BDNF are neuroprotective in the HD brain. Finally, we will describe the current experimental strategies under investigation that are aimed at increasing brain BDNF levels in animal models of HD, with a view to ultimately improving the clinical treatment of this condition.

2 Wild-Type Huntingtin and BDNF Gene Transcription

In this section we describe the evidence linking BDNF gene transcription to wild-type huntingtin as well as the data demonstrating that a well-known DNA regulatory sequence located within the BDNF promoter represents the first identified downstream molecular target of wild-type huntingtin's activity on the BDNF gene. We also discuss the mechanism by which wild-type huntingtin facilitates BDNF gene transcription and summarise the evidence showing that the same mechanism underlies the control of wild-type huntingtin over the transcription of other important neuronal genes.

2.1 In Vitro and In Vivo Evidence of a Link Between Wild-Type Huntingtin and BDNF

It was 2001 when huntingtin's ability to stimulate BDNF production was reported by means of a cell model of HD represented by immortalised ST14A cells stably transfected with human full-length wild-type or mutant huntingtin (Zuccato et al. 2001; Rigamonti et al. 2000). Enzyme-linked immunosorbent assays (ELISAs) of the different stable ST14A transfectants showed increased BDNF protein production in the cells overexpressing wild-type huntingtin in comparison with the mutant clones, which had a lower BDNF content than the mock-transfected ST14A cells. RNase protection assays further indicated that wild-type, but not

mutant huntingtin, facilitates BDNF production by acting at the level of BDNF gene transcription (Zuccato et al. 2001; Zuccato and Cattaneo 2007, 2009).

A second series of experiments showed that the pro-stimulatory effect of wild-type huntingtin on BDNF gene transcription depends on the activation of one specific BDNF promoter. At the beginning of 2000s the only data available about the structure and regulation of the BDNF gene were from work by Tonis Timmusk and colleagues at that time at Karolinska Institute in Stockholm, which identified four 5′ untranslated exons linked to separate promoters and one 3′ exon that encodes the BDNF protein (Timmusk et al. 1993). They also found that these promoters were alternatively used, generating a tissue-specific and stimulus-induced pattern of BDNF expression in the brain (Timmusk et al. 1993, 1995). It was later found that these different transcripts may also have different subcellular localisation and targets (Pattabiraman et al. 2005). Further studies from the same group published in 2007 clarified that the rodent BDNF gene contains a total of nine exons (I, II, III, IV, V, VI, VII, VIII and IX). The functional BDNF protein is produced following splicing at the 3′ end of exon IX, which contains the coding region (Aid et al. 2007) (Fig. 1). To evaluate whether the modulatory effect of huntingtin on BDNF gene transcription results from the preferential activation of one or more of these promoter regions, promoter reporter assays and polymerase chain reaction (PCR) for the specific mRNAs were performed (Zuccato et al. 2001). These experiments demonstrated that enhanced transcription from BDNF promoter II accounts for the increased BDNF level found in the presence of wild-type huntingtin, whereas transcription from BDNF promoter I, III and IV [the two last now renamed IV and VI, according to the new description of the gene by (Aid et al. 2007)] was unaffected (Zuccato et al. 2001). See Fig. 1.

This was further verified in vivo, in yeast-derived artificial chromosome (YAC) mice produced by Michael Hayden's group at the University of British Columbia and expressing increased full-length wild-type huntingtin with 18 glutamines (YAC18) (Hodgson et al. 1999). We have found that these mice carry higher BDNF protein levels as a consequence of the positive regulation on BDNF gene transcription by wild-type huntingtin. In particular, lysates from the cerebral cortex of these mice contained 47 ± 12 % more BDNF protein than that of their littermates and, consistently, there was 50 % increase in BDNF protein levels in the striatum (Zuccato et al. 2001). Increased transcription from BDNF promoter II accounted for the increased amount of BDNF protein in the cerebral cortex of YAC18 mice, whereas the transcriptional activity of other BDNF promoters was unchanged (Zuccato et al. 2001).

While extra copies of wild-type (but not mutant) huntingtin increase BDNF production in vitro and in vivo, one should expect that cells or brain tissues depleted of endogenous huntingtin are characterised by reduced BDNF levels. In 2003, we reported that BDNF mRNA levels were lower in the cerebral cortex of constitutive heterozygous huntingtin knockout mice (Zuccato et al. 2003). Similarly, the neuronal inactivation of huntingtin in conditional homozygous knockout mice (Dragatsis et al. 2000) led to a statistically significant reduction in BDNF mRNA levels in the cerebral cortex (Zuccato et al. 2007). Moreover, BDNF mRNA was progressively

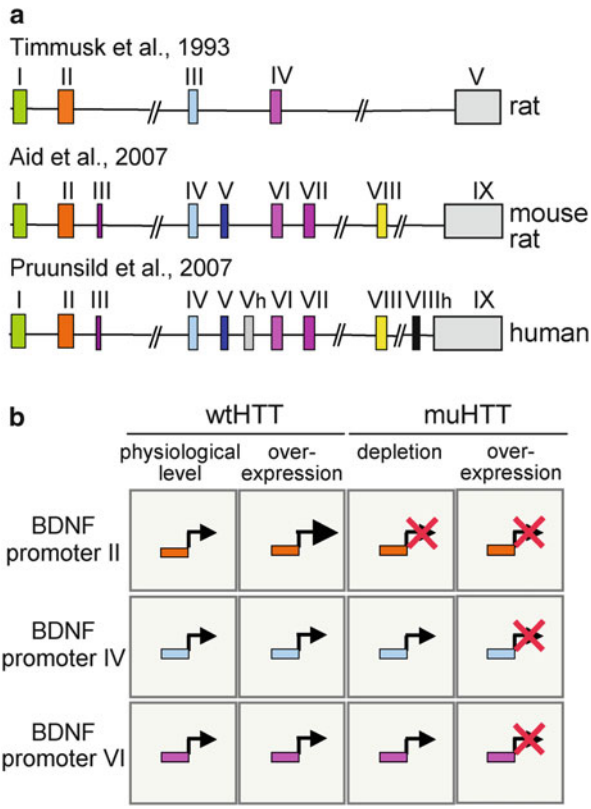

Fig. 1 (**a**) BDNF gene structure in humans and rodents proposed by different studies. Exons are shown as *boxes* and introns as *lines*. The BDNF coding region is indicated in *grey*. Homologous exons are highlighted with the same colour. (**b**) Transcription from BDNF promoter II is enhanced by wild-type huntingtin overexpression, whereas BDNF promoter IV and VI transcriptional activity is unaffected. Reduced wild-type huntingtin levels causes reduction of BDNF gene transcription from promoter II, while transcriptional activity of promoter IV and VI is unaffected. Mutant huntingtin overexpression reduces BDNF gene transcription from promoter II, IV and VI

reduced in mouse embryonic stem (ES) cells in which one or two alleles of the *Huntington disease gene homolog* (*Hdh)* have been inactivated via removal of exon 4 and 5 (Zuccato et al. 2007). This reduction in BDNF mRNA was attributable to a specific loss of BDNF mRNA II. These studies confirmed that loss of wild-type huntingtin specifically affects transcription from BDNF exon II promoter (Zuccato et al. 2003, 2007).

More recently, the group of David Rubinsztein at the University of Cambridge has used zebrafish to study wild-type huntingtin function. They demonstrated that loss of BDNF function is a major contributor to many of the developmental defects seen when huntingtin levels are knocked down in the embryo. BDNF mRNA levels were reduced in the huntingtin knockdown zebrafish, and these fishes also showed phenotypes that were very similar to those observed in the BDNF knockdown. Furthermore, the effects of huntingtin loss, which include brain atrophy, were attenuated by supplementation of the fish growth medium with recombinant BDNF protein (Diekmann et al. 2009; Henshall et al. 2009).

The data described above show that the ability of huntingtin to regulate BDNF expression is a component of its normal function which contributes to maintain the BDNF pool in the brain through a stimulatory action on BDNF promoter II.

2.2 The Involvement of REST/NRSF in Huntingtin's Activity in the CNS

The investigation of the mechanism by which wild-type huntingtin stimulates BDNF gene transcription has concentrated on BDNF promoter II. In 1998 a study by Tonis Timmusk highlighted that the BDNF promoter II contains a 21- to 23-bp DNA responsive element named repressor element 1/neuron-restrictive silencer element (RE1/NRSE), whose activity depends on its cognate transcription factor RE1 silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) (Timmusk et al. 1999). REST/NRSF is a master regulator of neuronal genes that is highly expressed in immature Central Nervous System (CNS) cells and at a much lower level in mature neurons, while remaining abundant in peripheral cells. Its role is to repress a large cohort of neuron-specific genes, through specific recruitment of a multi-subunit repressor complex to the RE1/NRSE (Ooi and Wood 2007).

In 2003, REST/NRSF was linked to HD with the discovery that wild-type but not mutant huntingtin inhibits the silencing activity of the RE1/NRSE within BDNF promoter II. In particular, wild-type huntingtin was found to retain REST/NRSF in the cytoplasm, thus reducing RE1/NRSE's activity and allowing BDNF gene transcription (Zuccato et al. 2003). Instead, mutated huntingtin causes the pathological entry of REST/NRSF into the nucleus where it can bind to the RE1/NRSE site and lead to BDNF repression (Zuccato et al. 2003).

In 2008, studies from Masahito Shimojo's laboratory at University of Kentucky College of Medicine demonstrated that huntingtin does not interact with REST/NRSF directly, but is part of a complex that contains huntingtin-associated protein 1 (HAP1) and REST-interacting LIM domain protein (RILP), a perinuclear protein that directly binds REST/NRSF and promotes its nuclear translocation. When huntingtin is mutated, REST/NRSF is released from the perinuclear protein complex and accumulates in the nucleus, where it binds to the RE1/NRSE sites within BDNF exon II and causing reduced BDNF gene transcription (Zuccato et al. 2003; Shimojo 2008) (Fig. 2).

2.3 Beyond BDNF: An Expanded Role for Wild-Type Huntingtin in Neuronal Gene Transcription

Bioinformatic studies from Noel Buckley's group at the University of Leeds indicated that the potential repertoire of REST/NRSF-regulated genes is extensive. In fact, in addition to the BDNF gene, the RE1/NRSE is found in thousands of neuronal genes including those encoding other growth factors, hormones, neuronal transcription factors, ion channels, proteins involved in axonal guidance, neurotransmitters, proteins involved in vesicle trafficking, fusion and synaptic transmission (Bruce et al. 2004). This suggested that wild-type huntingtin may play a broader role in regulating neuronal gene transcription via inhibition of the REST/NRSF–RE1/NRSE pathway.

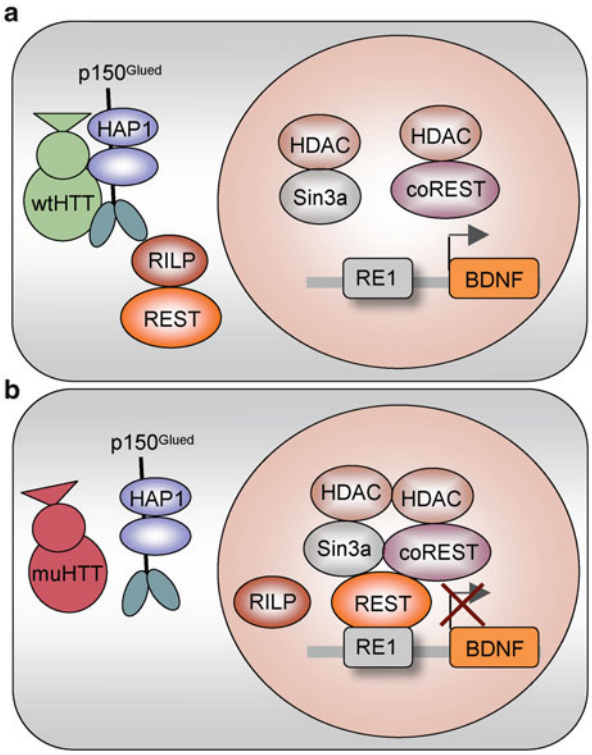

Fig. 2 (**a**) Regulation of BDNF gene transcription by huntingtin. Wild-type huntingtin (as part of a complex with HAP1, $p150^{Glued}$ and RILP) sequesters REST/NRSF in the cytoplasm, thereby preventing the formation of a co-repressor complex (involving sin3a, coREST and HDAC) at RE1/NRSE sites and allowing the BDNF gene to be transcribed. The binding between huntingtin and REST is indirect: $p150^{Glued}$, the large subunit of the dynactin complex, bridges the interaction between wild-type huntingtin and RILP, with the latter directly binding REST/NRSF. (**b**) The mutant huntingtin complex in HD is less capable of retaining REST/NRSF in the cytoplasm than the wild-type complex. REST/NRSF enters the nucleus and the repressor complex is able to form, leading to reduced transcription of the BDNF gene

Several experiments confirmed the above-mentioned hypothesis. ST14A cells and YAC18 mice overexpressing wild-type huntingtin showed increased levels of the mRNAs transcribed from many other RE1/NRSE-containing neuronal genes, in addition to BDNF (Zuccato et al. 2003). In particular, the levels of synapsin-1, cholinergic receptor nicotinic beta-polypeptide 2 and dynamin 1 mRNA were higher in the cerebral cortex of YAC18 mice, thus indicating that huntingtin may act as a general facilitator of neuronal gene transcription in the nervous system (Zuccato et al. 2003). Evidence in favour of a role of wild-type huntingtin in controlling RE1/NRSE-controlled neuronal gene transcription came also from chromatin immunoprecipation (ChIP) data showing that REST occupancy is significantly lower in cells and mice expressing wild-type huntingtin than in HD models (Zuccato et al. 2007). Consistently, depletion of endogenous huntingtin in cells

and mice is associated with increased occupancy of REST/NRSF at RE1/NRSE loci and reduced transcription from the same genes (Zuccato et al. 2003, 2007).

These results identify a key role for normal huntingtin in facilitating transcription of REST/NRSF-regulated genes essential for neuronal development and maintenance. Proper control of transcription of the BDNF gene is particularly important for the activity of the cortico-striatal synapse and for the survival of striatal and cortical neurons, but reduced wild-type huntingtin function in HD may have broader consequences on neuronal gene transcription through the mechanism described herein. These findings have also potential therapeutic implications and suggest that treatment of HD may benefit from the production of drugs that mimic wild-type huntingtin physiological activity on the REST/NRSF–RE1/NRSE regulon (Zuccato et al. 2003, 2007; Rigamonti et al. 2007; Conforti et al. 2012).

3 Reduced BDNF Gene Transcription in HD

A 1997 landmark discovery by Stanley J. Wiegand and colleagues at Regeneron Pharmaceuticals, in New York, showed that most of BDNF protein found in striatum is produced in the cerebral cortex and anterogradely transported along the cortico-striatal tract to the MSNs (Altar et al. 1997). MSNs depend on cortically derived BDNF for their survival and activity (Zuccato and Cattaneo 2007, 2009). Thus, it has been proposed that reduction in BDNF level in the cerebral cortex or in its delivery may contribute to striatal (and cortical) vulnerability in HD. The finding that wild-type huntingtin stimulates BDNF gene transcription and protein production has prompted analyses of BDNF levels in the brain of transgenic mice and patients with HD.

3.1 Evidence from HD Cell and Animal Models

A first indication of a specific molecular defect in BDNF protein and mRNA levels in HD came from experiments on striatum-derived ST14A cells overexpressing full-length wild-type or mutant huntingtin. Although cells overexpressing wild-type huntingtin produce more BDNF protein, the production of both BDNF mRNA and protein in mutant huntingtin cells was less than in control cells (Zuccato et al. 2001). A similar decrease was also found in mutant huntingtin knockin cells obtained from heterozygous and homozygous huntingtin knockin mice in which a 109 CAG triplet has been inserted in exon 1 of the murine *Huntington disease gene homolog (Hdh)* gene ($Hdh^{109/7}$ and $Hdh^{109/109}$) (Zuccato et al. 2001, 2003, 2007; Soldati et al. 2011; Trettel et al. 2000). Moreover, Josep Canals and collaborators at the University of Barcelona transiently expressed exon 1 of mutant human huntingtin with 47, 72 or 103 CAG repeats in a striatum-derived cell line and showed reduced BDNF content. They also indicated that the increase in CAG size did not exacerbate the BDNF phenotype (Canals et al. 2004). More recently, reduced level of BDNF mRNA has been reported also in a novel series of mouse neural stem (NS) cells lines that carry varying number of CAG repeats (20, 50, 111)

in the mouse huntingtin gene (Conforti et al. 2013). We revealed that reduction in BDNF mRNA level during neuronal differentiation is CAG dependent up to 111 CAG (Conforti et al. 2013). According to recent in vivo studies some HD phenotypes may be more promptly revealed in the presence of shorter CAG expansion (Dragatsis et al. 2009; Morton et al. 2009; Cummings et al. 2012).

Consistent with the in vitro data, many laboratories have found reduced BDNF levels in total brain or cortical and striatal samples from a large panel of mouse models of HD that show different degrees of similarity to the human condition. The first in vivo evaluation of BDNF levels in a mouse model of HD has been performed on YAC mice that express human full-length mutant huntingtin with 72 glutamines (YAC72) and was described to develop striatal degeneration of MSNs at 12 months of age. An approximately 30 % decrease in BDNF protein levels has been found in the cortex of 9-month-old YAC72 mice with no disease symptoms (Zuccato et al. 2001). Another study found reduced BDNF mRNA levels in YAC72 mice from the age of 3 months, thus confirming that BDNF gene transcription can be affected before the onset of disease symptoms in this animal model (Hermel et al. 2004). A 40 % reduction in BDNF content has also been detected in the hippocampus, a finding that may be consistent with observations of impaired spatial memory in HD mice, as well as reports of hippocampal cell proliferation and neurogenesis deficits (Gil et al. 2005; Grote et al. 2005; Lazic et al. 2004; Ben M'Barek et al. 2013). Although preliminary, these data may have a clinical correlate insofar as HD patients show cognitive abnormalities (Schmidtke et al. 2002). The battery of YAC mice includes also mice carrying 128 CAG repeats (Slow et al. 2003) which are especially interesting because they show an earlier disease onset with respect to YAC72 mice, with age dependent striatal and cortical degeneration, and development of well-characterised progressive motor and cognitive deficits (Zuccato et al. 2010). Recently, Baoji Xu and colleagues at Georgetown University Medical Center have reported similar BDNF mRNA levels in the cerebral cortex of symptomatic 16-month-old mice YAC128 compared to wild-type mice (Xie et al. 2010). In the same study levels of mature BDNF determined by western blot in YAC128 mice were similar in the cerebral cortex, but significantly reduced in the striatum when compared with control mice (Xie et al. 2010). It is surprising that BDNF mRNA level and protein do not change in YAC128 cortex at symptomatic stages, whereas significant BDNF reduction has been found in the cortex, striatum and hippocampus of YAC72 mice in the absence of neuropathological and behavioural phenotype (Zuccato et al. 2001; Hermel et al. 2004). Data from our group have shown that BDNF mRNA level, as determined by quantitative PCR, is approximately 30 % lower in the cortex of YAC128 mice from pre-symptomatic stages compared to controls (unpublished data). These different results may be due to the different techniques used for BDNF mRNA quantisation. Baoji Xu and colleagues used in situ hybridisation while we have used quantitative PCR.

In 2008 bacterial-derived artificial chromosome (BAC)-mediated transgenesis was used to develop mouse models of HD expressing full-length mutant huntingtin with 103 glutamine repeats (BACHD). These mice, produced by William Yang at the University of California Los Angeles, exhibit progressive motor deficits starting from 2-months of age, neuronal synaptic dysfunction and late onset selective

neuropathology, which includes significant cortical and striatal atrophy and numerous degenerating neurons in striatum (Gray et al. 2008). BACHD cortical tissues have been tested for the BDNF content and significant deficit in BDNF transcription was found at 8 and 6 months of age (Simmons et al. 2013; Gray et al. 2008). More recently, reduction in BDNF cortical mRNA has been revealed at earlier time points (2 and 4 months of age) (Conforti et al. 2012).

Other studies have shown reduced BDNF mRNA and protein levels in HD mice transgenic for the N-terminal portion of the mutant huntingtin. These mice are characterised by early onset of symptoms and a fast progression of the disease that makes them particularly useful to test BDNF levels along disease progression. The analyses usually cover an experimental window that is no longer than 24 weeks. The R6/2 line produced by Gill Bates group at King's College in London and expressing a 63 amino acid N-terminal fragment of mutant huntingtin with 150 glutamines (Mangiarini et al. 1996) has been tested independently by four groups. Zhang et al. have reported a 50 % reduction in BDNF protein in total brain from 12-week-old symptomatic (Zhang et al. 2003) while Wang et al., using animals of the same age, reported a 20 % decrease in perikarial BDNF mRNA in corticostriatal neurons located in layer V (which have projections to the striatum) (Wang et al., abstract 450.4/W11, Society for Neuroscience 36th Annual Meeting 2006). In line with the rapid disease progression—subtle motor and learning deficits appear after approximately 4–5 weeks and the animals usually die after 13–14 weeks—we found reduced BDNF mRNA levels in the cerebral cortex from early pre-symptomatic stages (Zuccato et al. 2005). Luthi-Carter et al. have shown that the same mice exhibit reduced BDNF gene transcription in the cerebellum from 8 weeks of age, possibly leading to cerebellar dysfunction and altered motor coordination (Luthi-Carter et al. 2002). In the last years, the reduction of BDNF in the brain of R6/2 mice has been confirmed by additional studies (Conforti et al. 2008; Apostol et al. 2008; Johnson et al. 2008; Mielcarek et al. 2011; Giampà et al. 2013).

Brain BDNF protein levels have been tested, but with conflicting results, in another transgenic mouse line, R6/1, created at the same time as R6/2. R6/1 mice show slower disease progression because of the smaller amount of expressed mutant huntingtin (Mangiarini et al. 1996). Spires et al. (2004) reported that BDNF protein levels were reduced in R6/1 striatum but not in the cerebral cortex at the age of 5 months (Spires et al. 2004), whereas Canals et al. found no deficiency in striatal BDNF protein levels at the age of 6 months (Canals et al. 2004). The latter authors suggested that the unchanged BDNF levels in R6/1 mice may be due to the low transgene level, as cells expressing low levels of an exogenous mutant huntingtin tract do not show a reduction in BDNF protein content (Canals et al. 2004). Pang et al. have reported similar BDNF protein levels in the striatum and hippocampus of 5-month-old controls and R6/1 mice, but increased levels were found in the frontal cortex and, in the same study, reduced BDNF mRNA levels in the striatum, anterior cortex and hippocampus was detected (Pang et al. 2006). Reduced BDNF mRNA level in the R6/1 hippocampus has been confirmed by a study from Zajac and colleagues (2010). These conflicting findings may be explained by the different methods used for BDNF protein quantification. Spires et al. (2004) used western blot, which differentiates mature BDNF (which is found

decreased) from the immature form (which remained unmodified), whereas Canals and Pang used ELISA, which is more quantitative but does not distinguish mature and immature BDNF. It is possible that the striatal level of mature BDNF protein is significantly decreased but levels of immature BDNF remain largely unchanged (Pang et al. 2006). Moreover, the reduced levels of BDNF mRNA in striatal neurons (which transcribe little or no BDNF) probably also affect the still uncertain BDNF levels in R6/1 mice, and so further investigations are necessary in this mouse model.

BDNF levels have also been tested in N171-82Q mice produced by David Borchelt laboratory at Johns Hopkins University and expressing a 517 amino acid N-terminal portion of huntingtin with 82 glutamine repeats driven by a mouse prion protein promoter (Duan et al. 2003; Schilling et al. 1999). Compared with the R6 mice, the N171-82Q model has fewer polyglutamine repeats resulting in a later onset of symptoms. ELISA assays showed that BDNF protein levels were significantly decreased by 70–80 % in the striatum and cortex of symptomatic 3-month-old N171-82Q mice (Duan et al. 2003). Quantitative PCR analyses have recently shown that BDNF mRNA is reduced in the cortex of N171-82Q mice at 4 months of age (Conforti et al. 2012). The above data indicate that R6/2 and N171-82Q mice are attractive tools for the study of pre-symptomatic therapies aimed at isolating drugs that increase BDNF levels.

BDNF levels have also been analysed in knockin mice that carry the HD mutation in the appropriate genomic context and express huntingtin protein at a physiological concentration, thus more reliably replicating the pathogenesis of HD. BDNF protein levels were first evaluated in a knockin mouse model produced by Marcy MacDonald at Massachusetts General Hospital in Boston and in which mouse exon 1 has been replaced with the human exon 1 carrying 111 CAG repeats (Wheeler et al. 1999). Immunoblots showed a less intense BDNF band in striatal and cortical extracts from homozygous mutant huntingtin knockin mice ($Hdh^{111/111}$) aged 5 months (Gines et al. 2003). Data from Borrell-Pages et al. indicating a small reduction in BDNF protein levels in total brain samples taken from 3-month-old homozygous knockin mice further support the notion of a BDNF deficit in this mouse model (Borrell-Pages et al. 2006). Support for an early BDNF reduction in the brain of mutant huntingtin knockin mice came also from a study by Simmons et al. who found that BDNF protein was reduced by 40–45 % in the hippocampus, cortex and striatum of 2-month-old $Hdh^{111/111}$ mice and from a work by our group highlighting reduced BDNF mRNA in cortex at 1 month of age (Lynch et al. 2007; Zuccato et al. 2007).

With a few exceptions that require further investigation, this evidence together speaks in favour of reduced BDNF level in HD cells and animal models and opens up the possibility that a similar dysfunction may be present in the human disease.

3.2 Reduced BDNF Promoter II Activity in HD

As previously described, wild-type—but not mutant—huntingtin stimulates BDNF gene transcription by acting at the level of BDNF promoter II. Several evidences indicate that the presence of a pathological CAG expansion in huntingtin abolishes the ability to sustain BDNF gene transcription from BDNF promoter II. Reduced

BDNF mRNA II levels are found in ST14A cells overexpressing full-length mutant huntingtin (Zuccato et al. 2001), as well as in heterozygous and homozygous mutant huntingtin knockin cells ($Hdh^{109/7}$ and $Hdh^{109/109}$) (Zuccato et al. 2003). Furthermore, reporter gene assays confirm that BDNF exon II promoter is 60 % less active in cells overexpressing mutant huntingtin than in parental cells (Zuccato et al. 2001). Earlier in vivo data support these observations and indicate that BDNF mRNA II levels are much reduced in the cerebral cortex and hippocampus of pre-symptomatic YAC72 mice expressing human full-length mutant huntingtin (Zuccato et al. 2001), and similar findings were reported in an independent study of the same YAC mice at 3 months of age (Hermel et al. 2004). Reduced BDNF mRNA II levels have been recently described also in cortical tissues from BAC-HD and in N171-82Q mice (Conforti et al. 2012). Cortical BDNF mRNA II levels are 25 % less in 8-week-old R6/2 mice than in controls and 60 % less in 12-week-old symptomatic R6/2 mice (Zuccato et al. 2005). Similar analyses by other authors have shown a significant depletion of wild-type huntingtin in 7-week-old R6/2 mice that parallels the timing of the reduced BDNF mRNA II level, thus suggesting that the decreased transcription from BDNF II promoter in this model may be due to the reduced level of endogenous huntingtin (Zhang et al. 2003).

The mechanism by which BDNF exon II promoter activity is reduced in HD has been described previously. As indicated, the RE1/NRSE silencer is the target of wild-type huntingtin on BDNF promoter II, and the wild-type protein inhibits its silencing activity by retaining the REST/NRSF transcription factor (which binds and activates the silencer) in the cell cytoplasm (Zuccato et al. 2003). ChIP assays have highlighted increased REST/NRSF binding at the RE1/NRSE of BDNF exon II in mutant huntingtin homozygous HD cells, in animal models (BAC-HD mice, R6/2 mice and homozygous mutant huntingtin knockin mice) as well as in the cerebral cortex of HD subjects, and this leads to increased activity of the silencer and to reduced BDNF mRNA II levels (Zuccato et al. 2007; Conforti et al. 2012) (Fig. 2).

Increased binding of REST/NRSF in the presence of mutant huntingtin is not confined to the RE1/NRSE of the BDNF gene. Increased REST/NRSF occupancy is evident in a cohort of RE1/NRSE-regulated genes in different cellular and animal HD models (Zuccato et al. 2007; Soldati et al. 2011; Johnson et al. 2008; Conforti et al. 2012; Soldati et al. 2013), resulting in repression of gene transcription. Furthermore, bioinformatic analyses of published microarray data of HD brain have shown that RE1/NRSE genes are preferentially repressed in HD patients (Johnson and Buckley 2009). This suggests that increased REST/NRSF repression can explain a significant fraction of gene dysregulation in the HD brain.

3.3 A Gained Toxic Activity of Mutant Huntingtin on BDNF Promoter IV and VI

In addition to reduced activity of BDNF promoter II, transcriptional activities of BDNF mRNA IV and VI are affected in HD cells and mice and contribute to reduction of the BDNF pool in HD brain. Short regions flanking promoters IV and

IV have been thoroughly characterised in terms of their regulatory elements of gene transcription. In HD cells, mouse and human tissue transcription from other BDNF promoters (BDNF promoter IV and VI) is also affected, suggesting that, in addition to reduced activity of BDNF promoter II (caused by loss of wild-type huntingtin activity), other mechanisms are in operation that lead to reduced BDNF gene transcription that are more specifically linked to mutant huntingtin's gain of toxic function (Zuccato et al. 2001, 2007; Zuccato and Cattaneo 2009) (Fig. 1). Information on these promoter exons is given below, followed by a summary of experiments indicating the deleterious effect of mutant huntingtin and speculation about the underlying mechanisms.

Early studies indicated that BDNF promoter I is physiologically activated at low levels and stimulated by the administration of kainic acid, which evokes calcium signals through different subtypes of glutamate receptors (Metsis et al. 1993; Zafra et al. 1990). For this reason BDNF exon I was originally defined as the inducible brain-specific promoter (Timmusk et al. 1993; Metsis et al. 1993). Recent studies by Liu et al. (2006) and by Aid et al. (2007) have shown that BDNF promoter I is subject to physiological activation as the mRNA transcribed from it can be detected in the cerebral cortex, cerebellum, hippocampus, thalamus and brain stem (Aid et al. 2007; Liu et al. 2006), but little is known about the mechanisms regulating the transcriptional activation of BDNF promoter exon I. It is known that BDNF promoter exon I has distal and proximal cyclic adenosine 3′, 5′ monophosphate (cAMP)/Ca^{2+} responsive elements (CRE), and a proximal CRE is overlapped by an upstream stimulatory factor (USF) binding element (Tabuchi et al. 2002). We also know that the proximal element is bound by CRE binding protein (CREB) and upstream stimulatory factor 1 and 2 (USF1/USF2) and responds to Ca^{2+} signals evoked via L-type voltage-dependent Ca^{2+} channels (L-VDCC) and *N*-methyl-D-aspartic acid (NMDA) (Tabuchi et al. 2000, 2002). A study from Hara and colleagues suggest that Ca^{2+} signal-induced transcription of BDNF promoter I is mediated by REST/NRSF (Hara et al. 2009). More recently, the group of Tonis Timmusk has identified a asymmetric E-box-like element named PasRE [basic helix-loop-helix (bHLH)-PAS transcription factor response element] in human BDNF promoter I and demonstrated that binding of this element by bHLH-PAS transcription factors ARNT2 (aryl hydrocarbon receptor nuclear translocator 2) and NPAS4 (neuronal PAS domain protein 4) is crucial for neuronal activity-dependent transcription from promoter I (Pruunsild et al. 2011).

More robust attempts have been made to elucidate the structure and activity of BDNF exon IV promoter [BDNF exon III, according to the nomenclature described in (Timmusk et al. 1993)], which is characterised by the three Ca^{2+} responsive elements CaRE1, CaRE2 and CaRE3/CRE. These regulatory elements are stimulated by Ca^{2+} signals evoked by *N*-methyl-D-aspartic acid (NMDA) glutamate receptor and involve CREB together with CaM kinase IV (Shieh et al. 1998; Tao et al. 1998). Moreover, CaRE1 and CaRE3/cAMP responsive element are bound by the neuronal calcium responsive transcription factor (CaRF), whereas CaRE2 activity depends on the binding of transcription factor USF1/USF2 (Tabuchi et al. 2002; Chen et al. 2003a). Two studies by Chen et al. and Martinowich

et al. have shown that methyl-CpG binding protein 2 (MeCP2), which binds methylated CpGs island on DNA and is involved in the long-term silencing of gene transcription, can selectively bind BDNF promoter exon IV and repress BDNF gene transcription (Chen et al. 2003b; Martinowich et al. 2003). Membrane depolarisation triggers the calcium-dependent phosphorylation and release of MeCP2 from BDNF promoter IV, thus facilitating transcription. Recently, it has been shown that for a full induction of human BDNF exon IV mRNA transcription, ARNT2 and NPAS4 binding to a PasRE sequence in promoter IV is needed (Pruunsild et al. 2011).

Unlike the other BDNF promoters analysed above, BDNF promoter VI [indicated as IV by (Timmusk et al. 1993)] contains glucocorticoid-responsive elements, and its activity is influenced by thyroid hormone (Koibuchi et al. 1999) and corticosterone (Hansson et al. 2006). Additional findings indicate that CaM kinase II mediates the activation of BDNF promoter VI by Ca^{2+} influx. Transient transfection and overexpression experiments have shown that two nuclear isoforms of CaM kinase II (delta 3 and alpha B) specifically activate only promoter VI (Takeuchi et al. 2000). Takeuchi et al. has shown that mitogen-activated protein kinase kinase (MEKK) and protein kinase A (PKA) can also upregulate the activity of BDNF promoter exon VI; in particular, CaM Kinase II and MEKK, respectively, activate the promoter linked to BDNF exon VI via CCAAT/enhancer binding protein beta (c/EBP/beta) and specificity protein 1 (Sp1) transcription factors (Takeuchi et al. 2002). More recent findings indicate that MEF2 and Sox11 are also implicated in the regulation of BDNF promoter IV (Lyons et al. 2012; Salerno et al. 2012).

The first indication about a possible effect of huntingtin on BDNF promoter I, IV and VI was reported in 2001 by our group. We found that ST14A neural cells overexpressing the mutant protein do not express BDNF mRNA I (Metsis et al. 1993), but we did find that transcription from BDNF promoter IV and VI, which are physiologically subject to activation in the CNS, was significantly reduced in the presence of the mutant protein. Consequently, BDNF mRNA VI and VI are also reduced in ST14A cells expressing mutant huntingtin, and their levels were also lower in heterozygous and homozygous mutant huntingtin knockin cells (Zuccato et al. 2001). Similar results have been obtained in mouse models of HD. YAC72 mice show a reduction in BDNF mRNA IV and VI levels starting at pre-symptomatic stages (Zuccato et al. 2001; Hermel et al. 2004). A similar pattern has been found in R6/2 mice, which express mutant huntingtin exon 1. In particular, BDNF exon VI mRNA level was the first to be affected (at 6 weeks of age), while defects in transcription from promoter IV occurred only at very late stages (12 weeks of age) (Zuccato et al. 2005). Transcription from BDNF promoter IV and VI was significantly reduced also in the brain of N171-82Q and in BAC-HD mice (Conforti et al. 2012).

The mechanism leading to the reduced expression of BDNF mRNA IV and VI in HD is still unknown. However, an impaired CRE pathway has been observed (Sugars et al. 2004; Sugars and Rubinsztein 2003) and, as BDNF promoter IV has a CRE element, it is possible that a dysfunction in CRE activity may account for its

reduced transcription. Various evidences indicate that crucial proteins in this event are CREB (which directly binds to the CRE element after phosphorylation by PKA at Ser133) and the CREB binding protein (CBP), which acts as a bridge between CREB and the transcriptional machinery. A finding by Joan Steffan and Leslie Thompson at the University of California Irvine indicates that mutant huntingtin can interact with both the glutamine-rich activation domain and the acetyl transferase domain of CBP (Steffan et al. 2001). They also found that a reduction in the acetyltransferase activity of CBP causes a reduction in histone acetylation (Steffan et al. 2001), thus leading to a more compact chromatin structure that is less accessible to transcription factors and potentially explaining the decrease in CRE-dependent transcription and reduction in BDNF mRNA IV levels. Although early findings suggested that CBP can be sequestered into mutant huntingtin aggregates (McCampbell et al. 2000; Nucifora et al. 2001), a study by Yu et al. showed that altered CRE-dependent gene expression may be due to the interactions of soluble mutant huntingtin with nuclear CBP, rather than to the depletion of this transcription factor by nuclear inclusions (Yu et al. 2002). CBP is therefore subtracted from the transcriptional machinery regulating the CRE element in BDNF promoter IV. Reduced CREB phosphorylation (Gines et al. 2003; Giampa et al. 2006) and reduced cAMP levels (Gines et al. 2003) may also contribute to reduced transcription from BDNF exon IV promoter in an HD background. Moreover, CRE-mediated transcription is also activated by TATA box binding protein (TBP)-associated factor, 130 kDa (TAFII130), and evidence from Dimitri Krainc originally at Massachusetts General Hospital indicates that TAFII130 interacts with mutant huntingtin, thus further impairing the transcriptional machinery at the CRE loci (Dunah et al. 2002). The reduced transcription from BDNF promoter linked to exon VI in HD (Zuccato et al. 2001, 2005) may be also explained on the basis of evidence showing that Sp1 participates in its activation (Takeuchi et al. 2002), whereas mutant huntingtin sequesters Sp1, thus blocking its physiological interaction with TAFII130 and causing reduced transcriptional activity (Dunah et al. 2002; Li et al. 2002).

In conclusion, reduced normal huntingtin activity is responsible for decreased transcription from promoter II, whereas reduced transcriptional activity at promoters IV and VI reflects mutant huntingtin-induced toxicity. The above has potential therapeutic implications insofar as it suggests the usefulness of restoring BDNF levels in HD. The BDNF promoters can be used as reporter assays of huntingtin activity in order to identify the contribution of the activity of the mutant protein versus the loss of normal huntingtin function during HD progression. In particular, they can be used to develop reporter assays for the isolation of molecules that mimic wild-type huntingtin on BDNF exon II promoter. Such an assay would have the advantage of reflecting the activity of a much larger number of promoters located in neuronal genes and containing the RE1/NRSE element, thus anticipating the possibility that active compounds would restore transcription from a large number of RE1/NRSE controlled neuronal genes. In parallel, BDNF exon IV and VI promoters can be used in reporter assays to identify drugs capable of reducing or blocking the ability of mutant huntingtin to inactivate BDNF gene transcription from the same promoters.

4 Huntingtin and BDNF Vesicles Transport

In 2004, the French group led by Frederic Saudou at the Centre Universitaire Orsay in Paris showed that full-length wild-type huntingtin stimulates BDNF vesicular trafficking in neuronal cells and that its transport can be attenuated by reducing the levels of wild-type huntingtin using RNA interference (Gauthier et al. 2004). Huntingtin is found predominantly in the cytoplasm of neurons, and it is enriched in compartments containing vesicle-associated proteins (DiFiglia et al. 1995); it is antero- and retrogradely transported in rat sciatic nerve axons, where it associates with vesicles and microtubules (Block-Galarza et al. 1997). It is also involved in fast axonal trafficking (Gunawardena et al. 2003) and in the transport of mitochondria (Trushina et al. 2004). Wild-type huntingtin regulates axonal transport by interacting with the scaffolding proteins of the motor complex on microtubules thereby enabling retrograde transport and perhaps anterograde transport (Block-Galarza et al. 1997; Gunawardena and Goldstein 2005).

In this section we describe the studies showing that huntingtin has a role in the control of BDNF vesicle transport and the underlying mechanisms while presenting the evidence indicating that BDNF vesicle transport is reduced in HD.

4.1 Huntingtin as a Scaffolding Protein That Drives BDNF Vesicles Transport

Saudou and colleagues tested the relationship between huntingtin and BDNF vesicle transport by a series of in vitro experiments that included cells overexpressing wild-type huntingtin and cells in which endogenous huntingtin has been reduced by means of RNA interference. The distribution and dynamics of BDNF vesicles were evaluated in real time by means of ultra-fast 3D videomicroscopy after the transfection of recombinant BDNF tagged with enhanced green fluorescent protein (eGFP), followed by deconvolution microscopy and the measurement of parameters such as the percentage of static vesicles, mean velocity and the pausing time of vesicles (Gauthier et al. 2004). These analyses revealed that BDNF vesicles move faster in the presence of exogenous wild-type huntingtin while their speed is lower when the level of huntingtin is reduced. This study revealed also that BDNF vesicle transport is mediated by microtubules and requires molecular motors, such as kinesin and dynein, i.e. proteins that move vital cargoes on microtubule tracks. Within axons, vesicles from the cell body are transported anterogradely by kinesin motors to nerve terminals and synapses, whereas dynein and some kinesin motors intervene to transport organelles in the retrograde direction. Wild-type huntingtin enhances BDNF transport to both the tips of the neurite and the cell body, suggesting a possible role for huntingtin in both the anterograde and retrograde transport of BDNF (Gauthier et al. 2004).

Biofractionation studies and immunoprecipitation experiments indicated that wild-type huntingtin is part of the motor complex that drives vesicles transport along microtubules. In particular, huntingtin was found to interact with 150 kDa dynein-associated polypeptide ($p150^{Glued}$) subunit of dynactin via HAP1, thereby stimulating

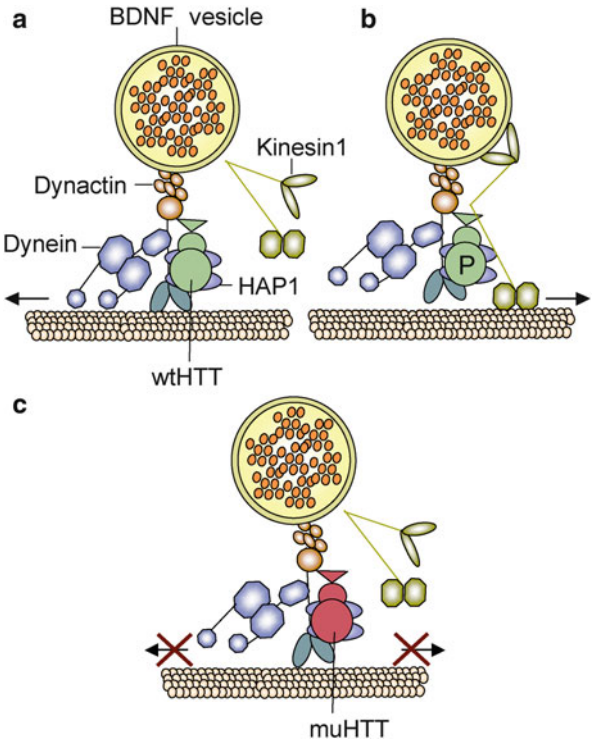

Fig. 3 The role of huntingtin in the intracellular transport of BDNF vesicles. Wild-type huntingtin forms part of a motor complex that controls BDNF vesicle intracellular transport along microtubules. *Arrows* indicate direction of transport (retrograde to the *left*, anterograde to the *right*). (**a**) when wild-type huntingtin is unphosphorylated, kinesin 1 molecules detach from the microtubules and vesicles undergo retrograde transport, mediated by dynein and dynactin. (**b**) when wild-type huntingtin is phosphorylated, kinesin 1 binds to the motor complex and microtubules, inducing a switch to anterograde transport. (**c**) mutant huntingtin is less readily phosphorylated than wild-type huntingtin and also binds more tightly to HAP1, reducing both anterograde and retrograde transport of the BDNF vesicles

BDNF transport. BDNF vesicle velocity decreased when HAP1 protein levels were reduced by RNA interference, whereas its overexpression caused the formation of BDNF vesicle clusters in which wild-type huntingtin and the $p150^{Glued}$ subunit of dynactin are recruited to activate BDNF vesicle transport (Gauthier et al. 2004) (Fig. 3).

Further elucidation of the molecular mechanisms that link wild-type huntingtin to BDNF vesicle transport came from the group of Sandrine Humbert at the Institute Curie in Paris and involves one of huntingtin post-translational modifications [reviewed in Zuccato et al. (2010)]. Humbert's group found that phosphorylation at Ser 421 by Akt kinase is crucial to control the direction of BDNF vesicles (Colin et al. 2008). When huntingtin is phosphorylated, BDNF anterograde transport is favoured, whereas when the phosphorylated status is reduced, BDNF vesicles undergo retrograde transport (Colin et al. 2008). Reduced phosphorylation of huntingtin at Ser 421 is observed in cellular and animal models

of HD and in post-mortem human tissue, and this is likely to impair the transport of BDNF vesicles although in vivo proofs are still missing (Colin et al. 2008; Warby et al. 2005) (Fig. 3). A recent finding from Humbert's group has shown that BDNF vesicle transport can also be regulated by phoshorylation of huntingtin at Ser 1181 and 1201 (Ben M'Barek et al. 2013). Particularly, it was found that unphosphorylated forms of the two residues cause increased anterograde and retrograde BDNF transport (Ben M'Barek et al. 2013).

4.2 The Impact of the HD Mutation on BDNF Vesicles Transport

Since 2004, different groups have tried to understand whether a pathological polyQ expansion affects BDNF vesicles transport in HD. The Saudou's group found that BDNF vesicle velocity is reduced in heterozygous and homozygous mutant huntingtin knockin cells and that proteins involved in other neurodegenerative diseases do not affect BDNF transport, indicating the selectivity of huntingtin involvement in the transport of BDNF vesicles (Gauthier et al. 2004). To test in vivo the possible alteration of BDNF transport in the brain, Saudou and colleagues analysed the composition of the microtubule transport machinery in brain homogenates from mutant huntingtin knockin mice ($Hdh^{109/109}$ mice) and human post-mortem brain tissue. As previously mentioned, huntingtin is involved in the motor complex that includes HAP1 and the $p150^{Glued}$ subunit of dynactin (Gauthier et al. 2004; Block-Galarza et al. 1997; Engelender et al. 1997; Li et al. 1995, 1998). The results of experiments using HD mice, as well as human post-mortem brain tissues, suggest that this motor complex is altered in HD. In particular, increased binding of mutant huntingtin to HAP1 reduced the association between HAP1/$p150^{Glued}$ dynactin and microtubules in heterozygous mutant huntingtin knockin mice (Gauthier et al. 2004). This suggests that the mechanism controlling retrograde transport is altered in the presence of the polyglutamine expansion in huntingtin. As most striatal BDNF comes from anterograde (and not retrograde) transport from the cerebral cortex, it was also investigated whether the association between kinesin and microtubules is also reduced and found this to be the case in in vitro experiments using homozygous mutant huntingtin knockin cells. On the basis of the consideration that, in yeast two hybrid experiments, HAP1 may be pulled down with a human kinesin-like protein, it was also suggested that the complex consisting of huntingtin/HAP1 and kinesin may be affected by the polyglutamine expansion, leading to impaired anterograde transport (Gauthier et al. 2004; McGuire et al. 2006).

The second study was from Her and Goldstein at the University of California San Diego. By using a knockin mouse model of HD, which carries a 150 CAG triplet repeat expansion in the huntingtin gene, (*Hdh*(CAG)150) this group reported impaired movement of BDNF vesicles along microtubules in striatal and hippocampal primary neurons, but not in cortical neurons, the main source of striatal BDNF (Her and Goldstein 2008). Contrary to previous findings of Saudou and colleagues, this study shows that the observed alteration of BDNF vesicles transport in HD is not attributable to a disruption of motor protein complexes in *Hdh*(CAG)150 knockin mice (Her and Goldstein 2008). To test whether this discrepancy could be caused by differences in the

HD mouse models used (*Hdh*(CAG)150 vs $Hdh^{109/109}$ used in the Gauthier's study), differences of age or methods, Her and Goldstein performed sucrose gradient fractionation of brain extracts of 14-months-old $Hdh^{109/109}$ using a 7.5–25 % sucrose gradient as previously described by the Saudou group (Gauthier et al. 2004). No gross change in the pattern of the dynein and dynactin complexes and of kinesin or HAP1 between mutant and control mice were found. This study indicates that different mechanisms may contribute to alter BDNF vesicle transport. Mutant huntingtin may form aggregates that impair cargo movement or physically block movement in axons (Chang et al. 2006; Orr et al. 2008). However, this is unlikely to occur in the Her and Goldstein experiments because no aggregates were observed in the presymptomatic primary neurons employed (Her and Goldstein 2008). More recently, a study from Wu and colleagues suggests a new mechanism involving huntingtin, HAP1 and its direct interaction with pro-BDNF. BDNF is synthesised as a precursor (pro-BDNF), sorted into the secretory pathway, transported along dendrites and axons and released in an activity-dependent manner. Wu et al. have shown that HAP1 may participate in axonal transport and activity-dependent release of pro-BDNF by directly interacting with pro-BDNF (Wu et al. 2010). Mutant huntingtin reduces the association of HAP1 with pro-BDNF, thus leading to decreased transport and release of BDNF in HD (Wu et al. 2010).

In 2006, Sandrine Humbert linked BDNF vesicle transport to heat shock protein DNAJ-containing protein 1b (HSJ1B). HSJB is an inhibitor of heat shock protein cognate 70 kDa (hsp70), which removes clathrin from clathrin-coated vesicles (Cheetham et al. 1996). Clathrin is the main component of the protein coats decorating the cytoplasmic face of vesicles budding from the plasma membrane, the trans-Golgi network and endosomes, and is important for regulating vesicle secretion and endocytosis (Gleeson et al. 2004). This study revealed that BDNF, HSJ1B and clathrin co-localise at the cis-Golgi. The overexpression of HSJ1B positively regulates the sorting of BDNF-containing vesicles from the Golgi/trans-Golgi network, thus increasing BDNF release. Increasing levels of HSJ1B enhance the co-localisation of BDNF and clathrin, whereas reducing HSJ1B by RNA interference dramatically decreases it (Borrell-Pages et al. 2006). Reduced HSJ1B levels have been found in HD patients. This suggests that formation of the clathrin coats on BDNF vesicles can be altered, leading to impairment in BDNF processing at the Golgi and reduced BDNF vesicle transport (Borrell-Pages et al. 2006).

The finding of an altered BDNF vesicle transport in HD needs more studies. However, the evidence available suggests that increasing endogenous BDNF levels may be of therapeutic interest. On these bases, several attempts have been made to understand if BDNF levels are consistently affected in the brain of HD patients.

5 BDNF in HD Patients

In this section we describe the studies aimed at investigating BDNF levels in HD patients. We present the available data about BDNF mRNA and protein levels in autoptic brain tissues and the studies that have tested the BDNF gene

polymorphisms as potential modifiers of age at HD onset. Finally, we will review the conflicting evidence related to BDNF measurement in human blood.

5.1 Studies on Post-mortem Tissues

In a preliminary study conducted in 2000 by Ferrer and colleagues at the University of Barcelona, a small selection of post-mortem HD brain samples was evaluated. Decreased BDNF levels were found in striatum but not in the cerebral cortex. In particular, the parietal cortex, temporal cortex, hippocampus, caudate and putamen of 4 grade III HD subjects were analysed and compared with samples from 6 - age-matched controls. Western blots indicated a decreased ranging of mature BDNF protein (14 kDa) from 53 to 82 % in the caudate and putamen of HD patients when compared with age-matched controls. BDNF levels were preserved in the cerebral (parietal and temporal) cortex and the hippocampus. Immunohistochemical studies of the same tissue samples confirmed the reduced BDNF immunoreactivity in HD striatum (Ferrer et al. 2000). Although the BDNF signal was decreased in striatal neurons, BDNF labelling was maintained in scattered fibres. The authors suggested that the reduced BDNF protein levels in HD striatum could be due to a selective reduction in striatal neurons rather than reduced BDNF input from the cerebral cortex (Ferrer et al. 2000). However, most of the BDNF found in striatum is notoriously derived from cerebral cortex.

The findings of a second study by the Saudou's group published in 2004 also showed that BDNF protein levels evaluated by western blot in the cerebral cortex of ten HD patients and seven controls were reduced to about 50 % in striatum, but not in the cerebral cortex, thus suggesting a defect in cortical BDNF transport to striatum in HD; the negligible patient-to-patient variations indicated the highly homogenous nature of the patient cohort (Gauthier et al. 2004). On the contrary, the third study (published by our group in 2001) found that the levels of BDNF protein (assessed by ELISA) and BDNF mRNA in cortex were consistent with those observed in the various transgenic mouse models of HD: there was a ~50 % decrease in BDNF levels in the frontoparietal cortex of two HD subjects (grade II) in comparison with 2 age-matched controls (Zuccato et al. 2001). It is highly likely that the differences in the results of these three studies were due to their different methods and the diversity of the analysed samples (including our own limited number of samples initially analysed), and would be eliminated by analysing a larger number of samples. To this end, in 2007 we have extended the study to a larger cohort of HD and control subjects and have provided new evidence indicating a significant reduction in BDNF mRNA and protein in the cortex of 20 HD subjects in comparison with 17 controls. Analyses of the BDNF isoforms showed also that transcription from BDNF promoter II and IV is downregulated in human HD cortex (Zuccato et al. 2008; Pruunsild et al. 2007).

This study supports the notion of impaired BDNF production in human HD cortex as a consequence of an expanded CAG tract in the HD gene and suggests that increasing BDNF level or its signalling may be beneficial.

5.2 BDNF Polymorphisms in HD

Given the extensive evidence linking BDNF to HD, the BDNF gene has been tested as a potential modifier of age at HD onset caused by the presence of the BDNF polymorphisms. One known polymorphism of the human BDNF gene is a valine-to-methionine substitution at position 66 (Val66Met BDNF) that is located in the 5′ pro-BDNF sequence encoding the precursor peptide (pro-BDNF), which is proteolytically cleaved to form the mature protein. This BDNF polymorphism does not affect mature BDNF protein function nor its rate of transcription, but it has been shown to dramatically alter the intracellular trafficking and packaging of pro-BDNF, and consequently the regulated secretion of the mature peptide (Chen et al. 2004; Egan et al. 2003). The BDNF Val66Met polymorphism is highly conserved across species and relatively common in the human population with a prevalence for heterozygotes of 20–30 % and a prevalence for the homozygote of ~4 % (Egan et al. 2003; Hariri et al. 2003; Neves-Pereira et al. 2002; Sen et al. 2003). Several genetic linkage and behavioural studies have shown that this polymorphism is associated with neuropsychiatric disorders, including Alzheimer's disease, Parkinson's disease, bipolar disorders, depression, obsessive compulsive disorder and schizophrenia, as well as with normal personality traits (Neves-Pereira et al. 2002, 2005; Momose et al. 2002; Sklar et al. 2002; Ventriglia et al. 2002).

In the case of HD, it was found that mutant huntingtin does not affect the transport of Val66Val BDNF nor of Val66Met BDNF from the endoplasmic reticulum to the Golgi apparatus. Instead, it specifically alters the post-Golgi trafficking of BDNF vesicles. In particular, the post-Golgi trafficking of Val66Val BDNF was significantly blocked in mutant huntingtin cells, whereas the transport of Val66Met BDNF was not affected. These data clearly indicate that the mutant protein affects solely the trafficking of Val66Val BDNF form, without causing a major retention of Val66Met BDNF in the Golgi apparatus (del Toro et al. 2006). However, this study does not exclude the possibility that patients withVal66Met BDNF polymorphisms manifest the disease earlier.

A first linkage studies from Jordi Alberch at the University of Barcelona reported a later age of onset in HD patients who were heterozygous for the Val66Met polymorphism compared to individuals who were homozygous for valine or methionine at this position, although this association was restricted to the group of patients with huntingtin CAG repeats between 42 and 49 (Alberch et al. 2005). However, four subsequent independent studies did not confirm an effect of Val66Met and other BDNF polymorphisms, representing the entire variability of the BDNF gene, on the age of onset of HD (Di Maria et al. 2006; Kishikawa et al. 2006; Mai et al. 2006; Metzger et al. 2006).

Collectively, these studies conclude that there is no convincing genetic link between BDNF polymorphisms and HD. As the Val66Met polymorphism influences BDNF transport from the Golgi region to the appropriate secretory granules, without affecting the transcriptional or biological activities of this molecule, we proposed that the lack of an association might indicate that the defect in BDNF transport has no impact on the age of disease onset, although it may still

affect disease progression. However, this evidence does not exclude the possibility that a defect in BDNF transcriptional activity may affect age of onset and/or disease progression (Zuccato and Cattaneo 2007).

5.3 BDNF in Blood: An Unsolved Issue

BDNF is highly concentrated in the nervous system but is also found in the blood of human and other mammals, where its function is poorly understood. The BDNF in blood derives not only from synthesis in mononuclear blood and endothelial cells but also from platelets release as well as, although to a very minor extent, from the passage through the brain blood barrier (Fujimura et al. 2002; Radka et al. 1996; Rasmussen et al. 2009; Pan et al. 1998; Pan and Kastin 1999). Although it is still unclear how BDNF expression and metabolism in human peripheral tissues are regulated, changes in serum BDNF levels in rats during development correlate to those in brain (Radka et al. 1996; Klein et al. 2011). Based on these findings and on the extensive data showing that BDNF is reduced in HD brain, it was proposed that peripheral BDNF could be used to measure the state of the disease and/or the effectiveness of a given treatment. A number of clinical studies in other pathological conditions revealed that BDNF protein can be measured in human plasma and serum. Although attempts at revealing BDNF protein levels in human HD blood have been performed (Ciammola et al. 2007) in our experience, the detection of BDNF in human blood samples remains extremely complex and variable and results can be easily affected by the experimental procedure (Marullo et al. 2010; Zuccato et al. 2011).

Also studies in rodents can be problematic and controversial. BDNF protein was detected in mouse and primate serum and found sensitive to pharmacological treatment with cystamine (Borrell-Pages et al. 2006). By contrast, earlier findings from Radka et al. (1996) further confirmed by Klein et al. (2011) indicated that BDNF protein is not detectable in either mouse serum or plasma with the most commonly used commercially available ELISA kit (Radka et al. 1996; Klein et al. 2011). However, BDNF mRNA can be monitored systematically by quantitative PCR in the blood of control and HD mice and correlates with disease progression (Conforti et al. 2008). Blood BDNF mRNA is also sensitive to pharmacological treatments as, for example, the acute and chronic treatment of R6/2 mice with CEP-1347, a mixed lineage kinase (MLK) inhibitor with neuroprotective and neurotrophic effects in mice, leads to increased total BDNF mRNA in blood and brain when compared to untreated R6/2 mice (Conforti et al. 2008; Apostol et al. 2008). BDNF mRNA levels in blood may represent a reliable tool to assess drug efficacy in pre-clinical trials in animals.

Table 1 Role of BDNF in HD: evidence from rodent models

Model	Observation	References
Emx1-BDNF knock-out mice	Complete inactivation of BDNF in wild-type mice forebrain leads to: – HD-like behavioral phenotype – Gene expression changes similar to the ones observed in the human HD caudate	Baquet et al. (2004), Strand et al. (2007)
BDNF$^{+/-}$ R6/1	Inactivation of one BDNF allele in HD mice leads to: – Earlier onset, worsening of the behavioural, motor phentype – Loss of striatal enkephalin-positive neurons	Canals et al. (2004)
CamKIIalpha BDNF Tg;R6/1 *CamKIIalpha BDNF Tg;YAC128*	Overexpression of BDNF in the brain of HD mice leads to: – Improvement of behavioral, motor phenotype – Improvement of neuropathology and BDNF-mediated signaling in HD mice	Gharami et al. (2008), Xie et al. (2010)

6 Experimental Manipulation of BDNF Levels and Its Impact on HD Progression

The data described above indicate a clear reduction in BDNF mRNA and protein levels in the cortex of subjects with HD, thus suggesting that the administration of BDNF may be a valid therapeutic option. In this section we present a number of studies involving genetically altered mice that have been performed to evaluating the effects of the modification of BDNF levels on disease onset and progression. These studies provided further support to the idea that cortical BDNF depletion and dysfunction are one of the critical factors in the pathology of HD and that BDNF administration could be beneficial to HD patients (Table 1).

6.1 Effect of BDNF Reduction

In a first set of experiments performed by the group of Kevin Jones at the University of Colorado, *empty spiracles homolog (Emx)*-BDNF knockout mice that are genetically engineered to be deficient in BDNF production in cortical neurons with little BDNF reduction in the thalamus, midbrain and hindbrain were produced. These mice gradually develop aspects of behavioural and anatomical abnormalities seen in mouse models of HD (Baquet et al. 2004). Cortical *Emx*-BDNF knockout mutants show significantly smaller striatal volumes due to reduced MSNs soma size, thinner dendrites and fewer dendritic spines than wild-type littermates. Similar results have been reported by Yves Barde group at the University of Basel that generated a new mouse line in which the BDNF gene has been globally inactivated in post-mitotic neurons of the CNS (Rauskolb et al. 2010). These data are in agreement with earlier studies demonstrating that BDNF stimulates the morphological differentiation of striatal neurons by increasing the length of their neurites, the number of branching

points on the neurites and the soma area (Ivkovic and Ehrlich 1999). Another study has confirmed reduced BDNF support as one major molecular pathway causing striatal dysfunctions in human HD (Strand et al. 2007). The aim of this specific work was to identify the animal model that best recapitulates the striatal gene expression profile of human HD. This study included the most widely used genetic models of HD, i.e. the R6/2 line, three mechanistically motivated HD models of mitochondrial dysfunction including 3-nitropropionic acid (3NP) treated rats, 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-alpha) and *Emx*-BDNF knockout mice. Remarkably, the authors found the *Emx*-BDNF knockout mice exhibited striatal gene expression abnormalities that are more similar to human HD than the other profiles, including those of mouse genetic HD models (Strand et al. 2007).

In a second experimental paradigm to explore the relevance of BDNF depletion in HD pathogenesis, inactivation of one BDNF allele was achieved in a transgenic mouse line expressing human huntingtin exon 1 with an expanded CAG repeat (i.e. R6/1 mouse) (Canals et al. 2004). These mice were reported to show a worsening of the HD phenotype as shown by anticipated age of onset and exacerbated behavioural deficits (Canals et al. 2004) and more accentuated cognitive and learning impairment before symptoms onset (Giralt et al. 2009).

These observations indicate that BDNF depletion may contribute to HD aetiology. The obvious clinical implication is that augmenting BDNF levels or activating downstream signalling pathways may be of therapeutic benefit.

6.2 Effect of BDNF Augmentation

Studies aimed at testing a possible neuroprotective role of BDNF in HD started in the early 1990s, soon after the discovery of the BDNF as potent pro-survival and pro-differentiative factor for developing mature neurons.

The first experiments to assess the effect of BDNF augmentation in vivo in HD mice were performed in chemically induced models. Before the isolation of the disease gene in 1993, HD animal models were produced by injecting excitotoxins into the striatum (Zuccato et al. 2010). BDNF delivery by protein infusion, intrastriatal injection of BDNF-expressing adenovirus, or grafting of BDNF-expressing cells conferred protection to striatal neurons exposed to excitotoxins (Zuccato and Cattaneo 2007). These early findings have been recently corroborated by a study in which the BDNF gene was delivered to the striatal neurons by use of adenoviral vectors. The authors found that transfer of low concentration of BDNF gene to striatal neurons using serotype adeno-associated viral vector (AAV) increased the BDNF protein level in the striatum and conferred protection to striatal neurons against excitotoxic insult, thus attenuating motor impairment (Bemelmans et al. 1999; Kells et al. 2004, 2008).

The impact of BDNF delivery has been then evaluated in genetic models of HD, which better recapitulate the human pathology. A first experiment was performed in vitro on cultured cells transfected with mutant huntingtin and showed that BDNF

conferred protection against death of neurons caused by mutant huntingtin (Saudou et al. 1998).

Later, four independent studies tried to establish whether BDNF could be neuroprotective also in vivo in HD transgenic mice. In one study, BDNF was delivered via osmotic minipump into the striatum of mice overexpressing exon 1 of human mutant huntingtin (R6/1 mice). It was found that daily treatment of BDNF for 1 week succeeded in increasing the expression of enkephalin, as well as in augmenting the number of enkephalin-expressing striatal neurons, the most severely affected cells in HD (Canals et al. 2004). The same study showed a slight improvement of the behavioural phenotype after BDNF administration. A more recent study has shown that chronic and systemic delivery of recombinant BDNF is beneficial also to R6/2 mice (Giampà et al. 2013). It was found that BDNF-treated R6/2 mice survived longer and displayed less severe signs of neurological and neuropathological dysfunctions than the vehicle treated ones (Giampà et al. 2013). To better address the potential of BDNF increase in the brain of HD mice, in two separate studies led by Baoji Xu at Georgetown University School of Medicine, the neurotrophin was constitutively overexpressed in R6/1 mice (Gharami et al. 2008) and YAC128 mice (Xie et al. 2010) by means of the promoter of the α-subunit of Ca^{2+}/calmodulin-dependent kinase II (CaM Kinase II). Such overexpression in the striatum and cerebral cortex of R6/1 mice substantially ameliorated motor dysfunction, reversed brain weight loss, restored tyrosine receptor kinase (TrkB) signalling in the striatum and reduced the formation of mutant huntingtin aggregates in neurons (Gharami et al. 2008). Similarly, BDNF overexpression in YAC128 mice prevented loss and atrophy of striatal neurons and motor dysfunction. Decreased spine density and abnormal spine morphology in striatal neurons of YAC128 mice were also reversed by increasing BDNF levels in the striatum (Xie et al. 2010). Evidence of a neuroprotective role of BDNF in HD came also from a study by Girald and colleagues that produced R6/2 mice overexpressing BDNF in astrocytes (Giralt et al. 2011). In the R6/2:p-GFAP BDNF animals, the decrease in striatal BDNF observed in R6/2 mice was prevented and mice showed an improvement in several motor coodination task and in synaptic plasticity (Giralt et al. 2011).

In 2007 the group of Steven Goldman at University of Rochester Medical Center used a different approach to increase BDNF level in R6/2 mice. BDNF was delivered to striatum by means of adenoviral vectors in combination with Noggin, a molecule that promotes neurogenesis and regulates striatal neuronal regeneration. The authors observed delayed motor impairment in the BDNF/Noggin treated R6/2 transgenic mice (Cho et al. 2007). In particular, these mice exhibited a significant slowing in latency to fall and in rotarod impairment relative to untreated R6/2 mice. Moreover, the BDNF/Noggin-treated mice survived an average of 16.8 % longer than the respective controls (Cho et al. 2007). These results suggest that the neurotrophic action of BDNF in combination with molecules that stimulate neurogenesis might confer considerable therapeutic potential for mitigating both neuropathological and motor function deficits in the brain of patients with HD (Cho et al. 2007; Benraiss et al. 2013).

7 Strategies to Increase BDNF Level In Vivo

The findings described above have generated considerable excitement about the possibility of establishing a "*BDNF therapy*" for neurodegenerative diseases. When designing therapeutic strategies based on BDNF administration, one important consideration is the level of BDNF receptor expression in the neurodegenerating brain. A study by Jordi Alberch group at the University of Barcelona has described a marked reduction in the number and activity of TrkB receptors levels in the striatum in mouse models of HD (Gines et al. 2006). Subsequent studies by our group have shown that TrkB mRNA levels are reduced in caudate tissue but not in the cortex, whereas the mRNA levels of T-Shc (a truncated TrkB isoform) and p75 neurotrophin receptor ($p75^{NTR}$) are increased in the caudate. More recently, it was also found that huntingtin can regulate TrkB transport and that the transport of TrkB is reduced in HD neurons (Liot et al. 2013). This indicates that, in addition to the reduction in BDNF mRNA and transport, there is also unbalanced neurotrophic receptor trafficking and signalling in HD (Zuccato et al. 2008; Liot et al. 2013). Overall, it remains likely that residual TrkB molecules in individuals with HD are still capable of efficiently transducing BDNF-dependent cell signalling (Canals et al. 2004), thereby justifying the effort to develop strategies aimed at increasing BDNF levels in the brain.

The first clinical trial that explicitly investigated the role of BDNF in neurodegenerative diseases was performed in patients with amyotrophic lateral sclerosis (ALS) (Bradley et al. 1995; Ochs et al. 2000; The BSG 1999). Methionyl human BDNF was infused subcutaneously or intrathecally and was well tolerated but failed to demonstrate a statistically significant effect of BDNF on the survival of patients with ALS (Ochs et al. 2000; The BSG 1999). It is possible that the promising results seen in animal models of disease have not translated well into clinical trials owing to the poor pharmacokinetics associated with the intact protein. In particular, BDNF has a short in vivo half-life, has a low blood–brain barrier penetrability and undergoes only limited diffusion in the brain parenchyma. However, there is a serious drawback associated with this intrathecal administration of BDNF. A steep concentration gradient is generated, originating from the point of infusion, which could lead to alteration of the infused tissue and the development of adverse effects such as edema (Gill et al. 2003). Moreover, the intrathecal delivery systems of recombinant BDNF need to be refilled repeatedly over time. The aforementioned problems and the limited neuroprotective effects observed led to the cessation of trials with BDNF.

For these reasons other approaches to efficiently deliver optimum doses of BDNF to the brain have been considered. Non-invasive approaches such as nanoparticle-, Trojan horse- and nose-to brain-mediated delivery of BDNF into the brain are being explored. Trojan horse technology involves conjugating BDNF to molecules that can readily cross the blood-brain barrier. Emerging evidence suggests that preferential uptake of BDNF into the CNS can be achieved by conjugating BDNF to ligands that bind to certain receptors in endothelial cells that facilitate trancytosis or to antibodies directed against these receptors

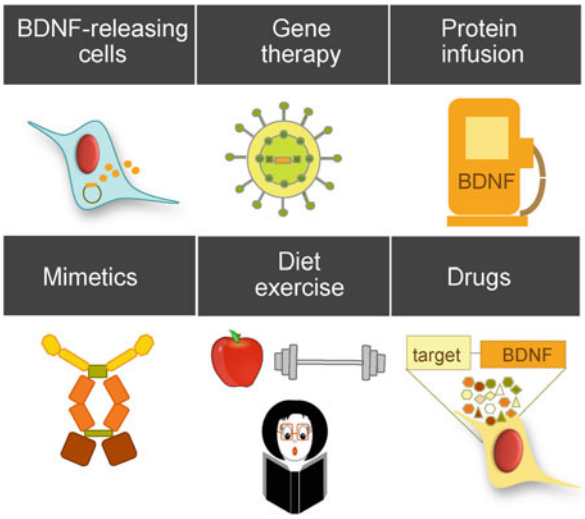

Fig. 4 Experimental therapeutic strategies for restoring BDNF function in HD

(Gabathuler 2010; Géral et al. 2013). Intranasal administration of BDNF protein is an alternative way to deliver BDNF into the CNS, and preliminary data in rodents indicate that the neurotrophin reaches the brain parenchyma (Alcala-Barraza et al. 2010; Jiang et al. 2011). The intranasal delivery method has great clinical potential due to simplicity of administration, noninvasive drug administration, relatively rapid CNS delivery, ability to repeat dosing easily, no requirement for drug modification and minimal systemic exposure. Additional approaches are represented by BDNF in vivo and ex vivo gene transfer while other strategies are aimed at stimulating the synthesis of endogenous BDNF (Zuccato and Cattaneo 2007, 2009). A number of drugs that enhance BDNF production in the brain are being studied, as well as the production of BDNF mimetics. Moreover, interesting new perspectives have arisen from the observation that physical exercise and diet markedly increase endogenous BDNF levels in the hippocampus and cerebral cortex (Zuccato and Cattaneo 2007, 2009). In this section we describe the current strategies that are under development to increase BDNF levels in the HD brain (Fig. 4).

7.1 Gene Therapy

Durable expression of BDNF or other neurotrophins such as glial cell line-derived neurotrophic factor (GDNF), from adenoviral, adeno-associated viral or lentiviral vectors, has been successfully tested and developed over the past decade in animal models of HD (Zuccato and Cattaneo 2007). Increasing BDNF levels through constant, local production following gene transfer has produced encouraging results in preclinical studies on mouse models of HD. Nevertheless, there are still a number of problems to be overcome if this approach is to be used in the clinic. The first

challenge is to regulate the amount of BDNF produced locally, as an excess of BDNF could have a deleterious effect on neuronal circuits, learning and memory (Croll et al. 1999). The second problem is that transduction is often associated with inflammation, which is usually accompanied by some vector toxicity, and together these effects prohibit long-term therapy on safety grounds. Another major problem is the risk of accidental insertional mutagenesis by viral vectors and subsequent tumour formation (Hacein-Bey-Abina et al. 2008). To overcome these problems, a large effort is currently underway to produce new viral vectors, which lack both pathogenicity and immunogenicity (Biffi and Naldini 2005). Methods utilising integration-deficient lentiviral vectors and nontoxic viral systems have been successfully used in other pathologies and are under scrutiny (Biffi et al. 2013; Aiuti et al. 2013; Yanez-Munoz et al. 2006). These approaches would allow the transduction of BDNF in a cell-specific and inducible manner.

7.2 Grafting of BDNF-Releasing Cells

To avoid concerns about the direct injection of a virus into the brain parenchyma, another possible strategy to increase BDNF levels in the brain is to graft cells engineered to stably express BDNF. In a first attempt, immortalised rat fibroblasts genetically engineered to secrete BDNF were implanted in the rat striatum 7 days before the striatal infusion of excitotoxic quantities of an NMDA-receptor agonist that causes widespread neuropathological deficits similar to those seen in the HD brain. Analysis of striatal damage 7 days after the lesion revealed that BDNF-secreting fibroblasts offered no protection (Frim et al. 1993). A later study showed that BDNF had only limited ability to protect the striatum from damage due to an excitotoxic lesion by transplanting putative neural stem cells that had been genetically modified to overexpress BDNF, which were injected in the same area 1 week later. One month after the lesion, striatal degeneration, lesion size and the loss of striatal dopamine- and cyclic AMP-regulated phosphoprotein 32 kDa (DARPP-32) positive neurons were only slightly improved by the BDNF-secreting cells (Martinez-Serrano and Bjorklund 1996). Subsequent attempts have been more successful probably because lower and safer BDNF doses have been released (Perez-Navarro et al. 1999, 2000; Ryu et al. 2004), including a particularly interesting study by Ryu et al. (2004). The authors investigated the ability of transplanted BDNF-overexpressing *bona fide* neural stem cells taken from human foetal brain to protect animals after 3NP administration, which causes striatal cells death similar to those seen in HD. The animals receiving the intrastriatal cell implantation 1 week before 3NP treatment showed significantly improved motor performance and less striatal neuron damage, whereas those transplanted 12 h after 3NP treatment did not show any improvement in motor performance or any protection of striatal neurons from the toxicity induced by 3NP (Ryu et al. 2004). More recently, mice grafted with primary astrocytes overexpressing BDNF have showed important and sustained behavioural improvements over time after quinolinate administration as compared with wild-type mice grafted with wild-

type astrocytes (Giralt et al. 2010). These findings suggested that astrocytes engineered to release BDNF can constitute a therapeutic approach for HD.

Since grafting of BDNF-releasing cells may still have some problems—xenogenic cells are at risk of being rejected and immortalised cells can cause tumour growth—researchers envisage encapsulating cells with new materials under development. These materials would serve as biological shields, preventing immune rejection and eliminating the need for immunosuppression (Emerich et al. 1997). There is also considerable interest in the development of stable, nontumorigenic human neural stem cell lines as well as mesenchymal stem cells that release BDNF (Conti and Cattaneo 2010; Hess and Borlongan 2008; Joyce et al. 2010; Rossi and Cattaneo 2002; Somoza et al. 2010; Olson et al. 2012).

7.3 BDNF Mimetics

As many of the issues surrounding BDNF efficacy and safety result from the need to deliver the neurotrophin close to the target site, investigators have considered the interesting possibility of using peptidomimetics, agonist antibodies and small molecules directed specifically to the BDNF receptors. These BDNF mimetics have been designed in accordance with the three-dimensional structure of BDNF, in particular, loops 1, 2 and 4, which are required for binding of BDNF to TrkB receptors. The synthetic molecules are also modified in such a way as to penetrate the blood–brain barrier more efficiently than BDNF (Longo et al. 2007; Massa et al. 2010; Pardridge 2006). Recently, Frank Longo of Stanford University and colleagues from the University of California at San Francisco screened over one million compounds and discovered four chemically distinct compounds which mimic BDNF being able to bind and activate selectively TrkB, but not the other Trks and not $p75^{NTR}$. One of the compounds was selected for further study, and it was used to treat various cell models of neurodegenerative disease, including HD, with promising results on cell survival (Massa et al. 2010; Simmons et al. 2013). BDNF mimetics applied locally or systemically may be a promising strategy to increase BDNF-mediated signalling in HD and, as a consequence, to induce neuroprotection effects, because it avoids the adverse effects associated with invasive methods of delivery or uncontrolled dosing, while improving upon the diffusion properties of BDNF.

7.4 Drug Increasing BDNF Levels and Their Effectiveness in HD

Current experiments are aimed at isolating compounds that increase endogenous BDNF level. Such a strategy would circumvent the problems related to invasive methods of BDNF delivery in humans, including achieving the correct dosage and maintaining stability of the neurotrophin. Several classes of compounds are able to increase BDNF levels in the brain of HD mice, leading to improvements of the neuropathology as well as of cognitive and behavioural deficits. Among them,

considerable attention has been received by selective serotonin reuptake inhibitors (SSRIs) and lithium. Furthermore, memantine and riluzole (a non-competitive inhibitor of ionotropic glutamate NMDA receptor), cystamine and cysteamine, ampakine (a positive modulator of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors), nicotinamide (an inhibitor of sirtuin 1/class III NAD (+)-dependent histone deacetylase) and the calcineurin inhibitor FK506 have been found to significantly restore BDNF levels in HD mice. We describe below a selection of these studies.

7.4.1 SSRIs

SSRIs facilitate the signalling of serotonin by inhibiting its reuptake. SSRIs may have protective effects on striatal and cortical neurons by activating cyclic AMP and CREB signals, which also lead to BDNF expression (Mostert et al. 2008; Tardito et al. 2006). A first attempt to test the effect of SSRIs on mouse models of HD involved the administration of paroxetine (5 mg/kg/day) to N171-82Q mice, which was found to delay the onset of behavioural symptoms and increase lifespan (Duan et al. 2004). Significant impairment of the behavioural phenotype was observed specifically at the level of motor function (Duan et al. 2004), and the weight loss previously reported in this model occurred significantly more slowly than in vehicle-treated HD mice. Histological analyses also revealed a decrease in brain atrophy. N171-82Q are normally hyperglycemic but paroxetine treatment reduced blood glucose levels, thus providing evidence that, in addition to neurodegenerative processes, it improves glucose metabolism in HD. Paroxetine also increased survival even when administered after the onset of motor dysfunction (Duan et al. 2004), thus suggesting the possibility that HD patients may benefit from SSRIs after they become symptomatic.

In 2008, Duan and colleagues confirmed the beneficial effects of SSRIs by demonstrating that sertraline prolongs survival, improves motor performance and ameliorates brain atrophy in two mouse models of HD represented by the R6/2 and N171-82Q (Duan et al. 2008; Peng et al. 2008). These beneficial effects of sertraline were associated with enhanced neurogenesis and increased BDNF levels in the brain (Duan et al. 2008; Peng et al. 2008).

These findings open the way to studies of the effects of paroxetine and sertraline in human HD patients, but previous studies have found no clinical benefit with the use of other SSRIs. There is a single case report of fluoxetine exacerbating chorea (Chari et al. 2003), and although another study found it a useful antidepressant, it failed to provide any substantial clinical benefit to non-depressed HD patients (Como et al. 1997). On the contrary successful treatment with sertraline in depressed HD patients has been reported. Moreover, sertraline is safe and well tolerated for long-term administration, including in HD patients (Ranen et al. 1996). This suggests that a clinical trial of SSRI treatment in order to retard disease progression in human HD may be warranted.

7.4.2 Lithium

Lithium induces the expression of BDNF and the subsequent activation of TrkB in cortical neurons (Fukumoto et al. 2001). Early studies by Wei et al. indicated that a subcutaneous lithium chloride (LiCl) injection for 16 days before quinolinic acid infusion considerably reduces the size of quinolinic acid-induced striatal lesions (Wei et al. 2001). It was later found that it can protect against polyglutamine toxicity in cell lines by inhibiting glycogen synthase kinase 3-beta (GSK-3beta), which is involved in apoptotic cell death, and increasing beta-catenin whose overexpression protects cells from mutant huntingtin-induced toxicity (Carmichael et al. 2002).

One year later, on the basis of lithium's reported neuroprotective and anti-depressive properties, other studies determined whether chronic LiCl treatment affects the progression of the phenotype in R6/2 mice, but found that it had variable effects on motor behaviour and did not improve survival (Wood and Morton 2003). A study by Senatorov et al. has suggested that lithium may be neuroprotective in the quinolinic acid-injection model of HD because of its ability to inhibit apoptosis and induce neuronal and astroglial progenitor proliferation or migration from the subventricular zone (SVZ) (Senatorov et al. 2004).

In 2008, David Rubinsztein at Cambridge University in the UK has shown that lithium enhances mammalian target of rapamycin (mTOR)-dependent and -independent autophagic processes in HD flies when administered in combination with mTOR inhibitor rapamycin, leading to protection against neurodegeneration (Sarkar et al. 2008). More recently, it has been reported that lithium induced brain and blood BDNF expression, improved striatal neuropathology, and behavioral abnormalities in YAC128 and N171-82Q mice (Chiu et al. 2011; Pouladi et al. 2012). Together with its favorable safety profile and pharmacokinetic properties, these findings support further development of lithium as a therapeutic agent in HD.

7.4.3 Memantine and Riluzole

Memantine is a medium-affinity non-competitive NMDA receptor antagonist that has been clinically used as a neuroprotective agent to treat Alzheimer's disease and Parkinson's disease. At clinically relevant doses, it markedly increases BDNF and TrkB mRNA levels in rat brain, and its effects on BDNF mRNA were reflected in changes in BDNF protein levels (Marvanova et al. 2001). Remarkably, two different studies demonstrated that memantine ameliorates neuropathological and behavioral phenotypes in HD mice (Okamoto et al. 2009, Milnerwood et al. 2010). These studies also suggest that the neuroprotective role of memantine depends on its ability to promote the CREB pathway which controls BDNF gene transcription (Okamoto et al. 2009; Milnerwood et al. 2010). Like memantine, riluzole (a neuroprotective drug commonly used in ALS) acts by blocking glutamatergic neurotransmission in the CNS. Interestingly, it has also been found to upregulate the levels of a number of key neurotrophic factors, including BDNF and GDNF (Katoh-Semba et al. 2002; Mizuta et al. 2001). These data suggest that the anti-excitotoxic activity of memantine and riluzole is accompanied by an increase in the endogenous BDNF production in the brain. On these bases a 2-year, multicentre

open-label study of 27 HD patients was carried out in order to investigate the effectiveness of memantine (up to 30 mg/day) in delaying disease progression. The results suggest that memantine treatment may be useful in doing so (Beister et al. 2004). Another open-label trial has found that riluzole causes transient motor improvement in human HD patients (Seppi et al. 2001; HSG 2003). These promising results have led to a 3-year, randomised controlled study conducted by the European Huntington's Disease Initiative Study Group led by Bernard Landwehrmeyer on 379 HD patients. The study, concluded in 2007, showed that, although riluzole was well tolerated, no neuroprotective or beneficial symptomatic effects were demonstrated (Landwehrmeyer et al. 2007). On the contrary, a study performed on a small number of HD patients ($n = 11$) has shown that riluzole protects HD patients from brain glucose hypometabolism and grey matter volume loss and increases production of BDNF (Squitieri et al. 2009).

7.4.4 Cystamine and Cysteamine

Transglutaminases (TGases) play a critical role in the pathogenesis of HD because they cross-link huntingtin and catalyse the formation of aggregates. As TGases activity is increased in HD brain, they represent an attractive target for possible therapeutic intervention in HD (Gentile and Cooper 2004; Hoffner and Djian 2005). Early findings indicated that cystamine, a competitive inhibitor of TGases activity, limits the aggregation of proteins with an expanded polyglutamine tract (de Cristofaro et al. 1999; Igarashi et al. 1998) and has also been shown to decrease apoptosis in cultured cells exposed to glutamate or an N-terminal fragment of mutant huntingtin (Ientile et al. 2003; Zainelli et al. 2005). Cystamine protects against 3NP striatal lesions in mice (Fox et al. 2004) and, more importantly, improved behaviour and survival in two independent therapeutic trials in R6/2 mice (Dedeoglu et al. 2002; Karpuj et al. 2002). Other findings indicated that cystamine reduces striatal volume loss and neuronal atrophy in YAC128 mice, but does not reverse progressive motor dysfunction or the downregulation of the striatal marker DARPP-32, whose expression is significantly reduced in this model (Van Raamsdonk et al. 2005). Recent evidence suggests that the improved survival and motor function in cystamine-treated R6/2 mice may not be solely due to TGase inhibition because R6/2 mice not expressing tissue transglutaminase also benefit from cystamine administration (Bailey and Johnson 2005). Other beneficial effects of cystamine include the inhibition of caspase-3 activity, increased cell levels of the anti-oxidant glutathione and cysteine (Fox et al. 2004; Lesort et al. 2003) and an increase in the expression of heat-shock proteins (Karpuj et al. 2002).

In 2006 cystamine and cysteamine (the Food and Drug Administration (FDA)-approved reduced form of cystamine) were linked to BDNF secretion, thus opening up the possibility that the neuroprotection observed in treated animals may be due to a cystamine-mediated increase in BDNF secretion (Borrell-Pages et al. 2006). In their study, Borrell-Pages et al. found that cystamine increases the levels of HSJ1B, which are low in HD patients. HSJ1B stimulates the BDNF secretory pathway through the formation of clathrin-coated vesicles containing BDNF. Therefore, the authors suggested that cystamine is neuroprotective because it increases BDNF

secretion from the Golgi. Cystamine and cysteamine are both neuroprotective in HD mice (Borrell-Pages et al. 2006). Tolerated cysteamine doses have been evaluated in HD patients, thus strengthening the case in favour of using cystamine and cysteamine as a therapeutic approach to HD. Although cysteamine can cross the blood–brain barrier, it takes larger doses to detect a variation in cysteamine or its metabolites in the brain (Bousquet et al. 2010). In 2011, Raptor Pharmaceutical Corporation initiated a collaboration with the Centre Hospitalier Universitaire (CHU) d'Angers in France to support a phase II clinical study of a delayed release preparation of cysteamine bitartrate in HD patients. Clinical researchers at the CHU d'Angers, in collaboration with the Curie Institute, have designed a 96 HD patients trial to investigate the efficacy of this new cysteamine delivery, using BDNF as a marker of efficacy (Gibrat and Cicchetti 2011). The trial has been recently concluded and results are pending.

7.4.5 FK506

BDNF vesicle transport depends on S421 phosphorylation and constitutive phosphorylation of mutant huntingtin restores impaired BDNF vesicle transport in HD (Colin et al. 2008; Zala et al. 2008). Pineda and colleagues found that pharmacological inhibition of calcineurin, the *bona fide* huntingtin S421 phosphatase, restored the BDNF transport defects observed in HD (Pineda et al. 2009). Particularly, FK506, an FDA-approved drug capable of crossing the blood–brain barrier, restored BDNF transport in two complementary models: rat primary neuronal cultures expressing mutant huntingtin and mouse cortical neurons from mutant huntingtin knockin mice ($Hdh^{Q111/Q111}$). This effect was the result of specific calcineurin inhibition, as calcineurin silencing restored both anterograde and retrograde transport in neurons from $Hdh^{Q111/Q111}$ mice (Pineda et al. 2009). These results indicate that drugs as FK506, which target a specific mechanism responsible for altered BDNF transport, may be of interest in HD.

7.4.6 Ampakine and Nicotinamide

Ampakine is a positive modulator of AMPA-type glutamate receptors. In 2009, Gary Lynch's group at the University of California Irvine showed that ampakine upregulates endogenous hippocampal BDNF levels, rescues neuronal plasticity and reduces learning problems in mutant huntingtin knockin mice (Simmons et al. 2009). A study from the same group published 2 years later has confirmed these data and showed that long-term ampakine treatment markedly slows the progression of striatal neuropathology and locomotor dysfunction in an additional mouse model of HD represented by the R6/2 transgenic line by increasing BDNF protein levels in the neocortex (Simmons et al. 2011). Ampakines are well tolerated in clinical trials and have shown efficacy in this study after brief exposures, suggesting that they may be useful for chronic treatment of the cognitive difficulties in the early stages of HD.

Nicotinamide is an inhibitor of sirtuin 1/class III NAD (+)-dependent histone deacetylase. The group of Anne Messer at the Albany Medical College has examined the effects of nicotinamide after administration to R6/1 mice. BDNF levels

were found to be significantly increased in the brain of R6/1 mice, and motor deficits associated to HD phenotype were significantly improved (Hathorn et al. 2011).

7.4.7 Towards the Identification of RE1/NRSE Modulators

Since some of the mechanisms of reduced BDNF gene transcription and protein transport have been elucidated, a valid option could be to increase BDNF levels by targeting specific mechanisms that are responsible for the BDNF dysfunction. Strategies that act specifically on a defined molecular dysfunction could be more effective than drugs that increase BDNF levels but do not specifically target a disease mechanism.

We have previously shown that the REST/NRSF–RE1/NRSE transcriptional system, important regulator of BDNF gene transcription, is impaired in HD, thus contributing to reduced BDNF levels in the disease as well as to reduced transcription of other REST/NRSF-regulated genes (Zuccato et al. 2003, 2007; Johnson and Buckley 2009; Hodges et al. 2006). These data opened to the development of therapeutic strategies that target the REST/NRSF–RE1/NRSE silencer complex. In vitro evidences suggested that this could be a feasible strategy. Overexpression of a dominant negative protein of REST/NRSF lacking any co-repressor domain resulted in attenuation of REST/NRSF binding at its target sites and restoration of the expression level of several target genes (Zuccato et al. 2007). A new study from Noel Buckley at King's College London has further demonstrated this concept. By delivering oligonucleotide decoys targeting REST/NRSF, REST/NRSF occupancy at several RE1/NRSE loci was reduced in mutant huntingtin knockin cells, thus restoring transcription of BDNF and other neuronal genes (Soldati et al. 2011). Compounds that specifically interfere with the REST/NRSF pathway in HD may represent a valid therapeutic approach to increase the transcription of REST/NRSF-regulated genes (Rigamonti et al. 2007; Leone et al. 2008). To this purpose Cell-based reporter assays to monitor RE1/NRSE activity in cultured brain cells with the final aim to identify compounds that specifically upregulate BDNF in HD have been developed (Rigamonti et al. 2007; Charbord et al. 2013; Conforti et al. 2013). Compounds identified in high-throughput screening as blockers of the RE1/NRSE silencing activity alleviate the REST/NRSF-dependent repression and, hence, ameliorate the global transcriptional repression in the disease (Conforti et al. 2013; Charbord et al. 2013). Other human pathologies exhibit abnormal REST activity, highlighting the importance of REST/NRSF-mediated regulation to the integrity of the cell. Abnormalities in REST/NRSF transcriptional activity have been demonstrated also in cardiac hypertrophy, ischaemia and Down syndrome (Rigamonti et al. 2009). Future therapeutics pointing at targeting REST/NRSF or the RE1/NRSE site might consequently be applied to an extended set of pathologies in addition to HD.

7.5 Other Interesting Perspectives for Increasing Endogenous BDNF

Physical exercise and diet cause a marked increase in BDNF levels in rat brain, particularly the hippocampus and cerebral cortex. Early studies showed that dietary restriction (DR) and physical exercise can have profound effects on brain functions and vulnerability to injury and disease (Spires et al. 2004; Mattson et al. 2003; Zoladz and Pilc 2010).

7.5.1 Diet and Environmental Enrichment

DR promotes neuronal survival by enhancing resistance against cell stress (Guo et al. 2000; Yu and Mattson 1999), reducing oxidative damage (Dubey et al. 1996), stimulating the production of new neurons (neurogenesis) and improving synaptic plasticity (Mattson et al. 2003). Data in mouse models of neurodegenerative diseases indicate that DR can protect neurons against neurodegeneration, suggesting that dietary changes may reduce disease severity (Mattson et al. 2003). When rats were kept on a periodic fasting/dietary restriction regimen for several months before the administration of 3NP acid to induce a striatal lesion, their motor function improved and more striatal neurons survived (Bruce-Keller et al. 1999). In DR condition BDNF levels increase in several brain regions (Duan et al. 2001a; Lee et al. 2002). The fact that beneficial effect of DR are mediated by BDNF came from studies showing that the infusion of a BDNF blocking antibody into the lateral ventricle of rats and mice significantly attenuated the neuroprotective effect of DR in the kainate model of seizure-induced hippocampal damage (Duan et al. 2001a, b). Other findings indicate that DR increases BDNF protein level in the cerebral cortex and striatum of HD mice (the N171-82Q line), which results in delayed disease onset and increased survival (Duan et al. 2003). DR reduces brain atrophy and the formation of huntingtin aggregates and diminishes caspase activation in N171-82Q mice, thus apparently blocking the toxic effects elicited by mutant huntingtin (Duan et al. 2003). DR may therefore be considered a potential early strategy (before the development of symptoms) for counteracting HD phenotypes and restoring normal brain BDNF levels.

Environmental enrichment also markedly delays the onset and progression of HD in transgenic mice. It involves providing the mice with environments containing regularly changed, complex and stimulating objects. The impact of such a strategy was reported for the first time in 2000, when it was shown that R6/1 mice exposed to environmental enrichment experienced a delayed disease onset and slower rate of disease progression, and had improved behavioural performances on motor tests (van Dellen et al. 2000). Further studies have indicated that environmental enrichment also slows disease progression in the more severe R6/2 mouse model of HD (Hockly et al. 2002), as well as in N171-82Q transgenic HD mice (Schilling et al. 2004). Environmental stimulation delays the onset of cognitive deficits (van Dellen et al. 2005), and its beneficial effects have also been demonstrated by studies of HD patients (Sullivan et al. 2001). The mechanisms by which these beneficial effects are mediated are still unclear, but there are a number

of plausible possibilities. Several studies indicated that environmental enrichment upregulates neurotrophins such as BDNF and nerve growth factor (NGF) in the hippocampus and cortex (Falkenberg et al. 1992; Keyvani et al. 2004; Pham et al. 1999a, b; Young et al. 1999). There is evidence that environmental enrichment or physical exercise upregulates the transcription of genes encoding neuronal proteins that are important for neuronal plasticity, learning and memory (Rampon and Tsien 2000). Enrichment is associated with increased synaptic signalling and the stimulation of second messenger systems; it also has an effect on neuronal morphology, as it is associated with increased spine density. The stimulatory role of enrichment and BDNF on neurogenesis (Bath et al. 2012) suggests that this may be an additional avenue for the therapeutic effects of environmental stimulation. Studies of R6/1 transgenic mice have shown that environmental enrichment rescues striatal and hippocampal BDNF protein deficits, leading to improvement of the disease phenotype (Spires et al. 2004; Pang et al. 2006). These observations suggest that the beneficial effect of enrichment may be partially mediated by increased BDNF levels.

Conclusions

BDNF seems to be necessary for the phenotypic maintenance and activity of mature, fully developed neurons, so it has been suggested that changes in its level or distribution could be important in the pathogenesis of neurodegenerative conditions in humans. The best example is given by HD. BDNF is crucial for cortical and striatal neurons, the most affected neuronal populations in the HD brain. The evidence described in this chapter points to BDNF deficit as one major contributor to HD pathogenesis.

Findings of the last decade indicated that the normal huntingtin protein, whose mutation causes HD, is involved in the physiological control of BDNF synthesis and transport in the brain. Wild-type huntingtin sustains cortical BDNF gene transcription and drives BDNF vesicles sorting in neuronal cells. Multiple experiments in HD cells and animal models indicated that BDNF production and possibly also its transport are impaired in the disease since early stages. Moreover, BDNF levels are reduced in the brain of HD patients and this is due to decreased normal huntingtin activity, but also to the toxicity of mutant huntingtin. The "*BDNF defect*" in HD has been documented by roughly 20 laboratories and corroborated by the elucidation of the underlying molecular mechanisms. BDNF measures are currently used as read-outs, both to test the quality of new cellular or animal models of HD as well as the efficacy of new compounds in pre-clinical studies.

Several groups are working to establish a "*BDNF therapy*" for the treatment of HD, but numerous methodological and safety issues will need to be addressed in patients before this approach can be widely adopted. In our opinion, one promising strategy is the use of BDNF mimetics directed to the BDNF receptors or small molecules that increase endogenous BDNF levels by acting on well-characterised molecular targets generated by the knowledge of the mechanisms underlying BDNF transcription and transport. We also believe that an important

problem to solve is the reliable and robust measurement of BDNF protein and mRNA levels in human material. It is true indeed that studies on postmortem samples, although quantitatively and systematically performed, may not mimic what happens in vivo. Moreover, in humans, BDNF synthesis is subjected to a wide range of influences (dietary restriction, physical exercise, circadian rhythms, stress) affecting the level of BDNF. The imprecise evaluation of the BDNF level in the diseased brain may lead to the administration of uncorrected doses of BDNF that could be inefficacious as well as deleterious. A better understanding of the timing of BDNF loss in patients and the precise measurement of its levels are crucial before proposing BDNF treatment as a beneficial and feasible therapeutic approach in the clinic.

References

Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T (2007) Mouse and rat BDNF gene structure and expression revisited. J Neurosci Res 85:525–535

Aiuti A, Biasco L, Scaramuzza S, Ferrua F, Cicalese MP, Baricordi C, Dionisio F, Calabria A, Giannelli S, Castiello MC, Bosticardo M, Evangelio C, Assanelli A, Casiraghi M, Di Nunzio S, Callegaro L, Benati C, Rizzardi P, Pellin D, Di Serio C, Schmidt M, Von Kalle C, Gardner J, Mehta N, Neduva V, Dow DJ, Galy A, Miniero R, Finocchi A, Metin A, Banerjee PP, Orange JS, Galimberti S, Valsecchi MG, Biffi A, Montini E, Villa A, Ciceri F, Roncarolo MG, Naldini L (2013) Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science 341(6148):1233151

Alberch J, Lopez M, Badenas C, Carrasco JL, Mila M, Munoz E, Canals JM (2005) Association between BDNF Val66Met polymorphism and age at onset in Huntington disease. Neurology 65:964–965

Alcala-Barraza SR, Lee MS, Hanson LR, McDonald AA, Frey WH 2nd, McLoon LK (2010) Intranasal delivery of neurotrophic factors BDNF, CNTF, EPO, and NT-4 to the CNS. J Drug Target 18:179–190

Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, Lindsay RM, Wiegand SJ (1997) Anterograde transport of brain-derived neurotrophic factor and its role in the brain. Nature 389:856–860

Apostol BL, Simmons DA, Zuccato C, Illes K, Pallos J, Casale M, Conforti P, Ramos C, Roarke M, Kathuria S, Cattaneo E, Marsh JL, Thompson LM (2008) CEP-1347 reduces mutant huntingtin-associated neurotoxicity and restores BDNF levels in R6/2 mice. Mol Cell Neurosci 39:8–20

Bailey CD, Johnson GV (2005) Tissue transglutaminase contributes to disease progression in the R6/2 Huntington's disease mouse model via aggregate-independent mechanisms. J Neurochem 92:83–92

Baquet ZC, Gorski JA, Jones KR (2004) Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. J Neurosci 24:4250–4258

Bath KG, Akins MR, Lee FS (2012) BDNF control of adult SVZ neurogenesis. Dev Psychobiol 54 (6):578–589. doi:10.1002/dev.20546

Beister A, Kraus P, Kuhn W, Dose M, Weindl A, Gerlach M (2004) The N-methyl-D-aspartate antagonist memantine retards progression of Huntington's disease. J Neural Transm Suppl (68):117–22

Bemelmans AP, Horellou P, Pradier L, Brunet I, Colin P, Mallet J (1999) Brain-derived neurotrophic factor-mediated protection of striatal neurons in an excitotoxic rat model of

Huntington's disease, as demonstrated by adenoviral gene transfer. Hum Gene Ther 10:2987–2997

Ben M'Barek K, Pla P, Orvoen S, Benstaali C, Godin JD, Gardier AM, Saudou F, David DJ, Humbert S (2013) Huntingtin mediates anxiety/depression-related behaviors and hippocampal neurogenesis. J Neurosci 33:8608–8620

Benraiss A, Toner MJ, Xu Q, Bruel-Jungerman E, Rogers EH, Wang F, Economides AN, Davidson BL, Kageyama R, Nedergaard M, Goldman SA (2013) Sustained mobilization of endogenous neural progenitors delays disease progression in a transgenic model of Huntington's disease. Cell Stem Cell 12:787–799

Biffi A, Montini E, Lorioli L, Cesani M, Fumagalli F, Plati T, Baldoli C, Martino S, Calabria A, Canale S, Benedicenti F, Vallanti G, Biasco L, Leo S, Kabbara N, Zanetti G, Rizzo WB, Mehta NA, Cicalese MP, Casiraghi M, Boelens JJ, Del Carro U, Dow DJ, Schmidt M, Assanelli A, Neduva V, Di Serio C, Stupka E, Gardner J, von Kalle C, Bordignon C, Ciceri F, Rovelli A, Roncarolo MG, Aiuti A, Sessa M, Naldini L (2013) Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science 341(6148):1233158

Biffi A, Naldini L (2005) Gene therapy of storage disorders by retroviral and lentiviral vectors. Hum Gene Ther 16:1133–1142

Block-Galarza J, Chase KO, Sapp E, Vaughn KT, Vallee RB, DiFiglia M, Aronin N (1997) Fast transport and retrograde movement of huntingtin and HAP 1 in axons. Neuroreport 8:2247–2251

Borrell-Pages M, Canals JM, Cordelieres FP, Parker JA, Pineda JR, Grange G, Bryson EA, Guillermier M, Hirsch E, Hantraye P, Cheetham ME, Neri C, Alberch J, Brouillet E, Saudou F, Humbert S (2006) Cystamine and cysteamine increase brain levels of BDNF in Huntington disease via HSJ1b and transglutaminase. J Clin Invest 116:1410–1424

Bousquet M, Gibrat C, Ouellet M, Rouillard C, Calon F, Cicchetti F (2010) Cystamine metabolism and brain transport properties: clinical implications for neurodegenerative diseases. J Neurochem 114:1651–1658

Bradley WG, Miami FL, The BDNF Trial Group (1995) A PhaseI/II study of recombinant human brain-derived neurotrophic factor in patients with amyotrophic lateral sclerosis. Ann Neurol 38:971

Bruce AW, Donaldson IJ, Wood IC, Yerbury SA, Sadowski MI, Chapman M, Gottgens B, Buckley NJ (2004) Genome-wide analysis of repressor element 1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) target genes. Proc Natl Acad Sci USA 101:10458–10463

Bruce-Keller AJ, Umberger G, McFall R, Mattson MP (1999) Food restriction reduces brain damage and improves behavioral outcome following excitotoxic and metabolic insults. Ann Neurol 45:8–15

Canals JM, Pineda JR, Torres-Peraza JF, Bosch M, Martin-Ibanez R, Munoz MT, Mengod G, Ernfors P, Alberch J (2004) Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. J Neurosci 24:7727–7739

Carmichael J, Sugars KL, Bao YP, Rubinsztein DC (2002) Glycogen synthase kinase-3beta inhibitors prevent cellular polyglutamine toxicity caused by the Huntington's disease mutation. J Biol Chem 277:33791–33798

Cattaneo E, Rigamonti D, Goffredo D, Zuccato C, Squitieri F, Sipione S (2001) Loss of normal huntingtin function: new developments in Huntington's disease research. Trends Neurosci 24:182–188

Cattaneo E, Zuccato C, Tartari M (2005) Normal huntingtin function: an alternative approach to Huntington's disease. Nat Rev Neurosci 6:919–930

Charbord J, Poydenot P, Bonnefond C, Feyeux M, Casagrande F, Brinon B, Francelle L, Aurégan G, Guillermier M, Cailleret M, Viegas P, Nicoleau C, Martinat C, Brouillet E, Cattaneo E, Peschanski M, Lechuga M, Perrier AL (2013) High throughput screening for

inhibitors of REST in neural derivatives of human embryonic stem cells reveals a chemical compound that promotes expression of neuronal genes. Stem Cells 31:1816–1828
Chang DT, Rintoul GL, Pandipati S, Reynolds IJ (2006) Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons. Neurobiol Dis 22:388–400
Chari S, Quraishi SH, Jainer AK (2003) Fluoxetine-induced exacerbation of chorea in Huntington's disease? A case report. Pharmacopsychiatry 36:41–43
Cheetham ME, Anderton BH, Jackson AP (1996) Inhibition of hsc70-catalysed clathrin uncoating by HSJ1 proteins. Biochem J 319(Pt 1):103–108
Chen WG, West AE, Tao X, Corfas G, Szentirmay MN, Sawadogo M, Vinson C, Greenberg ME (2003a) Upstream stimulatory factors are mediators of Ca2+−responsive transcription in neurons. J Neurosci 23:2572–2581
Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, Jaenisch R, Greenberg ME (2003b) Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science 302:885–889
Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J Neurosci 24:4401–4411
Chiu CT, Liu G, Leeds P, Chuang DM (2011) Combined treatment with the mood stabilizers lithium and valproate produces multiple beneficial effects in transgenic mouse models of Huntington's disease. Neuropsychopharmacology 36:2406–2421
Cho SR, Benraiss A, Chmielnicki E, Samdani A, Economides A, Goldman SA (2007) Induction of neostriatal neurogenesis slows disease progression in a transgenic murine model of Huntington disease. J Clin Invest 117:2889–2902
Ciammola A, Sassone J, Cannella M, Calza S, Poletti B, Frati L, Squitieri F, Silani V (2007) Low brain-derived neurotrophic factor (BDNF) levels in serum of Huntington's disease patients. Am J Med Genet B Neuropsychiatr Genet 144B:574–577
Colin E, Zala D, Liot G, Rangone H, Borrell-Pages M, Li XJ, Saudou F, Humbert S (2008) Huntingtin phosphorylation acts as a molecular switch for anterograde/retrograde transport in neurons. EMBO J 27:2124–2134
Como PG, Rubin AJ, O'Brien CF, Lawler K, Hickey C, Rubin AE, Henderson R, McDermott MP, McDermott M, Steinberg K, Shoulson I (1997) A controlled trial of fluoxetine in nondepressed patients with Huntington's disease. Mov Disord 12:397–401
Conforti P, Ramos C, Apostol BL, Simmons DA, Nguyen HP, Riess O, Thompson LM, Zuccato C, Cattaneo E (2008) Blood level of brain-derived neurotrophic factor mRNA is progressively reduced in rodent models of Huntington's disease: restoration by the neuroprotective compound CEP-1347. Mol Cell Neurosci 39:1–7
Conforti P, Mas Monteys A, Zuccato C, Buckley NJ, Davidson B, Cattaneo E (2012) In vivo delivery of DN:REST improves transcriptional changes of REST-regulated genes in HD mice. Gene Ther 20:678–685
Conforti P, Zuccato C, Gaudenzi G, Ieraci A, Camnasio S, Buckley NJ, Mutti C, Cotelli F, Contini A, Cattaneo E (2013) Binding of the repressor complex REST-mSIN3b by small molecules restores neuronal gene transcription in Huntington's disease models. J Neurochem 127:22–35
Conti L, Cattaneo E (2010) Neural stem cell systems: physiological players or in vitro entities? Nat Rev Neurosci 11:176–187
Croll SD, Suri C, Compton DL, Simmons MV, Yancopoulos GD, Lindsay RM, Wiegand SJ, Rudge JS, Scharfman HE (1999) Brain-derived neurotrophic factor transgenic mice exhibit passive avoidance deficits, increased seizure severity and in vitro hyperexcitability in the hippocampus and entorhinal cortex. Neuroscience 93:1491–1506
Cummings DM, Alaghband Y, Hickey MA, Joshi PR, Hong SC, Zhu C, Ando TK, André VM, Cepeda C, Watson JB, Levine MS (2012) A critical window of CAG repeat-length correlates

with phenotype severity in the R6/2 mouse model of Huntington's disease. J Neurophysiol 107:677–691

de Cristofaro T, Affaitati A, Cariello L, Avvedimento EV, Varrone S (1999) The length of polyglutamine tract, its level of expression, the rate of degradation, and the transglutaminase activity influence the formation of intracellular aggregates. Biochem Biophys Res Commun 260:150–158

Dedeoglu A, Kubilus JK, Jeitner TM, Matson SA, Bogdanov M, Kowall NW, Matson WR, Cooper AJ, Ratan RR, Beal MF, Hersch SM, Ferrante RJ (2002) Therapeutic effects of cystamine in a murine model of Huntington's disease. J Neurosci 22:8942–8950

del Toro D, Canals JM, Gines S, Kojima M, Egea G, Alberch J (2006) Mutant huntingtin impairs the post-Golgi trafficking of brain-derived neurotrophic factor but not its Val66Met polymorphism. J Neurosci 26:12748–12757

Di Maria E, Marasco A, Tartari M, Ciotti P, Abbruzzese G, Novelli G, Bellone E, Cattaneo E, Mandich P (2006) No evidence of association between BDNF gene variants and age-at-onset of Huntington's disease. Neurobiol Dis 24:274–279

Diekmann H, Anichtchik O, Fleming A, Futter M, Goldsmith P, Roach A, Rubinsztein DC (2009) Decreased BDNF levels are a major contributor to the embryonic phenotype of huntingtin knockdown zebrafish. J Neurosci 29:1343–1349

DiFiglia M, Sapp E, Chase K, Schwarz C, Meloni A, Young C, Martin E, Vonsattel JP, Carraway R, Reeves SA et al (1995) Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. Neuron 14:1075–1081

Dragatsis I, Levine MS, Zeitlin S (2000) Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. Nat Genet 26:300–306

Dragatsis I, Goldowitz D, Del Mar N, Deng YP, Meade CA, Liu L, Sun Z, Dietrich P, Yue J, Reiner A (2009) CAG repeat lengths $>$ or $=$335 attenuate the phenotype in the R6/2 Huntington's disease transgenic mouse. Neurobiol Dis 33:315–330

Duan W, Lee J, Guo Z, Mattson MP (2001a) Dietary restriction stimulates BDNF production in the brain and thereby protects neurons against excitotoxic injury. J Mol Neurosci 16:1–12

Duan W, Guo Z, Mattson MP (2001b) Brain-derived neurotrophic factor mediates an excitoprotective effect of dietary restriction in mice. J Neurochem 76:619–626

Duan W, Guo Z, Jiang H, Ware M, Li XJ, Mattson MP (2003) Dietary restriction normalizes glucose metabolism and BDNF levels, slows disease progression, and increases survival in huntingtin mutant mice. Proc Natl Acad Sci USA 100:2911–2916

Duan W, Guo Z, Jiang H, Ladenheim B, Xu X, Cadet JL, Mattson MP (2004) Paroxetine retards disease onset and progression in Huntingtin mutant mice. Ann Neurol 55:590–594

Duan W, Peng Q, Masuda N, Ford E, Tryggestad E, Ladenheim B, Zhao M, Cadet JL, Wong J, Ross CA (2008) Sertraline slows disease progression and increases neurogenesis in N171-82Q mouse model of Huntington's disease. Neurobiol Dis 30:312–322

Dubey A, Forster MJ, Lal H, Sohal RS (1996) Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse. Arch Biochem Biophys 333:189–197

Dunah AW, Jeong H, Griffin A, Kim YM, Standaert DG, Hersch SM, Mouradian MM, Young AB, Tanese N, Krainc D (2002) Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science 296:2238–2243

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112:257–269

Emerich DF, Winn SR, Hantraye PM, Peschanski M, Chen EY, Chu Y, McDermott P, Baetge EE, Kordower JH (1997) Protective effect of encapsulated cells producing neurotrophic factor CNTF in a monkey model of Huntington's disease. Nature 386:395–399

Engelender S, Sharp AH, Colomer V, Tokito MK, Lanahan A, Worley P, Holzbaur EL, Ross CA (1997) Huntingtin-associated protein 1 (HAP1) interacts with the p150Glued subunit of dynactin. Hum Mol Genet 6:2205–2212

Falkenberg T, Mohammed AK, Henriksson B, Persson H, Winblad B, Lindefors N (1992) Increased expression of brain-derived neurotrophic factor mRNA in rat hippocampus is associated with improved spatial memory and enriched environment. Neurosci Lett 138:153–156

Ferrer I, Goutan E, Marin C, Rey MJ, Ribalta T (2000) Brain-derived neurotrophic factor in Huntington disease. Brain Res 866:257–261

Fox JH, Barber DS, Singh B, Zucker B, Swindell MK, Norflus F, Buzescu R, Chopra R, Ferrante RJ, Kazantsev A, Hersch SM (2004) Cystamine increases L-cysteine levels in Huntington's disease transgenic mouse brain and in a PC12 model of polyglutamine aggregation. J Neurochem 91:413–422

Frim DM, Uhler TA, Short MP, Ezzedine ZD, Klagsbrun M, Breakefield XO, Isacson O (1993) Effects of biologically delivered NGF, BDNF and bFGF on striatal excitotoxic lesions. Neuroreport 4:367–370

Fujimura H, Altar CA, Chen R, Nakamura T, Nakahashi T, Kambayashi J, Sun B, Tandon NN (2002) Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Thromb Haemost 87:728–734

Fukumoto T, Morinobu S, Okamoto Y, Kagaya A, Yamawaki S (2001) Chronic lithium treatment increases the expression of brain-derived neurotrophic factor in the rat brain. Psychopharmacology (Berl) 158:100–106

Fusco FR, Chen Q, Lamoreaux WJ, Figueredo-Cardenas G, Jiao Y, Coffman JA, Surmeier DJ, Honig MG, Carlock LR, Reiner A (1999) Cellular localization of huntingtin in striatal and cortical neurons in rats: lack of correlation with neuronal vulnerability in Huntington's disease. J Neurosci 19:1189–1202

Fusco FR, Zuccato C, Tartari M, Martorana A, De March Z, Giampa C, Cattaneo E, Bernardi G (2003) Co-localization of brain-derived neurotrophic factor (BDNF) and wild-type huntingtin in normal and quinolinic acid-lesioned rat brain. Eur J Neurosci 18:1093–1102

Gabathuler R (2010) Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. Neurobiol Dis 37:48–57

Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H, Cordelieres FP, De Mey J, MacDonald ME, Lessmann V, Humbert S, Saudou F (2004) Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 118:127–138

Gentile V, Cooper AJ (2004) Transglutaminases – possible drug targets in human diseases. Curr Drug Targets CNS Neurol Disord 3:99–104

Géral C, Angelova A, Lesieur S (2013) From molecular to nanotechnology strategies for delivery of neurotrophins: emphasis on brain-derived neurotrophic factor (BDNF). Pharmaceutics 5:127–167

Gharami K, Xie Y, An JJ, Tonegawa S, Xu B (2008) Brain-derived neurotrophic factor overexpression in the forebrain ameliorates Huntington's disease phenotypes in mice. J Neurochem 105:369–379

Giampa C, DeMarch Z, D'Angelo V, Morello M, Martorana A, Sancesario G, Bernardi G, Fusco FR (2006) Striatal modulation of cAMP-response-element-binding protein (CREB) after excitotoxic lesions: implications with neuronal vulnerability in Huntington's disease. Eur J Neurosci 23:11–20

Giampà C, Montagna E, Dato C, Melone MA, Bernardi G, Fusco FR (2013) Systemic delivery of recombinant brain derived neurotrophic factor (BDNF) in the R6/2 mouse model of Huntington's disease. PLoS One 8(5):e64037

Gibrat C, Cicchetti F (2011) Potential of cystamine and cysteamine in the treatment of neurodegenerative diseases. Prog Neuropsychopharmacol Biol Psychiatry 35:380–389

Gil JM, Mohapel P, Araujo IM, Popovic N, Li JY, Brundin P, Petersen A (2005) Reduced hippocampal neurogenesis in R6/2 transgenic Huntington's disease mice. Neurobiol Dis 20:744–751

Gill SS, Patel NK, Hotton GR, O'Sullivan K, McCarter R, Bunnage M, Brooks DJ, Svendsen CN, Heywood P (2003) Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. Nat Med 9:589–595

Gines S, Seong IS, Fossale E, Ivanova E, Trettel F, Gusella JF, Wheeler VC, Persichetti F, MacDonald ME (2003) Specific progressive cAMP reduction implicates energy deficit in presymptomatic Huntington's disease knock-in mice. Hum Mol Genet 12:497–508

Gines S, Bosch M, Marco S, Gavalda N, Diaz-Hernandez M, Lucas JJ, Canals JM, Alberch J (2006) Reduced expression of the TrkB receptor in Huntington's disease mouse models and in human brain. Eur J Neurosci 23:649–658

Giralt A, Carretón O, Lao-Peregrin C, Martín ED, Alberch J (2011) Conditional BDNF release under pathological conditions improves Huntington's disease pathology by delaying neuronal dysfunction. Mol Neurodegener 6:71

Giralt A, Rodrigo T, Martin ED, Gonzalez JR, Mila M, Cena V, Dierssen M, Canals JM, Alberch J (2009) Brain-derived neurotrophic factor modulates the severity of cognitive alterations induced by mutant huntingtin: involvement of phospholipaseCgamma activity and glutamate receptor expression. Neuroscience 158:1234–1250

Giralt A, Friedman HC, Caneda-Ferron B, Urban N, Moreno E, Rubio N, Blanco J, Peterson A, Canals JM, Alberch J (2010) BDNF regulation under GFAP promoter provides engineered astrocytes as a new approach for long-term protection in Huntington's disease. Gene Ther 17:1294–1308

Gleeson PA, Lock JG, Luke MR, Stow JL (2004) Domains of the TGN: coats, tethers and G proteins. Traffic 5:315–326

Gray M, Shirasaki DI, Cepeda C, Andre VM, Wilburn B, Lu XH, Tao J, Yamazaki I, Li SH, Sun YE, Li XJ, Levine MS, Yang XW (2008) Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. J Neurosci 28:6182–6195

Grote HE, Bull ND, Howard ML, van Dellen A, Blakemore C, Bartlett PF, Hannan AJ (2005) Cognitive disorders and neurogenesis deficits in Huntington's disease mice are rescued by fluoxetine. Eur J Neurosci 22:2081–2088

Gunawardena S, Goldstein LS (2005) Polyglutamine diseases and transport problems: deadly traffic jams on neuronal highways. Arch Neurol 62:46–51

Gunawardena S, Her LS, Brusch RG, Laymon RA, Niesman IR, Gordesky-Gold B, Sintasath L, Bonini NM, Goldstein LS (2003) Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in Drosophila. Neuron 40:25–40

Guo Z, Ersoz A, Butterfield DA, Mattson MP (2000) Beneficial effects of dietary restriction on cerebral cortical synaptic terminals: preservation of glucose and glutamate transport and mitochondrial function after exposure to amyloid beta-peptide, iron, and 3-nitropropionic acid. J Neurochem 75:314–320

Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, Clappier E, Caccavelli L, Delabesse E, Beldjord K, Asnafi V, MacIntyre E, Dal Cortivo L, Radford I, Brousse N, Sigaux F, Moshous D, Hauer J, Borkhardt A, Belohradsky BH, Wintergerst U, Velez MC, Leiva L, Sorensen R, Wulffraat N, Blanche S, Bushman FD, Fischer A, Cavazzana-Calvo M (2008) Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 118:3132–3142

Hansson AC, Sommer WH, Metsis M, Stromberg I, Agnati LF, Fuxe K (2006) Corticosterone actions on the hippocampal brain-derived neurotrophic factor expression are mediated by exon IV promoter. J Neuroendocrinol 18:104–114

Hara D, Fukuchi M, Miyashita T, Tabuchi A, Takasaki I, Naruse Y, Mori N, Kondo T, Tsuda M (2009) Remote control of activity-dependent BDNF gene promoter-I transcription mediated by REST/NRSF. Biochem Biophys Res Commun 384:506–511

Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR (2003) Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci 23:6690–6694

Hathorn T, Snyder-Keller A, Messer A (2011) Nicotinamide improves motor deficits and upregulates PGC-1alpha and BDNF gene expression in a mouse model of Huntington's disease. Neurobiol Dis 41:43–50

HDCRG (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983

Henshall TL, Tucker B, Lumsden AL, Nornes S, Lardelli MT, Richards RI (2009) Selective neuronal requirement for huntingtin in the developing zebrafish. Hum Mol Genet 18:4830–4842

Her LS, Goldstein LS (2008) Enhanced sensitivity of striatal neurons to axonal transport defects induced by mutant huntingtin. J Neurosci 28:13662–13672

Hermel E, Gafni J, Propp SS, Leavitt BR, Wellington CL, Young JE, Hackam AS, Logvinova AV, Peel AL, Chen SF, Hook V, Singaraja R, Krajewski S, Goldsmith PC, Ellerby HM, Hayden MR, Bredesen DE, Ellerby LM (2004) Specific caspase interactions and amplification are involved in selective neuronal vulnerability in Huntington's disease. Cell Death Differ 11:424–438

Hess DC, Borlongan CV (2008) Stem cells and neurological diseases. Cell Prolif 41(Suppl 1):94–114

Hockly E, Cordery PM, Woodman B, Mahal A, van Dellen A, Blakemore C, Lewis CM, Hannan AJ, Bates GP (2002) Environmental enrichment slows disease progression in R6/2 Huntington's disease mice. Ann Neurol 51:235–242

Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, Elliston LA, Hartog C, Goldstein DR, Thu D, Hollingsworth ZR, Collin F, Synek B, Holmans PA, Young AB, Wexler NS, Delorenzi M, Kooperberg C, Augood SJ, Faull RL, Olson JM, Jones L, Luthi-Carter R (2006) Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet 15:965–977

Hodgson JG, Agopyan N, Gutekunst CA, Leavitt BR, LePiane F, Singaraja R, Smith DJ, Bissada N, McCutcheon K, Nasir J, Jamot L, Li XJ, Stevens ME, Rosemond E, Roder JC, Phillips AG, Rubin EM, Hersch SM, Hayden MR (1999) A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. Neuron 23:181–192

Hoffner G, Djian P (2005) Transglutaminase and diseases of the central nervous system. Front Biosci 10:3078–3092

HSG (2003) Dosage effects of riluzole in Huntington's disease: a multicenter placebo-controlled study. Neurology 61:1551–1556

Ientile R, Campisi A, Raciti G, Caccamo D, Curro M, Cannavo G, Li Volti G, Macaione S, Vanella A (2003) Cystamine inhibits transglutaminase and caspase-3 cleavage in glutamate-exposed astroglial cells. J Neurosci Res 74:52–59

Igarashi S, Koide R, Shimohata T, Yamada M, Hayashi Y, Takano H, Date H, Oyake M, Sato T, Sato A, Egawa S, Ikeuchi T, Tanaka H, Nakano R, Tanaka K, Hozumi I, Inuzuka T, Takahashi H, Tsuji S (1998) Suppression of aggregate formation and apoptosis by transglutaminase inhibitors in cells expressing truncated DRPLA protein with an expanded polyglutamine stretch. Nat Genet 18:111–117

Ivkovic S, Ehrlich ME (1999) Expression of the striatal DARPP-32/ARPP-21 phenotype in GABAergic neurons requires neurotrophins in vivo and in vitro. J Neurosci 19:5409–5419

Jiang Y, Wei N, Lu T, Zhu J, Xu G, Liu X (2011) Intranasal brain-derived neurotrophic factor protects brain from ischemic insult via modulating local inflammation in rats. Neuroscience 172:398–405

Johnson R, Buckley NJ (2009) Gene dysregulation in Huntington's disease: REST, microRNAs and beyond. Neuromolecular Med 11:183–199

Johnson R, Zuccato C, Belyaev ND, Guest DJ, Cattaneo E, Buckley NJ (2008) A microRNA-based gene dysregulation pathway in Huntington's disease. Neurobiol Dis 29:438–445

Joyce N, Annett G, Wirthlin L, Olson S, Bauer G, Nolta JA (2010) Mesenchymal stem cells for the treatment of neurodegenerative disease. Regen Med 5:933–946

Karpuj MV, Becher MW, Springer JE, Chabas D, Youssef S, Pedotti R, Mitchell D, Steinman L (2002) Prolonged survival and decreased abnormal movements in transgenic model of Huntington disease, with administration of the transglutaminase inhibitor cystamine. Nat Med 8:143–149

Katoh-Semba R, Asano T, Ueda H, Morishita R, Takeuchi IK, Inaguma Y, Kato K (2002) Riluzole enhances expression of brain-derived neurotrophic factor with consequent proliferation of granule precursor cells in the rat hippocampus. FASEB J 16:1328–1330

Kells AP, Fong DM, Dragunow M, During MJ, Young D, Connor B (2004) AAV-mediated gene delivery of BDNF or GDNF is neuroprotective in a model of Huntington disease. Mol Ther 9:682–688

Kells AP, Henry RA, Connor B (2008) AAV-BDNF mediated attenuation of quinolinic acid-induced neuropathology and motor function impairment. Gene Ther 15:966–977

Keyvani K, Sachser N, Witte OW, Paulus W (2004) Gene expression profiling in the intact and injured brain following environmental enrichment. J Neuropathol Exp Neurol 63:598–609

Kishikawa S, Li JL, Gillis T, Hakky MM, Warby S, Hayden M, MacDonald ME, Myers RH, Gusella JF (2006) Brain-derived neurotrophic factor does not influence age at neurologic onset of Huntington's disease. Neurobiol Dis 24:280–285

Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, Knudsen GM, Aznar S (2011) Blood BDNF concentrations reflect brain-tissue BDNF levels across species. Int J Neuropsychopharmacol 14:347–353

Koibuchi N, Fukuda H, Chin WW (1999) Promoter-specific regulation of the brain-derived neurotropic factor gene by thyroid hormone in the developing rat cerebellum. Endocrinology 140:3955–3961

Landwehrmeyer GB, Dubois B, de Yebenes JG, Kremer B, Gaus W, Kraus PH, Przuntek H, Dib M, Doble A, Fischer W, Ludolph AC (2007) Riluzole in Huntington's disease: a 3-year, randomized controlled study. Ann Neurol 62:262–272

Lazic SE, Grote H, Armstrong RJ, Blakemore C, Hannan AJ, van Dellen A, Barker RA (2004) Decreased hippocampal cell proliferation in R6/1 Huntington's mice. Neuroreport 15:811–813

Lee J, Seroogy KB, Mattson MP (2002) Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice. J Neurochem 80:539–547

Leone S, Mutti C, Kazantsev A, Sturlese M, Moro S, Cattaneo E, Rigamonti D, Contini A (2008) SAR and QSAR study on 2-aminothiazole derivatives, modulators of transcriptional repression in Huntington's disease. Bioorg Med Chem 16:5695–5703

Lesort M, Lee M, Tucholski J, Johnson GV (2003) Cystamine inhibits caspase activity. Implications for the treatment of polyglutamine disorders. J Biol Chem 278:3825–3830

Li XJ, Li SH, Sharp AH, Nucifora FC Jr, Schilling G, Lanahan A, Worley P, Snyder SH, Ross CA (1995) A huntingtin-associated protein enriched in brain with implications for pathology. Nature 378:398–402

Li SH, Gutekunst CA, Hersch SM, Li XJ (1998) Interaction of huntingtin-associated protein with dynactin P150Glued. J Neurosci 18:1261–1269

Li SH, Cheng AL, Zhou H, Lam S, Rao M, Li H, Li XJ (2002) Interaction of Huntington disease protein with transcriptional activator Sp1. Mol Cell Biol 22:1277–1287

Liot G, Zala D, Pla P, Mottet G, Piel M, Saudou F (2013) Mutant Huntingtin alters retrograde transport of TrkB receptors in striatal dendrites. J Neurosci 33:6298–6309

Liu QR, Lu L, Zhu XG, Gong JP, Shaham Y, Uhl GR (2006) Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. Brain Res 1067:1–12

Longo FM, Yang T, Knowles JK, Xie Y, Moore LA, Massa SM (2007) Small molecule neurotrophin receptor ligands: novel strategies for targeting Alzheimer's disease mechanisms. Curr Alzheimer Res 4:503–506

Luthi-Carter R, Hanson SA, Strand AD, Bergstrom DA, Chun W, Peters NL, Woods AM, Chan EY, Kooperberg C, Krainc D, Young AB, Tapscott SJ, Olson JM (2002) Dysregulation of gene expression in the R6/2 model of polyglutamine disease: parallel changes in muscle and brain. Hum Mol Genet 11:1911–1926

Lynch G, Kramar EA, Rex CS, Jia Y, Chappas D, Gall CM, Simmons DA (2007) Brain-derived neurotrophic factor restores synaptic plasticity in a knock-in mouse model of Huntington's disease. J Neurosci 27:4424–4434

Lyons MR, Schwarz CM, West AE (2012) Members of the myocyte enhancer factor 2 transcription factor family differentially regulate Bdnf transcription in response to neuronal depolarization. J Neurosci 32:12780–12785

Mai M, Akkad AD, Wieczorek S, Saft C, Andrich J, Kraus PH, Epplen JT, Arning L (2006) No association between polymorphisms in the BDNF gene and age at onset in Huntington disease. BMC Med Genet 7:79

Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates GP (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87:493–506

Martinez-Serrano A, Bjorklund A (1996) Protection of the neostriatum against excitotoxic damage by neurotrophin-producing, genetically modified neural stem cells. J Neurosci 16:4604–4616

Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, Fan G, Sun YE (2003) DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science 302:890–893

Marullo M, Zuccato C, Mariotti C, Lahiri N, Tabrizi SJ, Di Donato S, Cattaneo E (2010) Expressed Alu repeats as a novel, reliable tool for normalization of real-time quantitative RT-PCR data. Genome Biol 11:R9

Marvanova M, Lakso M, Pirhonen J, Nawa H, Wong G, Castren E (2001) The neuroprotective agent memantine induces brain-derived neurotrophic factor and trkB receptor expression in rat brain. Mol Cell Neurosci 18:247–258

Massa SM, Yang T, Xie Y, Shi J, Bilgen M, Joyce JN, Nehama D, Rajadas J, Longo FM (2010) Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. J Clin Invest 120:1774–1785

Mattson MP, Duan W, Guo Z (2003) Meal size and frequency affect neuronal plasticity and vulnerability to disease: cellular and molecular mechanisms. J Neurochem 84:417–431

McCampbell A, Taylor JP, Taye AA, Robitschek J, Li M, Walcott J, Merry D, Chai Y, Paulson H, Sobue G, Fischbeck KH (2000) CREB-binding protein sequestration by expanded polyglutamine. Hum Mol Genet 9:2197–2202

McGuire JR, Rong J, Li SH, Li XJ (2006) Interaction of Huntingtin-associated protein-1 with kinesin light chain: implications in intracellular trafficking in neurons. J Biol Chem 281:3552–3559

Metsis M, Timmusk T, Arenas E, Persson H (1993) Differential usage of multiple brain-derived neurotrophic factor promoters in the rat brain following neuronal activation. Proc Natl Acad Sci USA 90:8802–8806

Metzger S, Bauer P, Tomiuk J, Laccone F, Didonato S, Gellera C, Mariotti C, Lange HW, Weirich-Schwaiger H, Wenning GK, Seppi K, Melegh B, Havasi V, Baliko L, Wieczorek S, Zaremba J, Hoffman-Zacharska D, Sulek A, Basak AN, Soydan E, Zidovska J, Kebrdlova V, Pandolfo M, Ribai P, Kadasi L, Kvasnicova M, Weber BH, Kreuz F, Dose M, Stuhrmann M, Riess O (2006) Genetic analysis of candidate genes modifying the age-at-onset in Huntington's disease. Hum Genet 120:285–292

Mielcarek M, Benn CL, Franklin SA, Smith DL, Woodman B, Marks PA, Bates GP (2011) SAHA decreases HDAC 2 and 4 levels in vivo and improves molecular phenotypes in the R6/2 mouse model of Huntington's disease. PLoS One 6(11):e27746

Milnerwood AJ, Gladding CM, Pouladi MA, Kaufman AM, Hines RM, Boyd JD, Ko RW, Vasuta OC, Graham RK, Hayden MR, Murphy TH, Raymond LA (2010) Early increase in

extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. Neuron 65:178–190

Mizuta I, Ohta M, Ohta K, Nishimura M, Mizuta E, Kuno S (2001) Riluzole stimulates nerve growth factor, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor synthesis in cultured mouse astrocytes. Neurosci Lett 310:117–120

Momose Y, Murata M, Kobayashi K, Tachikawa M, Nakabayashi Y, Kanazawa I, Toda T (2002) Association studies of multiple candidate genes for Parkinson's disease using single nucleotide polymorphisms. Ann Neurol 51:133–136

Morton AJ, Glynn D, Leavens W, Zheng Z, Faull RL, Skepper JN, Wight JM (2009) Paradoxical delay in the onset of disease caused by super-long CAG repeat expansions in R6/2 mice. Neurobiol Dis 33:331–341

Mostert JP, Koch MW, Heerings M, Heersema DJ, De Keyser J (2008) Therapeutic potential of fluoxetine in neurological disorders. CNS Neurosci Ther 14:153–164

Neves-Pereira M, Mundo E, Muglia P, King N, Macciardi F, Kennedy JL (2002) The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. Am J Hum Genet 71:651–655

Neves-Pereira M, Cheung JK, Pasdar A, Zhang F, Breen G, Yates P, Sinclair M, Crombie C, Walker N, St Clair DM (2005) BDNF gene is a risk factor for schizophrenia in a Scottish population. Mol Psychiatry 10:208–212

Nucifora FC Jr, Sasaki M, Peters MF, Huang H, Cooper JK, Yamada M, Takahashi H, Tsuji S, Troncoso J, Dawson VL, Dawson TM, Ross CA (2001) Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. Science 291:2423–2428

Ochs G, Penn RD, York M, Giess R, Beck M, Tonn J, Haigh J, Malta E, Traub M, Sendtner M, Toyka KV (2000) A phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 1:201–206

Okamoto S, Pouladi MA, Talantova M, Yao D, Xia P, Ehrnhoefer DE, Zaidi R, Clemente A, Kaul M, Graham RK, Zhang D, Vincent Chen HS, Tong G, Hayden MR, Lipton SA (2009) Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. Nat Med 15:1407–1413

Olson SD, Pollock K, Kambal A, Cary W, Mitchell GM, Tempkin J, Stewart H, McGee J, Bauer G, Kim HS, Tempkin T, Wheelock V, Annett G, Dunbar G, Nolta JA (2012) Genetically engineered mesenchymal stem cells as a proposed therapeutic for Huntington's disease. Mol Neurobiol 45:87–98

Ooi L, Wood IC (2007) Chromatin crosstalk in development and disease: lessons from REST. Nat Rev Genet 8:544–554

Orr AL, Li S, Wang CE, Li H, Wang J, Rong J, Xu X, Mastroberardino PG, Greenamyre JT, Li XJ (2008) N-terminal mutant huntingtin associates with mitochondria and impairs mitochondrial trafficking. J Neurosci 28:2783–2792

Pan W, Kastin AJ (1999) Penetration of neurotrophins and cytokines across the blood–brain/blood-spinal cord barrier. Adv Drug Deliv Rev 36:291–298

Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ (1998) Transport of brain-derived neurotrophic factor across the blood–brain barrier. Neuropharmacology 37:1553–1561

Pang TY, Stam NC, Nithianantharajah J, Howard ML, Hannan AJ (2006) Differential effects of voluntary physical exercise on behavioral and brain-derived neurotrophic factor expression deficits in Huntington's disease transgenic mice. Neuroscience 141:569–584

Pardridge WM (2006) Molecular Trojan horses for blood–brain barrier drug delivery. Curr Opin Pharmacol 6:494–500

Pattabiraman PP, Tropea D, Chiaruttini C, Tongiorgi E, Cattaneo A, Domenici L (2005) Neuronal activity regulates the developmental expression and subcellular localization of cortical BDNF mRNA isoforms in vivo. Mol Cell Neurosci 28:556–570

Peng Q, Masuda N, Jiang M, Li Q, Zhao M, Ross CA, Duan W (2008) The antidepressant sertraline improves the phenotype, promotes neurogenesis and increases BDNF levels in the R6/2 Huntington's disease mouse model. Exp Neurol 210:154–163

Perez-Navarro E, Alberch J, Neveu I, Arenas E (1999) Brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4/5 differentially regulate the phenotype and prevent degenerative changes in striatal projection neurons after excitotoxicity in vivo. Neuroscience 91:1257–1264

Perez-Navarro E, Canudas AM, Akerund P, Alberch J, Arenas E (2000) Brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 prevent the death of striatal projection neurons in a rodent model of Huntington's disease. J Neurochem 75:2190–2199

Pham TM, Ickes B, Albeck D, Soderstrom S, Granholm AC, Mohammed AH (1999a) Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year. Neuroscience 94:279–286

Pham TM, Soderstrom S, Winblad B, Mohammed AH (1999b) Effects of environmental enrichment on cognitive function and hippocampal NGF in the non-handled rats. Behav Brain Res 103:63–70

Pineda JR, Pardo R, Zala D, Yu H, Humbert S, Saudou F (2009) Genetic and pharmacological inhibition of calcineurin corrects the BDNF transport defect in Huntington's disease. Mol Brain 2:33

Pouladi MA, Brillaud E, Xie Y, Conforti P, Graham RK, Ehrnhoefer DE, Franciosi S, Zhang W, Poucheret P, Compte E, Maurel JC, Zuccato C, Cattaneo E, Néri C, Hayden MR (2012) NP03, a novel low-dose lithium formulation, is neuroprotective in the YAC128 mouse model of Huntington disease. Neurobiol Dis 48:282–289

Pruunsild P, Kazantseva A, Aid T, Palm K, Timmusk T (2007) Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters. Genomics 90:397–406

Pruunsild P, Sepp M, Orav E, Koppel I, Timmusk T (2011) Identification of cis-elements and transcription factors regulating neuronal activity-dependent transcription of human BDNF gene. J Neurosci 31:3295–3308

Radka SF, Holst PA, Fritsche M, Altar CA (1996) Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay. Brain Res 709:122–301

Rampon C, Tsien JZ (2000) Genetic analysis of learning behavior-induced structural plasticity. Hippocampus 10:605–609

Ranen NG, Lipsey JR, Treisman G, Ross CA (1996) Sertraline in the treatment of severe aggressiveness in Huntington's disease. J Neuropsychiatry Clin Neurosci 8:338–340

Rasmussen P, Brassard P, Adser H, Pedersen MV, Leick L, Hart E, Secher NH, Pedersen BK, Pilegaard H (2009) Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. Exp Physiol 94:1062–1069

Rauskolb S, Zagrebelsky M, Dreznjak A, Deogracias R, Matsumoto T, Wiese S, Erne B, Sendtner M, Schaeren-Wiemers N, Korte M, Barde YA (2010) Global deprivation of brain-derived neurotrophic factor in the CNS reveals an area-specific requirement for dendritic growth. J Neurosci 30:1739–1749

Reiner A, Albin RL, Anderson KD, D'Amato CJ, Penney JB, Young AB (1988) Differential loss of striatal projection neurons in Huntington disease. Proc Natl Acad Sci USA 85:5733–5737

Rigamonti D, Bauer JH, De-Fraja C, Conti L, Sipione S, Sciorati C, Clementi E, Hackam A, Hayden MR, Li Y, Cooper JK, Ross CA, Govoni S, Vincenz C, Cattaneo E (2000) Wild-type huntingtin protects from apoptosis upstream of caspase-3. J Neurosci 20:3705–3713

Rigamonti D, Bolognini D, Mutti C, Zuccato C, Tartari M, Sola F, Valenza M, Kazantsev AG, Cattaneo E (2007) Loss of huntingtin function complemented by small molecules acting as repressor element 1/neuron restrictive silencer element silencer modulators. J Biol Chem 282:24554–24562

Rigamonti D, Mutti C, Zuccato C, Cattaneo E, Contini A (2009) Turning REST/NRSF dysfunction in Huntington's disease into a pharmaceutical target. Curr Pharm Des 15:3958–3967

Rosas HD, Salat DH, Lee SY, Zaleta AK, Pappu V, Fischl B, Greve D, Hevelone N, Hersch SM (2008) Cerebral cortex and the clinical expression of Huntington's disease: complexity and heterogeneity. Brain 131:1057–1068

Rossi F, Cattaneo E (2002) Opinion: neural stem cell therapy for neurological diseases: dreams and reality. Nat Rev Neurosci 3:401–409

Ryu JK, Kim J, Cho SJ, Hatori K, Nagai A, Choi HB, Lee MC, McLarnon JG, Kim SU (2004) Proactive transplantation of human neural stem cells prevents degeneration of striatal neurons in a rat model of Huntington disease. Neurobiol Dis 16:68–77

Salerno KM, Jing X, Diges CM, Cornuet PK, Glorioso JC, Albers KM (2012) Sox11 modulates brain-derived neurotrophic factor expression in an exon promoter-specific manner. J Neurosci Res 90:1011–1019

Sarkar S, Krishna G, Imarisio S, Saiki S, O'Kane CJ, Rubinsztein DC (2008) A rational mechanism for combination treatment of Huntington's disease using lithium and rapamycin. Hum Mol Genet 17:170–178

Saudou F, Finkbeiner S, Devys D, Greenberg ME (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 95:55–66

Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, Kotzuk JA, Slunt HH, Ratovitski T, Cooper JK, Jenkins NA, Copeland NG, Price DL, Ross CA, Borchelt DR (1999) Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. Hum Mol Genet 8:397–407

Schilling G, Savonenko AV, Coonfield ML, Morton JL, Vorovich E, Gale A, Neslon C, Chan N, Eaton M, Fromholt D, Ross CA, Borchelt DR (2004) Environmental, pharmacological, and genetic modulation of the HD phenotype in transgenic mice. Exp Neurol 187:137–149

Schmidtke K, Manner H, Kaufmann R, Schmolck H (2002) Cognitive procedural learning in patients with fronto-striatal lesions. Learn Mem 9:419–429

Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB, Burmeister M (2003) A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. Neuropsychopharmacology 28:397–401

Senatorov VV, Ren M, Kanai H, Wei H, Chuang DM (2004) Short-term lithium treatment promotes neuronal survival and proliferation in rat striatum infused with quinolinic acid, an excitotoxic model of Huntington's disease. Mol Psychiatry 9:371–385

Seppi K, Mueller J, Bodner T, Brandauer E, Benke T, Weirich-Schwaiger H, Poewe W, Wenning GK (2001) Riluzole in Huntington's disease (HD): an open label study with one year follow up. J Neurol 248:866–869

Shieh PB, Hu SC, Bobb K, Timmusk T, Ghosh A (1998) Identification of a signaling pathway involved in calcium regulation of BDNF expression. Neuron 20:727–740

Shimojo M (2008) Huntingtin regulates RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) nuclear trafficking indirectly through a complex with REST/NRSF-interacting LIM domain protein (RILP) and dynactin p150 Glued. J Biol Chem 283:34880–34886

Simmons DA, Rex CS, Palmer L, Pandyarajan V, Fedulov V, Gall CM, Lynch G (2009) Up-regulating BDNF with an ampakine rescues synaptic plasticity and memory in Huntington's disease knockin mice. Proc Natl Acad Sci USA 106:4906–4911

Simmons DA, Mehta RA, Lauterborn JC, Gall CM, Lynch G (2011) Brief ampakine treatments slow the progression of Huntington's disease phenotypes in R6/2 mice. Neurobiol Dis 41:436–444

Simmons DA, Belichenko NP, Yang T, Condon C, Monbureau M, Shamloo M, Jing D, Massa SM, Longo FM (2013) A small molecule TrkB ligand reduces motor impairment and neuropathology in R6/2 and BACHD mouse models of Huntington's disease. J Neurosci 33:18712–18727

Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM, Tsan G, Schaffner S, Kirov G, Jones I, Owen M, Craddock N, DePaulo JR, Lander ES (2002) Family-based association study of

76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. Mol Psychiatry 7:579–593

Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, Oh R, Bissada N, Hossain SM, Yang YZ, Li XJ, Simpson EM, Gutekunst CA, Leavitt BR, Hayden MR (2003) Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet 12:1555–1567

Soldati C, Bithell A, Conforti P, Cattaneo E, Buckley NJ (2011) Rescue of gene expression by modified REST decoy oligonucleotides in a cellular model of Huntington's disease. J Neurochem 116:415–425

Soldati C, Bithell A, Johnston C, Wong KY, Stanton LW, Buckley NJ (2013) Dysregulation of REST-regulated coding and non-coding RNAs in a cellular model of Huntington's disease. J Neurochem 124:418–430

Somoza R, Juri C, Baes M, Wyneken U, Rubio FJ (2010) Intranigral transplantation of epigenetically induced BDNF-secreting human mesenchymal stem cells: implications for cell-based therapies in Parkinson's disease. Biol Blood Marrow Transplant 16:1530–1540

Spires TL, Grote HE, Varshney NK, Cordery PM, van Dellen A, Blakemore C, Hannan AJ (2004) Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism. J Neurosci 24:2270–2276

Squitieri F, Orobello S, Cannella M, Martino T, Romanelli P, Giovacchini G, Frati L, Mansi L, Ciarmiello A (2009) Riluzole protects Huntington disease patients from brain glucose hypometabolism and grey matter volume loss and increases production of neurotrophins. Eur J Nucl Med Mol Imaging 36:1113–1120

Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL, Kazantsev A, Schmidt E, Zhu YZ, Greenwald M, Kurokawa R, Housman DE, Jackson GR, Marsh JL, Thompson LM (2001) Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. Nature 413:739–743

Strand AD, Baquet ZC, Aragaki AK, Holmans P, Yang L, Cleren C, Beal MF, Jones L, Kooperberg C, Olson JM, Jones KR (2007) Expression profiling of Huntington's disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. J Neurosci 27:11758–11768

Sugars KL, Rubinsztein DC (2003) Transcriptional abnormalities in Huntington disease. Trends Genet 19:233–238

Sugars KL, Brown R, Cook LJ, Swartz J, Rubinsztein DC (2004) Decreased cAMP response element-mediated transcription: an early event in exon 1 and full-length cell models of Huntington's disease that contributes to polyglutamine pathogenesis. J Biol Chem 279:4988–4999

Sullivan FR, Bird ED, Alpay M, Cha JH (2001) Remotivation therapy and Huntington's disease. J Neurosci Nurs 33:136–142

Tabuchi A, Nakaoka R, Amano K, Yukimine M, Andoh T, Kuraishi Y, Tsuda M (2000) Differential activation of brain-derived neurotrophic factor gene promoters I and III by Ca2+ signals evoked via L-type voltage-dependent and N-methyl-D-aspartate receptor Ca2+ channels. J Biol Chem 275:17269–17275

Tabuchi A, Sakaya H, Kisukeda T, Fushiki H, Tsuda M (2002) Involvement of an upstream stimulatory factor as well as cAMP-responsive element-binding protein in the activation of brain-derived neurotrophic factor gene promoter I. J Biol Chem 277:35920–35931

Takeuchi Y, Yamamoto H, Miyakawa T, Miyamoto E (2000) Increase of brain-derived neurotrophic factor gene expression in NG108-15 cells by the nuclear isoforms of Ca2+/calmodulin-dependent protein kinase II. J Neurochem 74:1913–1922

Takeuchi Y, Miyamoto E, Fukunaga K (2002) Analysis on the promoter region of exon IV brain-derived neurotrophic factor in NG108-15 cells. J Neurochem 83:67–79

Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME (1998) Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron 20:709–726

Tardito D, Perez J, Tiraboschi E, Musazzi L, Racagni G, Popoli M (2006) Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. Pharmacol Rev 58:115–134

The BSG (1999) A controlled trial of recombinant methionyl human BDNF in ALS: The BDNF Study Group (Phase III). Neurology 52:1427–1433

Timmusk T, Palm K, Metsis M, Reintam T, Paalme V, Saarma M, Persson H (1993) Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron 10:475–489

Timmusk T, Lendahl U, Funakoshi H, Arenas E, Persson H, Metsis M (1995) Identification of brain-derived neurotrophic factor promoter regions mediating tissue-specific, axotomy-, and neuronal activity-induced expression in transgenic mice. J Cell Biol 128:185–199

Timmusk T, Palm K, Lendahl U, Metsis M (1999) Brain-derived neurotrophic factor expression in vivo is under the control of neuron-restrictive silencer element. J Biol Chem 274:1078–1084

Trettel F, Rigamonti D, Hilditch-Maguire P, Wheeler VC, Sharp AH, Persichetti F, Cattaneo E, MacDonald ME (2000) Dominant phenotypes produced by the HD mutation in STHdh(Q111) striatal cells. Hum Mol Genet 9:2799–2809

Trushina E, Dyer RB, Badger JD 2nd, Ure D, Eide L, Tran DD, Vrieze BT, Legendre-Guillemin V, McPherson PS, Mandavilli BS, Van Houten B, Zeitlin S, McNiven M, Aebersold R, Hayden M, Parisi JE, Seeberg E, Dragatsis I, Doyle K, Bender A, Chacko C, McMurray CT (2004) Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. Mol Cell Biol 24:8195–8209

van Dellen A, Blakemore C, Deacon R, York D, Hannan AJ (2000) Delaying the onset of Huntington's in mice. Nature 404:721–722

van Dellen A, Grote HE, Hannan AJ (2005) Gene-environment interactions, neuronal dysfunction and pathological plasticity in Huntington's disease. Clin Exp Pharmacol Physiol 32:1007–1019

Van Raamsdonk JM, Pearson J, Bailey CD, Rogers DA, Johnson GV, Hayden MR, Leavitt BR (2005) Cystamine treatment is neuroprotective in the YAC128 mouse model of Huntington disease. J Neurochem 95:210–220

Ventriglia M, Bocchio Chiavetto L, Benussi L, Binetti G, Zanetti O, Riva MA, Gennarelli M (2002) Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. Mol Psychiatry 7:136–137

Warby SC, Chan EY, Metzler M, Gan L, Singaraja RR, Crocker SF, Robertson HA, Hayden MR (2005) Huntingtin phosphorylation on serine 421 is significantly reduced in the striatum and by polyglutamine expansion in vivo. Hum Mol Genet 14:1569–1577

Wei H, Qin ZH, Senatorov VV, Wei W, Wang Y, Qian Y, Chuang DM (2001) Lithium suppresses excitotoxicity-induced striatal lesions in a rat model of Huntington's disease. Neuroscience 106:603–612

Wheeler VC, Auerbach W, White JK, Srinidhi J, Auerbach A, Ryan A, Duyao MP, Vrbanac V, Weaver M, Gusella JF, Joyner AL, MacDonald ME (1999) Length-dependent gametic CAG repeat instability in the Huntington's disease knock-in mouse. Hum Mol Genet 8:115–122

Wood NI, Morton AJ (2003) Chronic lithium chloride treatment has variable effects on motor behaviour and survival of mice transgenic for the Huntington's disease mutation. Brain Res Bull 61:375–383

Wu LL, Fan Y, Li S, Li XJ, Zhou XF (2010) Huntingtin-associated protein-1 interacts with pro-brain-derived neurotrophic factor and mediates its transport and release. J Biol Chem 285:5614–5623

Xie Y, Hayden MR, Xu B (2010) BDNF overexpression in the forebrain rescues Huntington's disease phenotypes in YAC128 mice. J Neurosci 30:14708–14718

Yanez-Munoz RJ, Balaggan KS, MacNeil A, Howe SJ, Schmidt M, Smith AJ, Buch P, MacLaren RE, Anderson PN, Barker SE, Duran Y, Bartholomae C, von Kalle C, Heckenlively JR, Kinnon C, Ali RR, Thrasher AJ (2006) Effective gene therapy with nonintegrating lentiviral vectors. Nat Med 12:348–353

Young D, Lawlor PA, Leone P, Dragunow M, During MJ (1999) Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. Nat Med 5:448–453

Yu ZF, Mattson MP (1999) Dietary restriction and 2-deoxyglucose administration reduce focal ischemic brain damage and improve behavioral outcome: evidence for a preconditioning mechanism. J Neurosci Res 57:830–839

Yu ZX, Li SH, Nguyen HP, Li XJ (2002) Huntingtin inclusions do not deplete polyglutamine-containing transcription factors in HD mice. Hum Mol Genet 11:905–914

Zafra F, Hengerer B, Leibrock J, Thoenen H, Lindholm D (1990) Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. EMBO J 9:3545–3550

Zainelli GM, Dudek NL, Ross CA, Kim SY, Muma NA (2005) Mutant huntingtin protein: a substrate for transglutaminase 1, 2, and 3. J Neuropathol Exp Neurol 64:58–65

Zajac MS, Pang TY, Wong N, Weinrich B, Leang LS, Craig JM, Saffery R, Hannan AJ (2010) Wheel running and environmental enrichment differentially modify exon-specific BDNF expression in the hippocampus of wild-type and pre-motor symptomatic male and female Huntington's disease mice. Hippocampus 20:621–636

Zala D, Colin E, Rangone H, Liot G, Humbert S, Saudou F (2008) Phosphorylation of mutant huntingtin at S421 restores anterograde and retrograde transport in neurons. Hum Mol Genet 17:3837–3846

Zhang Y, Li M, Drozda M, Chen M, Ren S, Mejia Sanchez RO, Leavitt BR, Cattaneo E, Ferrante RJ, Hayden MR, Friedlander RM (2003) Depletion of wild-type huntingtin in mouse models of neurologic diseases. J Neurochem 87:101–106

Zoladz JA, Pilc A (2010) The effect of physical activity on the brain derived neurotrophic factor: from animal to human studies. J Physiol Pharmacol 61:533–541

Zuccato C, Cattaneo E (2007) Role of brain-derived neurotrophic factor in Huntington's disease. Prog Neurobiol 81:294–330

Zuccato C, Cattaneo E (2009) Brain-derived neurotrophic factor in neurodegenerative diseases. Nat Rev Neurol 5:311–322

Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR, Timmusk T, Sipione S, Cattaneo E (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science 293:493–498

Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, Cataudella T, Leavitt BR, Hayden MR, Timmusk T, Rigamonti D, Cattaneo E (2003) Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nat Genet 35:76–83

Zuccato C, Liber D, Ramos C, Tarditi A, Rigamonti D, Tartari M, Valenza M, Cattaneo E (2005) Progressive loss of BDNF in a mouse model of Huntington's disease and rescue by BDNF delivery. Pharmacol Res 52:133–139

Zuccato C, Belyaev N, Conforti P, Ooi L, Tartari M, Papadimou E, MacDonald M, Fossale E, Zeitlin S, Buckley N, Cattaneo E (2007) Widespread disruption of repressor element-1 silencing transcription factor/neuron-restrictive silencer factor occupancy at its target genes in Huntington's disease. J Neurosci 27:6972–6983

Zuccato C, Marullo M, Conforti P, MacDonald ME, Tartari M, Cattaneo E (2008) Systematic assessment of BDNF and its receptor levels in human cortices affected by Huntington's disease. Brain Pathol 18:225–238

Zuccato C, Valenza M, Cattaneo E (2010) Molecular mechanisms and potential therapeutical targets in Huntington's disease. Physiol Rev 90:905–981

Zuccato C, Marullo M, Vitali B, Tarditi A, Mariotti C, Valenza M, Lahiri N, Wild EJ, Sassone J, Ciammola A, Bachoud-Lèvi AC, Tabrizi SJ, Di Donato S, Cattaneo E (2011) Brain-derived neurotrophic factor in patients with Huntington's disease. PLoS One 6(8):e22966

Motoneuron Disease

M. Sendtner

Abstract

Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) represent the two major forms of motoneuron disease. In both forms of disease, spinal and bulbar motoneurons become dysfunctional and degenerate. In ALS, cortical motoneurons are also affected, which contributes to the clinical phenotype. The gene defects for most familial forms of ALS and SMA have been discovered and they point to a broad spectrum of disease mechanisms, including defects in RNA processing, pathological protein aggregation, altered apoptotic signaling, and disturbed energy metabolism. Despite the fact that lack of neurotrophic factors or their corresponding receptors are not found as genetic cause of motoneuron disease, signaling pathways initiated by neurotrophic factors for motoneuron survival, axon growth, presynaptic development, and synaptic function are disturbed in ALS and SMA. Better understanding of how neurotrophic factors and downstream signaling pathways interfere with these disease mechanisms could help to develop new therapies for motoneuron disease and other neurodegenerative disorders.

Keywords

Amyotrophic lateral sclerosis • Superoxide dismutase • Mitochondria • Endoplasmic reticulum stress • RNA metabolism • Survival motoneuron protein • Frontotemporal lobar degeneration • TDP-43 gene • Ciliary neurotrophic factor • Cardiotropin-1 • Leukemia-inhibitory factor • Neurotrophins • BDNF • Glial-derived neurotrophic factor • Neurodegeneration

M. Sendtner (✉)
Institute for Clinical Neurobiology, University of Würzburg, Versbacherstr. 5, 97078 Würzburg, Germany
e-mail: Sendtner_M@ukw.de

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_15,

1 Introduction

During development of higher vertebrates, many types of neurons are generated in excess, and about half of the newly generated neurons undergo cell death. Spinal and bulbar motoneurons have been a central focus of research to understand the underlying mechanisms. These neurons become postmitotic at early stages of development; they grow out axons and make functional contacts with skeletal muscle, before this phase of physiological cell death occurs. Pioneering work by Viktor Hamburger and Rita Levi-Montalcini has shown that limiting amounts of survival factors from target tissue play a central regulatory role in this context, and this observation has been the basis for the discovery of neurotrophic factors in the twentieth century. It has long been suspected that dysregulation of neurotrophic signaling could also underlie the degeneration of motoneurons in amyotrophic lateral sclerosis and spinal muscular atrophy, the two major forms of human motoneuron disease. During the last three decades, gene defects underlying monogenetic forms of these disorders have been identified, and none of these gene defects point to a lack of neurotrophic factors or defective receptors as cause of motoneuron disease. Motoneurons that are isolated from mouse embryos and cultured in vitro also depend on neurotrophic factors for their survival. These cultures are a useful tool for studying signaling pathways for motoneuron survival, but also signaling for axon growth, presynaptic differentiation, dendrite growth and stabilization of neurites, and synaptic contacts. These parameters represent targets of motoneuron disease processes, and recent research has indicated that neurotrophic factor signaling also interferes with these mechanisms. The analysis of disease processes and mechanisms how neurotrophic factors interfere could help to develop new therapeutic strategies for amyotrophic laterals sclerosis and spinal muscular atrophy.

2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder causing dysfunction and death of lower motoneurons in the spinal cord and brain stem and of upper motoneurons in the motor cortex (Kiernan et al. 2011). This results in progressive dysfunction of neuromuscular innervation that normally causes death due to respiratory failure. The incidence of ALS is approximately 2 per 100,000 individuals worldwide, the mean age of onset is 55–60 years, and the disease more commonly affects men than women. Average survival from symptom onset is approximately 3 years, although some forms of the disease also have a much slower disease course, allowing patients to survive for several decades (Wood-Allum and Shaw 2010). Traditionally, ALS has been considered as a pure motor disorder. However, it has become increasingly evident that also other types of neurons are affected and that some forms of ALS are coupled with prefrontal dementia or with degeneration of dopaminergic neurons. Even in patients in which dysfunction of the motor systems predominates the clinical phenotype, histopathological alterations

are also found in many types of neurons, including hippocampus and basal ganglia (Al-Sarraj et al. 2011). Therefore, ALS is now regarded as a more general neurodegenerative disorder in which the motor phenotype predominates the clinical picture.

In more than 90 % of all cases, ALS appears sporadic. Only 5–10 % of all cases are familial. In these cases, an autosomal-dominant inheritance is predominant (Andersen and Al-Chalabi 2011). Even this subset of familial ALS is highly heterogeneous on a genetic basis, and the so far identified genetic defects underlying familial forms of ALS point to multifactorial pathogenic processes (Table 1).

3 fALS with Mutations in the SOD-I Gene

The first identified gene defect which accounts for about 10–20 % of familial ALS were point mutations in the gene for Cu2+/Zn2+-dependent superoxide dismutase (SOD-I) (NM 000454) (Rosen et al. 1993). So far, more than 50 different mutations in this gene have been identified. Clinically, there seems to be no clear correlation between disease onset or severity with specific mutation in the SOD-I gene (Andersen and Al-Chalabi 2011), and the clinical appearance of ALS does not differ from the majority of sporadic cases in this disease. Some types of mutations in the SOD-I gene are prone to cause a severe and rapid course of disease, in particular the A4V mutation; other forms, i.e., E21G, G37R, D40A, G93C, I104F, L144S, and I151C, are associated with survival times that can exceed 10 years (reviewed in Ferraiuolo et al. 2011). Not all of these mutations are associated with loss of enzymatic function. In particular the G37A mutation which has been intensively studied in transgenic mouse models, but also the A90V or D91A mutations, does not primarily affect enzyme activity (reviewed in Al-Chalabi et al. 2012).

The SOD-I protein plays a central role in detoxifying superoxide radicals from the cell and preventing the generation of hydroxyl radicals that react with a great variety of molecules, in particular polyunsaturated fatty acids in cell membranes, but also proteins and nucleic acids. Cerebrospinal fluid (CSF), but also serum and urine, show elevated markers of free radical damage in patients with ALS (Smith et al. 1998; Simpson et al. 2004), and this does not only apply to patients and mouse models of fALS with mutations in the SOD-I gene. Interestingly, knockout of the SOD-I gene in mice does not result in motoneuron disease (Reaume et al. 1996), whereas transgenic overexpression of mutant SOD-I in general causes rapid and severe forms of the disease (Gurney et al. 1994). Mouse models overexpressing A4V or G93A mutant SOD-I molecules have most commonly been used in studies on the pathophysiological consequences of these mutations. The observation that SOD-I gene knockout does not lead to motoneuron disease in mice and that many of the mutations identified in patients with fALS do not show reduced enzyme activity points to pathogenic mechanisms other than loss of enzyme activity. These include actions of the mutant protein in cell types that appear not primarily affected such as microglia and astrocytes. Mutant SOD-I in microglia increases NADPH

Table 1 Established ALS associated genes

Genetic subtype	Gene	Chromosomal locus	Mouse	Onset/ inheritance	Phenotype	References
ALS1	*SOD1*	21q22.1	Chr 16	Adult/AD	Cognitive impairment (rare)	Rosen et al. (1993)
ALS2	*Als2* *Alsin*	2q33.2	Chr 1	Juvenile/AR		Hadano et al. (2001)
ALS3	*Unknown*	18q21		Adult/AD		Hand et al. (2002)
ALS4	*SETX* *Senataxin*	9q34	Chr 2	Juvenile/AD	Cerebellar ataxia, motor neuropathy	Chen et al. (2004)
ALS5	*SPG11* *Spatacsin, Spastic paraplegia 11*	15q21.1	Chr 2	Juvenile/AR		Orlacchio et al. (2010)
ALS6	*FUS* *Fused in sarcoma*	16p11.2	Chr 7	Adult/AD	Frontotemporal dementia (FTD), Parkinsonism	Vance et al. (2009), Kwiatkowski et al. (2009)
ALS7	*Unknown*	20p13		Adult/AD		Sapp et al. (2003)
ALS8	*VAPB (vesicle associated membrane protein) associated protein and C*	20q13.3	Chr 2	Adult/AD		Nishimura et al. (2004)
ALS9	*ANG* *Angiogenin ribonuclease, RNAseA family 5*	14q11.2	Chr14	Adult/AD	Parkinsonism	Greenway et al. (2006) Rayaprolu et al. (2012)
ALS10	*TARDBP* *TAR DNA-binding protein*	1p36.2	Chr4	Adult/AD	FTD, Parkinsonism	Sreedharan et al. (2008), Kabashi et al. (2008)
ALS11	*FIG4* *SAC domain containing lipid phosphatase*	6q21	Chr10	Adult/AD	Charcot–Marie–Tooth disease 4J	Chow et al. (2009)
ALS12	*OPTN* *Optineurin*	10p13	Chr 2	Adult/AD and AR		Maruyama et al. (2010)
ALS13	*ATXN2* *Ataxin 2*	12q24	Chr 5	Adult/AD		Elden et al. (2010)

Associated or putatively associated with ALS						
	C9Orf72 *Chromosome 9 open reading frame 72*	9p21.2	Chr 4	Adult/AD, sporadic	Also in FTD, common	Renton et al. (2011), DeJesus-Hernandez et al. (2011)
ALS14	*VCP* *Valosin containing protein*	9p13.3	Chr 4	Adult/AD	Rare, described in one family, FTD, Paget disease, inclusion body myopathy	Johnson et al. (2010)
ALS15	*UBQLN2* *Ubiquilin 2*	Xp11.23–p11.1	Chr X	Juvenile and Adult/x linked	Rare, described in one family	Deng et al. (2011)
ALS16	*SIGMAR1* *Non opioid intracellular receptor 1*	8p13.3	Chr 4	Juvenile/AR	Rare, described in few families	Luty et al. (2010)
ALS17	*CHMP2B* *Charged multivesicular body protein 2B*	3p11	Chr 16	Adult/AD sporadic	Also in FTD, rare	Parkinson et al. (2006)
ALS18	*PFN1* *Profilin 1*	17p13.3	Chr 11	Adult/AD sporadic	Rare	Wu et al. (2012)

oxidase-mediated superoxide production (Harraz et al. 2008), resulting in prolonged ROS production. Mutant SOD-I protein has been found to interact with chromogranins (Urushitani et al. 2006) and by this way appears to be released from astrocytes and interneurons. Thus, extracellular mutant SOD-I can activate microglia and possibly also promote direct toxic effects on motoneurons. In chimeric mice expressing mutant SOD-I in astrocytes, motoneurons degenerate and show ALS pathology (Clement et al. 2003). Furthermore, mice in which the mutant allele encoding SOD-I G37R is deleted from motoneurons using Cre-lox technology (Boillee et al. 2006) show delayed disease onset, but no alteration in the disease course once first symptoms have become apparent. Also in cell culture, toxic effects of astrocytes expressing mutant SOD-I have been demonstrated when these cells are cocultured with primary motoneurons from embryonic mouse (Nagai et al. 2007) or human stem cell-derived motoneurons (Di Giorgio et al. 2008). This toxic effect of mutant astrocytes has been shown to involve the deregulation of glutamate receptor 2 (GluR2) in motoneurons, as a consequence of mutant SOD-I expression in astrocytes (Van Damme et al. 2007). Taken together, these findings indicate that non-neuronal cells expressing mutant forms of SOD-I exert toxic effects on motoneurons and contribute to disease.

Both in patients and mouse models with mutations in the SOD-I gene, protein inclusions are found in motoneurons and other types of neurons, but these inclusions differ from inclusions found in the vast majority of sporadic ALS patients and other forms of familial ALS because they do not include the TDP43 protein (Maekawa et al. 2009). Some of the protein aggregates that include the mutant SOD-I protein are associated with mitochondria and thus could contribute to mitochondrial dysfunction. The mutated SOD-I protein seems to aggregate in vacuoles in the mitochondrial intermembrane space (Wong et al. 1995), and this finding together with other reports showing interaction of mutant SOD-I protein with bcl-2 (Pasinelli et al. 2004) gives further support to the idea that the mutant SOD-I protein causes mitochondrial dysfunction and defective respiratory chain activity. These findings also correlate with observations that the calcium buffering capacity is impaired in mitochondria isolated from neural tissues of SOD-I mutant mice (Damiano et al. 2006; Grosskreutz et al. 2010). The altered calcium homeostasis caused by this defect might also correlate with susceptibility for glutamate-mediated excitotoxicity and ER stress, which is also observed in motoneurons from SOD-I mutant mouse models.

Mitochondrial dysfunction and morphological alterations such as vacuolation occur early during presymptomatic disease stages in mouse models, and they are thought to contribute to defective axonal transport of mitochondria (De Vos et al. 2007). It is thought that a reduction in the mitochondrial content in axon terminals could be a major mechanism for dying-back axonopathy, which is generally observed in ALS.

Because of the multitude of pathological mechanisms that apparently contribute to motoneuron disease in SOD-I mutant mouse and cell culture models, no clear conclusions can be drawn on which signaling pathways downstream of neurotrophic factor receptors are most important to interfere with pathomechanisms

in this form of familial ALS. Overexpression of mutant SOD-I in motoneurons causes cytoplasmic aggregation of the enzyme, and neurons with such aggregates subsequently undergo apoptotic cell death (Durham et al. 1997). When SOD-I G93A mice are crossed with bcl-2-overexpressing mice, onset of disease is delayed (Kostic et al. 1997), suggesting that inhibition of classical pathways for apoptotic cell death interferes with the disease. However, bcl-2 overexpression cannot prevent disease. Similar observations were made when SOD-I G93A mutated mice were crossed with mice overexpressing a dominant-negative ICE isoform, which prevents caspases from activation of cell death pathways (Friedlander et al. 1997). Thus, interference with classical apoptotic signaling pathways apparently has some impact, but the effects are not sufficient to prevent disease in this mouse model of familial motoneuron disease. This indicates that interference with motoneuron cell death programs is not sufficient for therapy and that additional pathomechanisms, ranging from dysfunction of neuromuscular transmission to destabilization of axonal processes and depletion of dendritic synaptic inputs, also need to be targeted, at least in this form of motoneuron disease.

4 Inclusions and Altered RNA Metabolism in ALS: TDP-43, FUS, C9orf72

Alterations in RNA metabolism have been suspected to contribute to ALS pathophysiology for a long time. Since the discovery that fragile X syndrome is caused by altered expression of FMR-1, a member of a large family of RNP proteins that are involved in RNA binding and transport (Ashley et al. 1993), the potential impact of defective RNA processing to neurodegeneration has increasingly become a focus of interest. However, the mechanisms how altered RNA metabolism could contribute to the pathomechanisms of ALS have only become more concrete in the last few years. An important finding in this context was the identification of the TAR DNA-binding protein-43 (TDP-43) protein as a major component of ubiquitin-positive cellular inclusions (Neumann et al. 2006). These inclusions have the appearance of threads, skeins, or compact bodies and are located in nuclei and soma of neurons, including proximal dendrites and axons. TDP-43-positive inclusions have also been found in other neurodegenerative disorders such as FTLD (Buratti and Baralle 2008). They have also been described in *postmortem* brain of patients with Huntington's disease (Schwab et al. 2008), Alzheimer's disease, and dementia with Lewy body inclusions (Higashi et al. 2007).

The cellular function of TDP-43 is not fully understood. TDP-43 is a 414-amino-acid protein of the hnRNP family (Krecic and Swanson 1999), with two RNA recognition motifs (RRM1 and RRM2) and a C-terminal glycine-rich domain, and thus resembles many other RNA-binding proteins such as fused in sarcoma (FUS) and hnRNP-R, the latter having been previously identified to interact with the survival motoneuron (Smn) protein (Rossoll et al. 2002), the central protein of the Smn complex that is deficient in spinal muscular atrophy.

After the identification of TDP-43 protein as the major component of proteinaceous inclusions in sporadic ALS and other neurodegenerative disorders, mutations in the gene encoding for TDP-43 were found in some ALS patients (Rutherford et al. 2008; Mackenzie et al. 2010), and also in patients with frontotemporal lobar degeneration (FTLD, new nomenclature FTLD-TDP). About 4 % of patients with familial ALS and 1.5 % of patients with sporadic ALS have mutations in the TDP-43 gene. All of the so far identified mutations in familial ALS are autosomal dominant, and most of them encode for a missense mutation within the C-terminal domain which encodes the glycine-rich domain (Pesiridis et al. 2009), a part of the protein that is important for interaction with other proteins and molecules, but it does not directly interact with RNA. Based on these data, it has been suggested that the mutations in the C-terminus are sufficient to induce neurodegeneration.

The TDP-43-positive inclusions are strongly ubiquitinylated and phosphorylated. It is not the full-length TDP-43 which is found in inclusions, but a truncated 20–25 kDa C-terminal fragment (Pesiridis et al. 2011). So far it is still unclear whether the associated loss of TDP-43 function, due to the cleavage of the N-terminus, which contains the RRM1 domain, is causative for neurodegeneration or a loss of function due to depletion of TDP-43 from the nucleus and other cytoplasmic regions where TDP-43 functions are necessary for neuronal maintenance or gain of function by the cleaved fragments.

TDP-43 interactions with RNA have been studied in detail, and these studies have revealed functions of TDP-43 in several aspects of RNA metabolism. The RRM1 domain of this protein binds to single-stranded RNA (Buratti and Baralle 2001), in particular to regions containing UG repeats. These UG regions are contained in many RNAs, and this fits with the observation that several thousand different RNA species can interact with the protein (Tollervey et al. 2011; Polymenidou and Lagier-Tourenne 2011), in particular intronic regions, but also 3′untranslated regions (UTRs), and also noncoding RNAs. The association of TDP-43 with intronic sequences and its predominant nuclear localization implicates TDP-43 in early steps of pre-mRNA processing in the nucleus. These functions could include transcriptional regulation, alternative splicing (Buratti et al. 2001), and in particular micro-RNA (miRNA) processing. Defects in miRNA malfunction have been shown to result in motoneuron disease (Haramati et al. 2010). Thus, TDP-43 (Buratti and Baralle 2010a) and other members of the hnRNP protein family (Pascale and Govoni 2012) like FUS (Morlando et al. 2012) could also contribute to motoneuron maintenance by regulating miRNA function.

Whether motoneuron injury is caused by loss of such nuclear function of TDP-43 is still not fully understood. Among the mRNAs that interact with TDP-43 are those encoding for FUS, VCP (Sephton et al. 2011), and TDP-43 mRNA itself (Buratti and Baralle 2010b). TDP-43 regulates processing of its own transcript by interaction of the protein with the 3′UTR of TDP-43 mRNA, leading to alternative splicing of the 3′UTR. As a consequence, high levels of TDP-43 cause reduced translation of TDP-43 mRNA. These functions involve interaction of TDP-43 with other proteins that bind to the C-terminus, in particular other members of the hnRNP family. These include hnRNP-A2/B1 (Buratti et al. 2005), hnRNP-

A1, hnRNP-A3, and hnRNP-C1-C2 (Ling et al. 2010), but possibly also other members of this large family (Freibaum et al. 2010).

Blocking TDP-43 expression with antisense oligonucleotides in adult mouse brain alters the expression levels of more than 600 mRNA transcripts and changes splicing of more than 900 transcripts (Polymenidou and Lagier-Tourenne 2011), including such transcripts which are also relevant for motoneuron function, such as choline acetyl transferase, and transcripts for other RNA-binding proteins for which mutations lead to degeneration, such as FUS and progranulin.

Several animal models have been developed to study TDP-43 dysfunction, including mouse models in which the gene is knocked out or overexpressed in mutant form, but also *Drosophila*, zebrafish, and *C. elegans* models (reviewed in Wegorzewska and Baloh 2011). Many models available so far cannot give final hints about the pathomechanistic contributions of mutant TDP-43 to motoneuron disease, because those overexpressing TDP-43 might also lead to dysregulation of RNAs simply as a consequence of the TDP-43 overexpression itself, and a good example for this problem is the processing of the TDP-43 mRNA by TDP-43. Another potential problem is the interaction of the TDP-43 with intronic sequences, which are highly different between species, and interaction of TDP-43 with intronic sequences in human genes might not be found in mouse, fish, and *C. elegans* models, because the intronic sequences differ more than coding sequences between these species. Moreover, by overexpression of mutant TDP-43, the RRM1 domains are mostly preserved, allowing functions in pre-mRNA processing that either resemble the physiological function or alter these functions in a dominant-negative manner, for example, when the C-terminal mutations lead to altered distribution of the protein. Thus, the normal function of TDP-43 in regulating expression of mRNA levels of cyclin-dependent kinase 6 (Ayala et al. 2008), histone deacetylase 6 (Fiesel et al. 2010, 2011), low molecular weight neurofilament (Strong et al. 2007), or other transcripts with essential functions in motoneurons could contribute to the pathophysiology and generate additional pathological features in these mouse models that do not necessarily exist in humans with mutant TDP-43.

So far, the mechanisms that lead to altered subcellular distribution of TDP-43 and translocation between the nucleus and the cytoplasm are not fully understood. Different types of cell stress lead to TDP-43 redistribution from the nucleus to the cytoplasm (Moisse et al. 2009a, b), and the protein is then found within stress granules (Freibaum et al. 2010; Dewey et al. 2011; Kiebler and Bassell 2006), which are thought to stabilize mRNAs and prevent translation under these specific cellular conditions (Kiebler and Bassell 2006). This function seems to be central for understanding the role of TDP-43 in motoneuron disease. After axotomy, TDP-43 translocates to cytosolic compartments, and this translocation seems to be functionally connected with caspase-3 activation (Moisse et al. 2009a). Also after oxidative insult, TDP-43 is recruited to stress granules (Colombrita et al. 2009). There are also reports indicating that TDP-43 could interact with SOD-I and 14-3-3 proteins in the cytosol and thus modulate the stability of mRNAs such as the neurofilament-L chain mRNA (Volkening et al. 2009). Moreover, stress granule dynamics seems to be influenced by TDP-43 (Dewey et al. 2011; McDonald et al. 2011) under

conditions such as oxidative stress or sorbitol-induced osmotic stress. It is still unclear at which stage of disease such stress granules occur and whether TDP-43 inclusion in stress granules is a consequence of other pathophysiological mechanisms, whether such stress granules are fully reversible, or whether such stress granules can give rise to insoluble proteinaceous aggregates. As a common observation made under different types of cell stress, TDP-43 seems to be redistributed from the nucleus to the cytosol. Sporadic ALS patients with slow progression of the disease have been reported to exhibit a relatively low number of TDP-43 inclusions (Nishihira et al. 2009), and this points to a correlation between the number of these aggregates in motoneurons and severity of disease. Thus, TDP-43 aggregates apparently do not protect neurons from degeneration. It remains to be shown whether these TDP-43 aggregates are toxic and contribute to the neurodegenerative process.

The TDP-43 protein also interacts with another RNA-binding protein named fused in sarcoma (FUS) (Zinszner et al. 1994) or translocated in liposarcoma (TLS) (Freibaum et al. 2010). TDP-43 and FUS are related (Drepper et al. 2011) and both are members of the hnRNP protein family with two RRM (RRM1 and RRM2) motifs. This protein is also involved in transcriptional regulation and mRNA processing. Mutations in the FUS genes are found in 4 % of fALS cases (Kwiatkowski et al. 2009) and only rarely (probably less than 1 %) in sporadic ALS cases (Ferraiuolo et al. 2011; Mackenzie et al. 2010). Similar to TDP-43, most mutations associated with ALS are found in the C-terminal regions containing the glycine-rich domain. Some of these mutations seem to disrupt a nuclear translocation signaling, thus leading to cytoplasmic accumulation of the FUS protein within cytoplasmic granules (Ito et al. 2011; Dormann et al. 2010). Alternatively, these mutations in the C-terminus could also disturb protein interaction in particular with other members of the hnRNP family, so that the altered subcellular distribution of the FUS protein in these ALS patients could also be caused by such defects. Similar to TDP-43, it is still not resolved whether loss of a physiological function of FUS due to instability of the protein, cellular misdistribution, or decreased stability causes motoneuron degeneration or a toxic gain of function.

Gene knockout mice have been generated that lack FUS gene function (Kuroda et al. 2000). These mice show male sterility and increased sensitivity to ionizing radiation, but no phenotype that could help to understand the role of this protein in neurons and in neurodegeneration.

The last major gap in the identification of gene defects responsible for familial ALS was closed by two independent groups identifying a hexanucleotide (GGGGCC) repeat expansion in the first intron of the C9ORF72 gene on human chromosome 5 as a frequent genetic cause of ALS (Renton et al. 2011; DeJesus-Hernandez et al. 2011). These mutations, which are associated with both frontotemporal dementia and ALS, cause disease with high penetrance with autosomal-dominant inheritance. There are first indications that this pathophysiology also influences RNA metabolism and that the expanded pre-mRNA also binds to members of the hnRNP family, in particular hnRNP-A3 (Mori et al. 2013a). The repeat domain forms a G-quadruplex structure in the corresponding mRNA (Fratta

et al. 2012), exactly the same as those found in specific mRNAs that are highly sorted in neurons such as the mRNA for PSD95 and CaMKIIa (Subramanian et al. 2011; Drepper and Sendtner 2011). It is possibly that this structure encoded by mutant C9orf72 transcripts disturbs transport and sorting of mRNAs from the nucleus to the cytoplasm and subsequently into axons and dendrites. As an alternative disease mechanism, the formation of proteinaceous aggregates has been suggested. The (GGGGCC) repeat expansion seems to be translated, and the corresponding protein products are found as poly-(Gly-Ala) or poly-(Gly-Pro) or poly-(Gly-Arg) dipeptide repeat proteins in nuclei and the cytoplasm of neuronal cells (Ash et al. 2013; Mori et al. 2013b). These are presumably generated by novel translation initiation sites allowing the expanded GGGGCC repeat to be translated into proteins. Whether altered RNA metabolism by interaction of the corresponding mRNAs with hnRNP proteins or the formation of a quadruplex structure is the primary pathomechanism, or the formation of protein aggregates, and how these mechanisms relate to TDP-43 and FUS pathomechanisms is currently unknown.

Besides TDP-43, FUS, and C9orf72, two other proteins play a role in neurodegenerative disorders like FTLD and in ALS: progranulin and sortilin: Reduced progranulin levels and activity are thought to be of broad relevance for these diseases (Hu et al. 2010). Recently, sortilin was identified as a key progranulin-binding partner on the surface of cortical neurons. In the stressed nervous system, progranulin is not expressed in neurons, but in nearby glial cells. Sortilin rapidly internalizes progranulin to lysosomes. Mice that do not express Sortilin exhibit high levels of extracellular progranulin. Importantly, mice with a progranulin deficiency similar to that seen in FTLD were fully normalized with regard to progranulin levels when sortilin was deleted. These findings implicate sortilin-mediated progranulin endocytosis in FTLD and ALS pathophysiology and identify sortilin binding as a potential therapeutic site to alter progranulin pathology. Sortilin is also a co-receptor for the p75 neurotrophin receptor (P75NTR) and modifies a broad spectrum of actions through this receptor. In addition, sortilin is also involved in subcellular transport of complexes including BACE and other membrane proteins relevant for APP processing. Therefore, the interaction of p75NTR with sortilin could be a modifier for proganulin actions and thus modify disease mechanisms in those forms of motoneuron disease that suffer from altered progranulin metabolism.

5 Spinal Muscular Atrophy

Spinal muscular atrophy is the most common form of motoneuron disease in children and young adults (Hausmanowa-Petrusewicz 1978; Crawford and Pardo 1996). In contrast to amyotrophic lateral sclerosis, more than 90 % of all cases of this disease are caused by homozygous deletion or in rare cases mutation of the *SMN1* gene on human chromosome 5. In contrast to most forms of familial ALS, this form of motoneuron disease is autosomal recessive and represents one of the rare cases where loss of function of a specific gene and the corresponding protein is responsible for the disease. The Smn protein is a central component of a complex

that is necessary for assembly of spliceosomal snRNP particles (Pellizzoni 2007) and the regeneration of such particles in the nucleus. This so-called Smn complex has been characterized in much detail, the interaction partners of Smn called gemins have been identified, and the structural basis of the interaction and of the function of these components in the assembly of snRNP particles investigated. However, the Smn protein is also localized in axons (Rossoll et al. 2002) and axon terminals of motoneurons that are very distant from the cell body in which the assembly of spliceosomal snRNP particles normally occurs. This has led to the conclusions that, in addition to its role in the assembly of snRNP particles, the Smn protein could serve an additional function in RNA metabolism in axons and axon terminals (Sendtner 2001; Burghes and Beattie 2009).

In contrast to the human genome which contains two copies of *SMN* called *SMN1* and *SMN2*, both of which are expressed, the mouse genome only contains one copy of the Smn gene. Conventional gene knockout of *Smn* in the mouse is embryonic lethal at early developmental stages (Schrank et al. 1997), before blastocysts form. This is consistent with an essential role of the Smn protein in the assembly of spliceosomes: Abolishing pre-mRNA splicing and nuclear processing is considered not to be compatible with life. Interestingly, when the *SMN2* gene, which is still present in patients with this disease, is overexpressed on a Smn knockout background in mice (Monani et al. 2000), these mice develop to term and then show typical signs of the disease. The *SMN1* and *SMN2* genes differ only by five nucleotide exchanges (Wirth 2000), two of them within exons. A translationally silent cytosine to thymidine exchange at position 6 of exon 7 is responsible for the skipping of exon 7 in the majority of transcripts from the *SMN2* gene. It has been shown that this mutation abolishes an exonic splice enhancer site (Cartegni and Krainer 2002) and generates a new exonic splicing silencer domain (Kashima and Manley 2003) for the last coding exon of the *SMN2* gene. Therefore, at least 80 % of the resulting SMN protein from *SMN2* transcripts lack the C-terminal 16 amino acids which are replaced by four amino acids encoded by exon 8 sequences. As a consequence, the corresponding protein is unstable (Cho and Dreyfuss 2010), the truncated SMN protein with altered C-terminus cannot self-associate, and thus it is less active in forming SMN complexes and probably also less active in a putative axonal function.

A large variety of animal models has been generated in which the consequences of Smn deficiency have been investigated (reviewed in Burghes and Beattie 2009). In all of these organisms, complete loss of Smn is lethal, and the time point of lethality depends on the levels of maternal Smn protein. For example, this explains why death in Smn-deficient *Drosophila melanogaster* (Chan et al. 2003) occurs later during development than in early mouse embryos. Interestingly, expression of a high number of *SMN2* gene copies in *Smn*−/− mice completely reverses the phenotype (Monani et al. 2000). Such mice appear healthy, indicating that high expression of SMN2 can fully restore function.

Overexpression of Smn via the prion promoter only in the nervous system in mice with low Smn expression in non-neuronal tissues also has a major effect on survival of these animals (Gavrilina et al. 2008). Thus, low levels of functional Smn

proteins produced from at least two copies of *SMN2* gene are sufficient for normal function in most organs and cell types. Apparently, motoneurons need more SMN protein than other types of neurons and non-neuronal cell types, and this could explain why the disease expresses itself as a relatively pure motoneuron disease. Interestingly, efforts to restore Smn expression in muscle in mouse models with reduced Smn expression had much less effect than restoring Smn in neurons (Gavrilina et al. 2008). This correlates with observations that isolated motoneurons from *Smn−/−SMN2tg* mice already show a clear dysmorphic phenotype in cell culture (Rossoll et al. 2003). Survival of Smn-deficient motoneurons in cell culture is normal: No difference in neuronal numbers is observed in the presence or absence of neurotrophic factors when *Smn−/−SMN2tg* and control motoneurons are compared in culture. However, axon growth is altered (Rossoll et al. 2003). Within the first 3 days in culture, these motoneurons show normal axon growth, but further extension of the axons between day 3 and 7 is severely disturbed (Jablonka et al. 2007). Axonal growth cones are smaller, and a specific lack of actin protein is observed in axon terminals. This correlates with the finding that actin mRNA levels are highly reduced in axons of Smn-deficient motoneurons (Rossoll et al. 2003).

The Smn protein itself does not interact with specific mRNAs such as the β-actin mRNA. However, the Smn protein does not only bind to components of the classical Smn complex (Gubitz et al. 2004), but also with other proteins of the hnRNP family, in particular hnRNP-R (Rossoll et al. 2002; Mourelatos et al. 2001). There are also reports that the Smn protein interacts with TDP-43 (Wang et al. 2002) and also with the FUS protein (Yamazaki et al. 2012). However, in the case of TDP-43 and FUS, it is still not fully resolved whether these interactions occur directly in postnatal motoneurons, or whether the Smn interacts with hnRNP complexes containing TDP-43, FUS, and other members of the hnRNP family. Studies aimed at identifying TDP-43-binding partners in nuclear and cytosolic extracts point to the fact that the TDP-43 and FUS proteins are normally present in large protein complexes involving several members of the hnRNP family (Ling et al. 2010; Freibaum et al. 2010). Therefore, it is possible that Smn does not directly interact with each of these proteins, but with different preference to individual members of the hnRNP family. This needs further experimental analyses.

Smn and hnRNP-R proteins are co-localized in axons of motoneurons (Rossoll et al. 2002). The hnRNP-R protein is capable of directly interacting with the β-actin mRNA, and this interaction is reduced when the Smn-binding domain of hnRNP-R is deleted (Rossoll et al. 2003). The consequences of reduced interaction of Smn with hnRNP-R are not known. However, the observation that Smn-deficient motoneurons show reduced β-actin translocation into axons indicates that the Smn protein could play a role for axonal translocation of this and probably also other mRNAs into axons. Knockdown of hnRNP-R in isolated motoneurons or in zebrafish embryos (Glinka et al. 2010) leads to a similar phenotype as Smn deficiency. This points to an involvement of Smn in the formation and function of hnRNP complexes for axonal translocation of specific mRNAs. Whether Smn plays an essential role in the assembly of hnRNP complexes in the nucleus, in

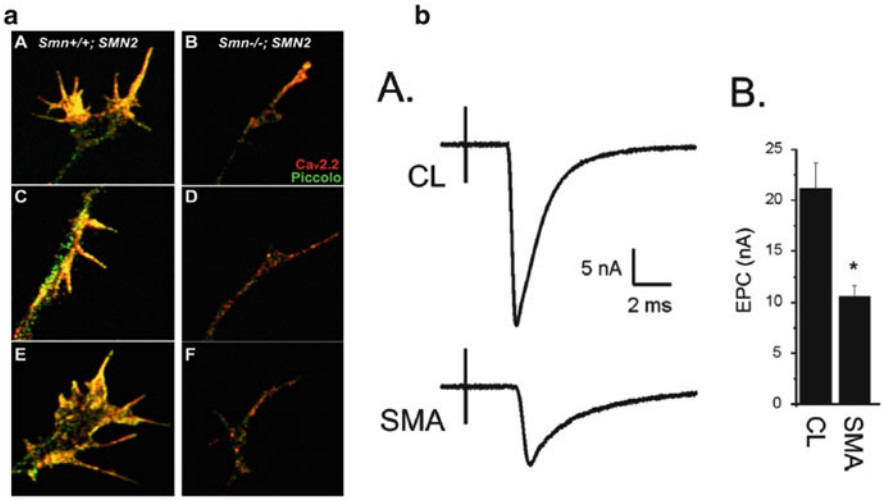

Fig. 1 Axonal defects in Smn-deficient motoneurons. (**a**) *Smn−/−SMN2*tg motoneurons show defects in formation of presynaptic structures. They lack the accumulation of voltage-gated calcium channels (Cav2.2) in the tip of axonal growth cones where active zones form. This is also reflected by reduced colocalization with other proteins of the active zone, such as piccolo (*green*). Reproduced from Jablonka et al. (2007) (**b**) Diminished neuromuscular endplate currents (EPC) in tibialis anterior muscle of postnatal Smn-deficient (SMA) mice. The deficit in neurotransmission is caused by a deficit in release of synaptic vesicles. *CL* control; $*P < 0.01$. Reproduced from Kong et al. (2009)

nuclear exports of such mRNA transport complexes, and in the translocation of these protein/mRNA complexes to axons, and whether it also has a role in regulating the translation of these mRNAs in the axon terminals, remains to be determined.

Reduced axon growth has also been observed in zebrafish embryos in which Smn has been knocked down by Morpholino technologies (McWhorter et al. 2003). Axons are shorter and many of them are truncated or branched, so that they do not reach their physiological target muscles. There is no evidence that axon growth is reduced in Smn-deficient mouse models in vivo (McGovern et al. 2008). However, motor axons grow out very early during embryonic development, and reduced speed of axon elongation could be compensated in vivo, so that even motoneurons with reduced axon growth rates in cell culture reach their target and make synaptic connections. Smn-deficient motoneurons in cell culture show altered growth behavior on laminin-β2/merosin (Jablonka et al. 2007). Wild-type motoneurons normally exhibit reduced axon growth on the synapse-specific form of laminin, but Smn-deficient motoneurons do not. This is due to altered distribution of $Ca_V2.2$ voltage-gated Ca^{2+} channels in axon terminals of Smn-deficient motoneurons (Fig. 1). The altered distribution of these voltage-gated Ca^{2+} channels correlates with altered excitability and altered Ca^{2+} influx after the initiation of action potentials in the cell body of Smn-deficient motoneurons (Jablonka et al. 2007).

Only a small proportion of action potential-like depolarizations in isolated Smn-deficient motoneurons leads to Ca^{2+} transients in axon terminals of motoneurons from the SMA mouse model. These alterations predict defects in presynaptic function and neurotransmission at the neuromuscular endplates. Indeed, such defects are also observed in mouse models. Reduced folds at neuromuscular junctions have been observed in Smn-deficient mouse models during postnatal development, and neurotransmission at the synapses is also altered (Kong et al. 2009; Torres-Benito et al. 2012; Ruiz et al. 2010) (Fig. 1).

Additional defects affecting excitability and neurotransmission have been discovered in Smn-deficient mouse and *Drosophila* models. In Smn-deficient mice, synaptic input to spinal motoneurons is reduced (Mentis et al. 2011), and the majority of the proprioceptive sensory afferents that normally make direct synaptic contact with spinal motoneurons are defective. It is still not clear whether this sensory defect is a consequence of altered excitablilty of motoneurons or reflects a primary defect in sensory neurons (Gogliotti et al. 2012). Such alterations in sensory afferent have also been observed in fly models. Smn deficiency in *Drosophila melanogaster* leads to aberrant splicing of stasimon in cholinergic sensory neurons and interneurons (Imlach et al. 2012; Lotti et al. 2012), due to severely impaired U12 splicing in Smn-deficient neurons, including neural cell types other than motoneurons. This leads to decreased excitation of motoneurons and thus possibly to malfunction and degeneration.

Taken together, the cellular basis of spinal muscular atrophy is complex. Smn deficiency on the one side could lead to altered splicing of gene products that are important for the function of neurons that project to motoneurons and are necessary for giving them excitatory signals. Furthermore, Smn deficiency in motoneurons could impair axon growth and presynaptic differentiation, resulting in impaired neurotransmission at neuromuscular endplates. In any case, therapy has to focus on restoration of Smn function, and this could be through increasing full-length Smn expression in neurons, i.e., through strategies that improve the inclusion of exon 7-encoded domains from the SMN2 gene, or in increasing promoter activity for the SMN2 gene with the aim to increase overall transcript levels of the Smn mRNA (Sendtner 2010). In addition, strategies to restore physiological innervation of motoneurons, motoneuron excitability, and neurotransmission at neuromuscular endplates appear essential. Such strategies could go beyond increasing the levels of functional Smn protein in motoneurons. Evidence for this has been given by depleting PTEN in Smn-deficient motoneurons in cell culture and in vivo in mouse models. PTEN depletion leads to a normalization of axon elongation, increases axonal growth cone size, and restores excitability of Smn-deficient motoneurons (Ning et al. 2010). These changes are associated with increased pAKT and p70S6 levels in Smn-deficient motor axons. This treatment also restores actin protein levels in axonal growth cones of Smn-deficient motoneurons. In vivo, the injection of siPTEN constructs in limb muscles of Smn-deficient motoneurons increases motoneuron survival (Ning et al. 2010). The hypothesis that defective actin cytoskeleton in axon terminals contributes to disease is also supported by genetic evidence in humans. Plastin-3, a protein that stabilizes filamentous actin, has

been shown as modifier of SMA in patients (Oprea et al. 2008). This genetic observation in patients has recently been confirmed by a corresponding mouse model (Ackermann et al. 2013), and this opens perspectives for therapeutic strategies that stabilize the actin cytoskeleton in presynaptic compartments of neuromuscular endplates as another target for therapy development.

6 Developmental Motoneuron Cell Death

Neuronal cell death is often considered as a pathological feature, disregarding that many neurons undergo cell death during normal development. Although some observations on this phenomenon go back to the early twentieth century, it was the work of Viktor Hamburger (1934, 1975) and other pioneer researchers who discovered the principles and physiological meaning of this phenomenon. Spinal motoneurons played a central role in this discovery process. Viktor Hamburger and his colleagues showed that developmental motoneuron cell death is guided by influences provided from target tissue. Removal of limb buds in developing chick embryos enhances massively developmental motoneuron cell death and transplantation of an additional limb reduces the number of dying motoneurons. This kind of plasticity does not only allow the individual organism to react to deviations from genetically determined developmental programs, it also allows plasticity to generate an individual architecture of the nervous system in response to environmental cues, and thus might have contributed during evolution to the generation of a highly complex nervous system in higher vertebrates. On the other hand, the complex nature of such regulatory mechanisms also implies vulnerability and any disturbance of the regulatory processes theoretically could lead to pathological losses of neurons and neuronal function. Since the cloning of BDNF (Leibrock et al. 1989) and CNTF (Stockli et al. 1989) in 1989, a broad variety of neurotrophic factors were identified that can support motoneuron survival. At least three neurotrophins, brain derived neurotrophic factor, neurotrophin-4, and neurotrophin-3, but not NGF, support motoneuron survival. The CNTF/LIF family, which mediates pro-survival actions through a cytokine receptor involving LIFR-β and gp130, also includes several members, besides CNTF leukemia-inhibitory factor (LIF), cardiotrophin-1 (CT-1) (Pennica et al. 1996), and cardiotrophin-1-like cytokine (CLC) (Elson et al. 2000).

Survival of cultured motoneurons is also supported by members of the glial-derived neurotrophic factor (GDNF) gene families. Factors supporting motoneuron survival include GDNF (Henderson et al. 1994), neurturin (Klein et al. 1997), persephin (Milbrandt et al. 1998), and artemin (Baloh et al. 1998), and these molecules mediate their survival effects through C-Ret-tyrosine kinase and specific α-receptors. Motoneurons are also supported by insulin-like growth factor 1 and 2 (Arakawa et al. 1990). In cultures of isolated embryonic chick spinal motoneurons, the survival-promoting effect of IGF is relatively low. However, when IGFs are combined with other neurotrophic factors such as CNTF, this leads to supra-additive survival effects, indicating that neurotrophic factors

potentate each other. The relatively low survival effect of IGF-1 on chick embryonic motoneurons could be due to cell culture conditions that include serum in culture medium with inhibitory insulin-like growth factor-binding proteins. Not very much is known on how insulin-like growth factor-binding proteins modulate the action of IGFs on motoneuron survival during development and in the adult.

Also other types of pluripotent growth factors support motoneuron survival such as members of the FGF family (Arakawa et al. 1990; Hughes et al. 1993a), members of the vascular endothelial growth factor (VEGF) family (Poesen et al. 2008; Azzouz et al. 2004; Carmeliet and Storkebaum 2002), or hepatocyte growth factor family (Yamamoto et al. 1997). HGF is a heterodimer protein with similarities to plasminogen. However, it lacks the enzymatic activity of plasminogen (Weidner et al. 1991). Interestingly, only lumbar motoneurons from 5-day-old chick embryos survive with HGF, but not motoneurons from thoracic or cervical spinal cord (Novak et al. 2000). In developing chick embryos, the c-met tyrosine kinase is expressed in lumbar but not in thoracic motoneurons between embryonic day 5 and 10 during the period of physiological motoneuron cell death. Additional experiments have shown that the expression of c-met in lumbar motoneurons seems to be regulated by target tissue-derived factors other than HGF. This was concluded from experiments showing that the massive cell death of motoneurons in the lumbar spinal cord after limb removal cannot be rescued by HGF treatment because the receptor was downregulated by target deprivation. HGF thus represents another neurotrophic factor that influences survival of only specific subpopulations of motoneurons and needs cooperation with other signals in order to exert a survival-promoting effect. Together with the observation that IGF acts in a supra-additive way with other factors on cultured motoneurons, this supports the conclusion that motoneuron survival during development is regulated by a complex orchestra of many factors that play together in supporting survival, presynaptic differentiation, and maturation of neuromuscular endplates, regulating preservation and stabilization of axons and by this way also the long-term functionality of these cells in the nervous system.

Such interactions are also observed experimentally after lesion in peripheral nerves. For example, when the facial nerve is transected in newborn rats, individual application of CNTF (Sendtner et al. 1990) or BDNF (Sendtner et al. 1992a) supports survival, but does not prevent atrophy of motoneuron cell bodies. Atrophy is significantly reduced when both factors are applied (Gravel et al. 1997). Not all of these factors that support survival of isolated embryonic motoneurons are also expressed in developing skeletal muscle. For example, CNTF is not expressed in muscle. The high expression found in adult mice is confined to myelinating Schwann cells, and expression of this factor only starts in the postnatal period in rodents when the period of physiological cell death is over. Similarly, only low quantities of BDNF are found in skeletal muscle (Hughes et al. 1993b). Levels of BDNF expression are much higher in Schwann cells after nerve lesion (Meyer et al. 1992). Gene knockout experiments have been performed and it has been shown that depletion of BDNF and/or NT-4 does not increase developmental cell death of motoneurons (Liu et al. 1995). The same is true in animal models lacking

CNTF and/or LIF (Holtmann et al. 2005). Only in mouse models in which GDNF is depleted (Oppenheim et al. 2000) or cardiotrophin-1 (Oppenheim et al. 2001), subsets of motoneurons are lost during this physiological cell death period. Also these genetic data point to a collaboration of several neurotrophic factors in developmental maintenance and regulation of survival during the period of physiological cell death.

These data also show that Schwann cells play a role in motoneuron maintenance. Mice in which Schwann cell-derived CNTF and LIF are eliminated show progressive loss of motoneurons and of motoneuron functions, which correlates with loss of muscle strength in adult mice (Holtmann et al. 2005). Similarly, mice deficient for erb-B3, the receptor for glial growth factor (GGF), which exhibit severely disturbed development of Schwann cells, show as a consequence significant reduction (79 %) in motoneurons (Riethmacher et al. 1997). Thus, Schwann cells apparently do not only play a role as source of survival and maintenance factor in the adult peripheral nervous system, but apparently also during development. Developing Schwann cells either play a role in helping motoneurons to contact skeletal muscle and to become functionally active, which then leads to upregulation of neurotrophic support from skeletal muscle, or alternatively, they could provide trophic support in addition to that of motoneurons, and only those motoneurons that receive sufficient signals from developing Schwann cells and muscles are supported, and those that do not receive the support are eliminated. When limb buds are completely removed from chick embryos, motoneuron survival is severely impaired (Oppenheim 1985). Similar observation is made when only skeletal muscle is destroyed (Grieshammer et al. 1998), indicating that the remaining Schwann cells are not sufficient to support survival, and therefore, additional support from muscle is necessary.

7 Interactions of Neurotrophic Signaling with Pathomechanisms of Motoneuron Disease

The identification of underlying gene defects for most of the familial forms of spinal muscular atrophy and amyotrophic lateral sclerosis has pointed to a large variety of disease mechanisms. There are two major groups of pathomechanisms that have emerged: On the one side, dysfunctional RNA processing in spinal muscular atrophy and familial forms with mutations in TDP-43, FUS, and abnormal protein aggregates and dysfunctional signaling pathways for mitochondrial metabolisms due to mutations in the SOD-I genes and potentially also the c9Orf72 gene. These two groups of pathomechanisms do not exclude each other: TDP-43 C-terminal fragments are a major component of inclusions in most cases of ALS, including the majority of sporadic ALS. Axonal swellings containing protein aggregates and dysmorphic mitochondria are commonly found in motoneurons and also in mouse models of motoneuron disease such as pmn mutant mice (Bommel et al. 2002; Selvaraj et al. 2012) (Fig. 2). None of the gene products named above are directly connected to neurotrophic factors or their receptors. Therefore,

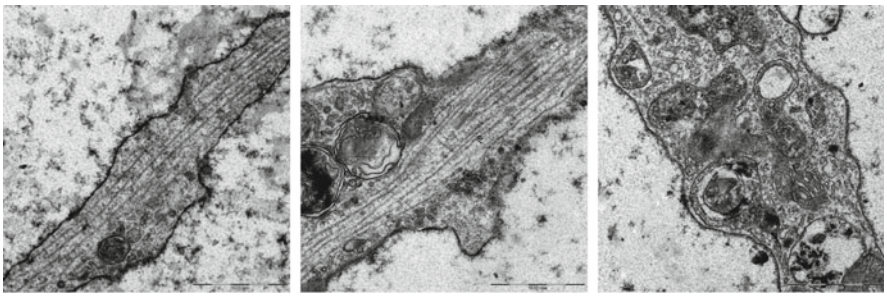

Fig. 2 Axonal swellings in isolated motoneurons from progressive motor neuropathy (pmn) mutant mice. Based on a mutation in the *TBCE* gene (Bommel et al. 2002) motoneurons develop axonal swellings containing protein aggregates and dysmorphic mitochondria. *Scale bar*: 1,000 nm

deficiency of individual neurotrophic factors that lead to enhanced developmental cell death, i.e., in the case of CT-1, GDNF, or HGF, or progressive postnatal motoneuron loss after depletion of CNTF, LIF, or IGF-1, apparently does not seem to be a primary cause of motoneuron disease, at least on a genetic level. Nevertheless, the signaling pathways exerted downstream from receptor for neurotrophic factors for motoneuron survival, for axon maintenance and regeneration, and for presynaptic function and stabilization of neuromuscular endplates apparently seem to be disturbed are not fully functional in motoneuron disease, and several possibilities exist that need to be considered.

First, neurotrophic factors could play a role in compensating for neurodegeneration of spinal motoneurons by promoting sprouting. The central role of neurotrophic factors in axonal and terminal sprouting has been known for a long time (Caroni 1997). Indeed, in a mouse model of a mild form of spinal muscular atrophy, *Smn*+/− mice that exhibit a reduction of Smn protein by only 50 % and thus resemble mild forms of spinal muscular atrophy in humans do not show an overt phenotype (Simon et al. 2010). Nevertheless, there is progressive loss of motoneurons that reaches more than 50 % at an age of 1 year in this mouse model. For comparison, *Smn*−/−*SMN2tg* mice, the mouse model for the severe form of SMA type I only exhibits a loss of about 20 % above control (Monani et al. 2000) when mice are terminally sick and completely paralyzed early after birth. This indicates that loss of motor function does not necessarily correlate with the loss of motoneurons, in particular not in cases with slowly progressive forms of motoneuron disease that allow remaining motoneurons to sprout and compensate for lost neurons by reinnervating denervated skeletal muscle fibers. Indeed, massive sprouting occurs in *Smn*+/− mice and this explains the lack of any loss of muscle strength in these mice. Electrophysiological analysis shows an increase of motor units by a factor of at least 2, and morphological analysis provides evidence for massive sprouting, including terminal sprouting and axonal sprouting to reinnervate neuromuscular endplates in different muscle groups. This type of sprouting depends

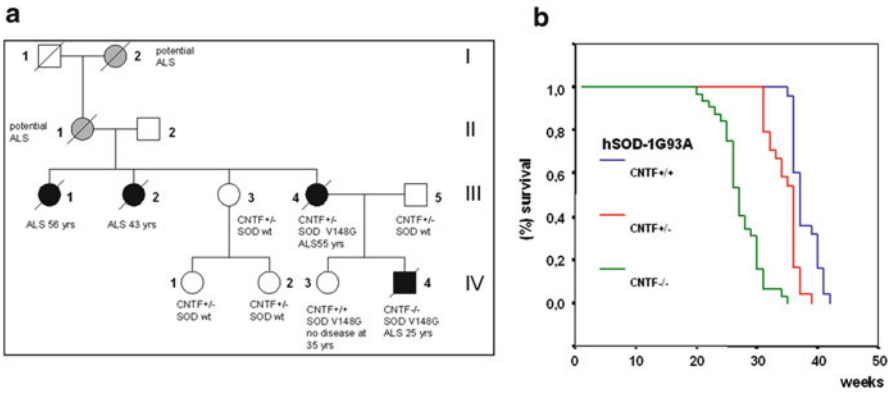

Fig. 3 Modifier effect of *CNTF* in a family with fALS patients (SOD V148G) and in SOD G93A tg mice: (**a**) A family with autosomal-dominant ALS with SOD-I V148G mutation showed highly variable disease onset, ranging from 25 to 56 years. Search for candidate modifier gene defects revealed a homozygous *CNTF* null mutation in the patient with early onset at 25 years. (**b**) Depletion of CNTF from SOD G93A mice confirms that CNTF deficiency leads to earlier disease onset in mice. Reproduced from (Giess et al. 2002)

on neurotrophic factors provided from myelinating Schwann cells, in particular CNTF. When these *Smn*+/− mice are crossbred with CNTF-deficient mice, sprouting does not occur, and the compensatory increase in motor unit size detected by electromyographical analysis is also not found. Thus, neurotrophic signaling could help to compensate for loss of motoneuron function over prolonged periods, and it is possible that this contributes to the observation that most forms of ALS only become apparent at higher age.

This is also suggested by experiments when SOD-1 G93A mice are crossbred with CNTF-deficient mice (Giess et al. 2002) (Fig. 3). When CNTF is lacking, disease onset occurs earlier, providing evidence that this and probably also other factors contribute to plasticity that helps animal models or individuals with SOD-I gene defects to maintain motor function before disease finally becomes apparent. Also in patients with ALS, the presence or absence of CNTF seems to play a role. Due to an abundant polymorphism in the splice acceptor site of exon 2 of the CNTF gene, about 2 % of the population worldwide is homozygous *CNTF* deficient and express only a truncated CNTF protein without function. Average disease onset in such patients is at least 10 years earlier, and in one family with an SOD-I mutation, the additional homozygous deletion of CNTF leads to very early disease onset, whereas other members of the same family with the same SOD-I mutation develop the disease only 20 years later (Giess et al. 2002).

The question is open as to which parameters determine the time point when compensation is lost and disease becomes apparent. In SOD-I mutant mice, depletion of synaptic vesicles in presynaptic motor terminals at neuromuscular junctions precedes the loss of presynaptic branches (Pun et al. 2006) and the progressive degeneration of the motoneurons. Interestingly, when CNTF was injected, the

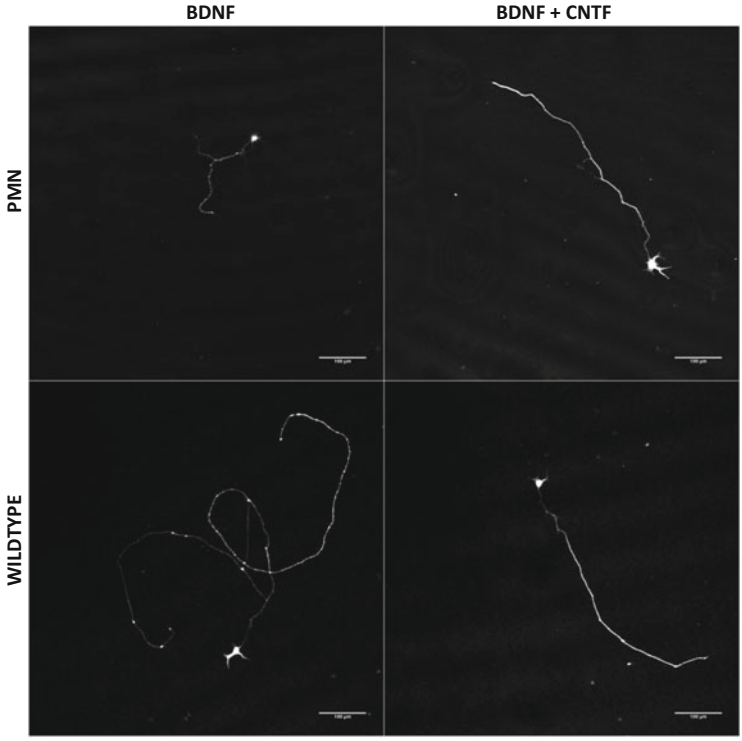

Fig. 4 CNTF rescues defective axon elongation in pmn mutant motoneurons (Bommel et al. 2002; Selvaraj et al. 2012; Sendtner et al. 1992b): Wild-type and pmn mutant motoneurons were cultured for 5 days in the presence of BDNF or BDNF+CNTF and stained with alpha-tubulin. Pmn mutant motoneurons cultured with BDNF have shorter axons when compared to wild-type controls. CNTF application restores axon elongation in pmn mutant motoneurons. *Scale bar*: 100 μm

depletion of synaptic vesicles and pruning of nerve terminals are delayed. Even more interestingly, the injection of the neurotrophic factor GDNF was without effect in this context. This is interesting insofar as both CNTF and GDNF are potent survival factors for developing motoneurons, but apparently, they seem to differ with respect to their function in maintaining nerve terminals.

Such differences between different groups of neurotrophic factors have also been observed in other mouse models of motoneuron disease. For example, in pmn mutant mice, which develop a motoneuron disease on the basis of a gene defect in tubulin-specific chaperone-E gene (Bommel et al. 2002), CNTF can delay disease onset and prolong survival (Sendtner et al. 1992b), whereas treatment with GDNF (Sagot et al. 1996a) or BDNF cannot. The mutation in the TBCE gene leads to instability of microtubules that correspond to defective axon growth in isolated pmn mutant motoneurons in cell culture (Fig. 4).

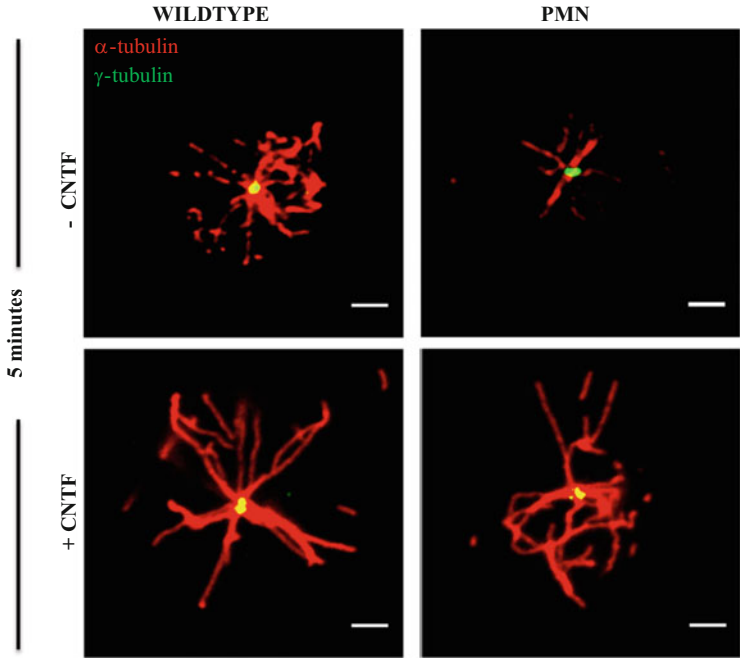

Fig. 5 CNTF promotes microtubule polymerization: Primary motoneurons isolated from wild-type and *pmn* mutant embryos were treated with nocodazole for 6 h to depolymerize the microtubule network. Nocodazole was washed out and microtubule regrowth was analyzed at 5 min after CNTF application. Polymerized microtubules were labeled with antibodies against α-tubulin (*red*) and microtubule organization center was labeled with antibodies against γ-tubulin (*green*). Number of microtubules and length of microtubules formed in *pmn* mutant motoneurons were significantly less when compared to wild-type motoneurons. Application of CNTF increased the number of microtubules and length of microtubules formed in *pmn* mutant motoneurons (Selvaraj et al. 2012). *Scale bar*: 2 μm

Survival of pmn mutant motoneurons is primarily not affected, but axons are shorter and exhibit swellings that contain dysmorphic filaments and accumulations of mitochondria (Fig. 2). Microtubule stability is altered in pmn mutant motoneurons (Selvaraj et al. 2012): There is an increase of tyrosinated highly dynamic microtubules, and this correlates with reduced axonal transport of mitochondria. Interestingly, CNTF, but not GDNF or BDNF, can rescue this axonal phenotype. The CNTF effect is mediated by the activation of STAT-3, which exerts a local, non-transcriptional function in the axon via interaction with Stathmin, a microtubule-destabilizing protein. Destabilization of microtubules in cultured motoneurons shows that the capacity to regrow microtubules is highly reduced in isolated motoneurons from this mouse model of motoneuron disease and that CNTF treatment can help pmn mutant motoneurons to regrow stable microtubules (Fig. 5). Similarly, Stathmin knockdown also rescues the phenotype. Stabilization of microtubules with Taxol has a similar effect. Treatment of pmn mutant mice with CNTF delays disease onset (Sendtner et al. 1992b), but transgenic overexpression of bcl-2 (Sagot et al. 1996b) or

treatment with GDNF only rescues cell bodies without any effect on axons, and the consequence is that onset and course of the disease are not altered by bcl-2 overexpression or GDNF treatment. Thus, the local effect of neurotrophic signaling on axon stability, in the case of CNTF, via STAT-3 and Stathmin seems to be more important for modulating disease than the classical neurotrophic signaling pathways for neuronal survival, and it is possible that similar effects are also contributing to the modulatory effect of CNTF in fALS with mutations in the SOD-I gene.

Some neurotrophic factors, in particular members of the neurotrophin family and their receptors, could also mediate additional effects via the p75 neurotrophin receptor ($p75^{NTR}$). This transmembrane protein shares structural and functional similarities with other transmembrane molecules of the FAS/APO-1 CD95 and TNF-receptor-1 family (Chao 2003). In a variety of cellular contexts in vitro and in vivo, $p75^{NTR}$ mediates cell death after binding of neurotrophins and in particular pro-neurotrophins, in particular when Trk receptors are not expressed, and binding of pro-neurotrophins to $p75^{NTR}$ has been shown in a variety of physiological contexts to destabilize neurites and cause neuronal cell death. Injection of neutralizing antibodies against $p75^{NTR}$ into the eye of early chick embryos has shown that early developmental cell death of retinal ganglion cells can be mediated through this receptor (Frade et al. 1996). Some mediators of $p75^{NTR}$ signaling also specifically destabilize axons (Plachta et al. 2007). Activation of $p75^{NTR}$ upregulates expression of the sugar-binding protein galectin-1. Increased amounts of galectin-1 destroy neuronal processes, both in cell culture and in vivo. The $p75^{NTR}$ receptor is highly upregulated in degenerating motoneurons in ALS (Kerkhoff et al. 1991; Seeburger et al. 1993), and it is likely that activation of this receptor contributes to the degenerative effects, in particular pruning of neuromuscular synapses and degeneration of neural processes (Singh et al. 2008).

In summary, neurotrophic factors modulate motoneuron disease on several levels. On the one side, they play a role in compensating the loss of motoneurons at early stages by sprouting, by stabilizing neuromuscular synapses, by stabilizing axons, and also by acting on motoneuron survival. On the other side, $p75^{NTR}$ signaling could contribute to degenerative mechanisms responsible for denervation of neuromuscular endplates and axon destruction, possibly even motoneuron cell death at later stages of disease. This offers many options how neurotrophic factors and their signaling pathways could be used as targets for therapy. So far, clinical trials with CNTF, IGF-1, and BDNF have not been successful in motoneuron disease (Thoenen and Sendtner 2002), but this is mainly due to side effects in the case of CNTF and adverse pharmacokinetic properties in the case of BDNF and possible also IGF-1. Future developments to overcome these problems could help to develop new therapies for motoneuron disease. Similarly, approaches that inhibit potential destructive signaling through $p75^{NTR}$ could also be of benefit for treatment of motoneuron disease. These strategies could be even more efficient when combined with therapies aiming at counteracting the consequences of primary causes of motoneuron disease, such as Smn deficiency in spinal muscular atrophy, or of altered TDP-43, Fus, or C9orf72 processing in familial or sporadic forms of motoneuron disease.

References

Ackermann B et al (2013) Plastin 3 ameliorates spinal muscular atrophy via delayed axon pruning and improves neuromuscular junction functionality. Hum Mol Genet 22(7):1328–1347

Al-Chalabi A et al (2012) The genetics and neuropathology of amyotrophic lateral sclerosis. Acta Neuropathol 124:339–352

Al-Sarraj S et al (2011) p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. Acta Neuropathol 122:691–702

Andersen PM, Al-Chalabi A (2011) Clinical genetics of amyotrophic lateral sclerosis: what do we really know? Nat Rev Neurol 7:603–615

Arakawa Y, Sendtner M, Thoenen H (1990) Survival effect of ciliary neurotrophic factor (CNTF) on chick embryonic motoneurons in culture: comparison with other neurotrophic factors and cytokines. J Neurosci 10:3507–3515

Ash PE et al (2013) Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. Neuron 77(4):639–646

Ashley CT Jr, Wilkinson KD, Reines D, Warren ST (1993) FMR1 protein: conserved RNP family domains and selective RNA binding. Science 262:563–566

Ayala YM, Misteli T, Baralle FE (2008) TDP-43 regulates retinoblastoma protein phosphorylation through the repression of cyclin-dependent kinase 6 expression. Proc Natl Acad Sci U S A 105:3785–3789

Azzouz M et al (2004) VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. Nature 429:413–417, 1995 Sep 28; 377(6547):340–344

Baloh RH et al (1998) Artemin, a novel member of the GDNF ligand family, supports peripheral and central neurons and signals through the GFRalpha3-RET receptor complex. Neuron 21:1291–1302

Boillee S et al (2006) Onset and progression in inherited ALS determined by motor neurons and microglia. Science 312:1389–1392

Bommel H et al (2002) Missense mutation in the tubulin-specific chaperone E (Tbce) gene in the mouse mutant progressive motor neuronopathy, a model of human motoneuron disease. J Cell Biol 159:563–569

Buratti E, Baralle FE (2001) Characterization and functional implications of the RNA binding properties of nuclear factor TDP-43, a novel splicing regulator of CFTR exon 9. J Biol Chem 276:36337–36343

Buratti E, Baralle FE (2010) TDP-43 regulates its mRNA levels through a negative feedback loop. EMBO J 30:277–288

Buratti E, Baralle FE (2008) Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. Front Biosci 13:867–878

Buratti E, Baralle FE (2010a) The multiple roles of TDP-43 in pre-mRNA processing and gene expression regulation. RNA Biol 7(4):420–429

Buratti E, Baralle FE (2010b) TDP-43 regulates its mRNA levels through a negative feedback loop. EMBO J 30: 277–288

Buratti E et al (2001) Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. EMBO J 20:1774–1784

Buratti E et al (2005) TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through its C-terminal tail: an important region for the inhibition of cystic fibrosis transmembrane conductance regulator exon 9 splicing. J Biol Chem 280:37572–37584

Burghes AH, Beattie CE (2009) Spinal muscular atrophy: why do low levels of survival motor neuron protein make motor neurons sick? Nat Rev Neurosci 10:597–609

Carmeliet P, Storkebaum E (2002) Vascular and neuronal effects of VEGF in the nervous system: implications for neurological disorders. Semin Cell Dev Biol 13:39–53

Caroni P (1997) Intrinsic neuronal determinants that promote axonal sprouting and elongation. Bioessays 19:767–775

Cartegni L, Krainer AR (2002) Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1. Nat Genet 30:377–384
Chan YB et al (2003) Neuromuscular defects in a Drosophila survival motor neuron gene mutant. Hum Mol Genet 12:1367–1376
Chao MV (2003) Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci 4:299–309
Chen YZ et al (2004) DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). Am J Hum Genet 74(6):1128–1135
Cho S, Dreyfuss G (2010) A degron created by SMN2 exon 7 skipping is a principal contributor to spinal muscular atrophy severity. Genes Dev 24:438–442
Chow CY et al (2009) Deleterious variants of FIG4, a phosphoinositide phosphatase, in patients with ALS. Am J Hum Genet 84(1):85–88
Clement AM et al (2003) Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science 302:113–117
Colombrita C et al (2009) TDP-43 is recruited to stress granules in conditions of oxidative insult. J Neurochem 111:1051–1061
Crawford TO, Pardo CA (1996) The neurobiology of childhood spinal muscular atrophy. Neurobiol Dis 3:97–110
Damiano M et al (2006) Neural mitochondrial Ca2+ capacity impairment precedes the onset of motor symptoms in G93A Cu/Zn-superoxide dismutase mutant mice. J Neurochem 96:1349–1361
De Vos KJ et al (2007) Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. Hum Mol Genet 16:2720–2728
DeJesus-Hernandez M et al (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72:245–256
Deng HX et al (2011) Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 477(7363):211–215
Dewey CM et al (2011) TDP-43 is directed to stress granules by sorbitol, a novel physiological osmotic and oxidative stressor. Mol Cell Biol 31:1098–1108
Di Giorgio FP, Boulting GL, Bobrowicz S, Eggan KC (2008) Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. Cell Stem Cell 3:637–648
Dormann D et al (2010) ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import. EMBO J 29:2841–2857
Drepper C, Sendtner M (2011) A new postal code for dendritic mRNA transport in neurons. EMBO Rep 12:614–616
Drepper C, Herrmann T, Wessig C, Beck M, Sendtner M (2011) C-terminal FUS/TLS mutations in familial and sporadic ALS in Germany. Neurobiol Aging 32(3):548.e1–4
Durham HD, Roy J, Dong L, Figlewicz DA (1997) Aggregation of mutant Cu/Zn superoxide dismutase proteins in a culture model of ALS. J Neuropathol Exp Neurol 56:523–530
Elden AC et al (2010) Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 466(7310):1069–1075
Elson GC et al (2000) CLF associates with CLC to form a functional heteromeric ligand for the CNTF receptor complex. Nat Neurosci 3:867–872
Ferraiuolo L, Kirby J, Grierson AJ, Sendtner M, Shaw PJ (2011) Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. Nat Rev Neurol 7:616–630
Fiesel FC et al (2010) Knockdown of transactive response DNA-binding protein (TDP-43) downregulates histone deacetylase 6. EMBO J 29:209–221
Fiesel FC, Schurr C, Weber SS, Kahle PJ (2011) TDP-43 knockdown impairs neurite outgrowth dependent on its target histone deacetylase 6. Mol Neurodegener 6:64
Frade JM, RodriguezTebar A, Barde YA (1996) Induction of cell death by endogenous nerve growth factor through its p75 receptor. Nature 383:166–168

Fratta P et al (2012) C9orf72 hexanucleotide repeat associated with amyotrophic lateral sclerosis and frontotemporal dementia forms RNA G-quadruplexes. Sci Rep 2:1016
Freibaum BD, Chitta RK, High AA, Taylor JP (2010) Global analysis of TDP-43 interacting proteins reveals strong association with RNA splicing and translation machinery. J Proteome Res 9:1104–1120
Friedlander RM, Brown RH, Gagliardini V, Wang J, Yuan J (1997) Inhibition of ICE slows ALS in mice. Nature 388:31
Gavrilina TO et al (2008) Neuronal SMN expression corrects spinal muscular atrophy in severe SMA mice while muscle-specific SMN expression has no phenotypic effect. Hum Mol Genet 17:1063–1075
Giess R et al (2002) Early onset of severe familial amyotrophic lateral sclerosis with a SOD-1 mutation: potential impact of CNTF as a candidate modifier gene. Am J Hum Genet 70:1277–1286
Glinka M et al (2010) The heterogeneous nuclear ribonucleoprotein-R is necessary for axonal beta-actin mRNA translocation in spinal motor neurons. Hum Mol Genet 19:1951–1966
Gogliotti RG et al (2012) Motor neuron rescue in spinal muscular atrophy mice demonstrates that sensory-motor defects are a consequence, not a cause, of motor neuron dysfunction. J Neurosci 32:3818–3829
Gravel C, Götz R, Lorrain A, Sendtner M (1997) Adenoviral gene transfer of ciliary neurotrophic factor and brain-derived neurotrophic factor leads to longterm survival of axotomized motoneurons. Nat Med 3:765–770
Greenway MJ et al (2006) ANG mutations segregate with familial and 'sporadic' amyotrophic lateral sclerosis. Nat Genet 38(4):411–413
Grieshammer U et al (1998) Muscle-specific cell ablation conditional upon Cre-mediated DNA recombination in transgenic mice leads to massive spinal and cranial motoneuron loss Neuronal cell death. Neuron 20:633–647
Grosskreutz J, Van Den Bosch L, Keller BU (2010) Calcium dysregulation in amyotrophic lateral sclerosis. Cell Calcium 47:165–174
Gubitz AK, Feng W, Dreyfuss G (2004) The SMN complex. Exp Cell Res 296:51–56
Gurney ME et al (1994) Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. Science 264:1772–1775
Hadano S et al (2001) A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nat Genet 29(2):166–173
Hamburger V (1934) The effects of wing bud extirpation on the development of the central nervous system in chick embryos. J Exp Zool 68:449–494
Hamburger V (1975) Cell death in the development of the lateral column of the chick embryo. J Comp Neurol 160:535–546
Hand CK et al (2002) A novel locus for familial amyotrophic lateral sclerosis, on chromosome 18q. Am J Hum Genet 70(1):251–256
Haramati S et al (2010) miRNA malfunction causes spinal motor neuron disease. Proc Natl Acad Sci U S A 107:13111–13116
Harraz MM et al (2008) SOD1 mutations disrupt redox-sensitive Rac regulation of NADPH oxidase in a familial ALS model. J Clin Invest 118:659–670
Hausmanowa-Petrusewicz I (1978) In: Spinal muscular atrophy: infantile and juvenile type. National Library of Medicine & The National Science Foundation, Washington DC
Henderson CE et al (1994) GDNF: a potent survival factor for motoneurons present in peripheral nerve and muscle. Science 266:1062–1064
Higashi S et al (2007) Concurrence of TDP-43, tau and alpha-synuclein pathology in brains of Alzheimer's disease and dementia with Lewy bodies. Brain Res 1184:284–294
Holtmann B et al (2005) Triple knock-out of CNTF, LIF, and CT-1 defines cooperative and distinct roles of these neurotrophic factors for motoneuron maintenance and function. J Neurosci 25:1778–1787

Hu F et al (2010) Sortilin-mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. Neuron 68:654–667
Hughes RA, Sendtner M, Goldfarb M, Lindholm D, Thoenen H (1993a) Evidence that fibroblast growth factor 5 is a major muscle derived survival factor for cultured spinal motoneurons. Neuron 10:369–377
Hughes RA, Sendtner M, Thoenen H (1993b) Members of several gene families influence survival of rat motoneurons in vitro and in vivo. J Neurosci Res 36(6):663–671
Imlach WL et al (2012) SMN is required for sensory-motor circuit function in Drosophila. Cell 151:427–439
Ito D, Seki M, Tsunoda Y, Uchiyama H, Suzuki N (2011) Nuclear transport impairment of amyotrophic lateral sclerosis-linked mutations in FUS/TLS. Ann Neurol 69:152–162
Jablonka S, Beck M, Lechner BD, Mayer C, Sendtner M (2007) Defective Ca2+ channel clustering in axon terminals disturbs excitability in motoneurons in spinal muscular atrophy. J Cell Biol 179:139–149
Johnson JO et al (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. Neuron 68(5):857–864
Kabashi E et al (2008) TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nat Genet 40(5):572–574
Kashima T, Manley JL (2003) A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. Nat Genet 34:460–463
Kerkhoff H, Jennekens FGI, Troost D, Veldman H (1991) Nerve growth factor receptor immunostaining in the spinal cord and peripheral nerves in amyotrophic lateral sclerosis. Acta Neuropathol (Berl) 81:649–656
Kiebler MA, Bassell GJ (2006) Neuronal RNA granules: movers and makers. Neuron 51:685–690
Kiernan MC et al (2011) Amyotrophic lateral sclerosis. Lancet 377:942–955
Klein RD et al (1997) A GPI-linked protein that interacts with Ret to form a candidate neurturin receptor. Nature 387:717–721
Kong L et al (2009) Impaired synaptic vesicle release and immaturity of neuromuscular junctions in spinal muscular atrophy mice. J Neurosci 29:842–851
Kostic V, Jackson-Lewis V, de Bilbao F, Dubois-Dauphin M, Przedborski S (1997) Bcl-2: prolonging life in a transgenic mouse model of familial amyotrophic lateral sclerosis. Science 277:559–562
Krecic AM, Swanson MS (1999) hnRNP complexes: composition, structure, and function. Curr Opin Cell Biol 11:363–371
Kuroda M et al (2000) Male sterility and enhanced radiation sensitivity in TLS(-/-) mice. EMBO J 19:453–462
Kwiatkowski TJ Jr et al (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 323:1205–1208
Leibrock J et al (1989) Molecular cloning and expression of brain-derived neurotrophic factor. Nature 341:149–152
Ling SC et al (2010) ALS-associated mutations in TDP-43 increase its stability and promote TDP-43 complexes with FUS/TLS. Proc Natl Acad Sci U S A 107:13318–13323
Liu X, Ernfors P, Wu H, Jaenisch R (1995) Sensory but not motor neuron deficits in mice lacking NT4 and BDNF. Nature 375:238–241
Lotti F et al (2012) An SMN-dependent U12 splicing event essential for motor circuit function. Cell 151:440–454
Luty AA et al (2010) Sigma nonopioid intracellular receptor 1 mutations cause frontotemporal lobar degeneration-motor neuron disease. Ann Neurol 68(5):639–649
Mackenzie IRA, Rademakers R, Neumann M (2010) TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. Lancet Neurol 9:995–1007
Maekawa S et al (2009) TDP-43 is consistently co-localized with ubiquitinated inclusions in sporadic and Guam amyotrophic lateral sclerosis but not in familial amyotrophic lateral sclerosis with and without SOD1 mutations. Neuropathology 29:672–683

Maruyama H et al (2010) Mutations of optineurin in amyotrophic lateral sclerosis. Nature 465 (7295):223–226
McDonald KK et al (2011) TAR DNA-binding protein 43 (TDP-43) regulates stress granule dynamics via differential regulation of G3BP and TIA-1. Hum Mol Genet 20:1400–1410
McGovern VL, Gavrilina TO, Beattie CE, Burghes AH (2008) Embryonic motor axon development in the severe SMA mouse. Hum Mol Genet 17:2900–2909
McWhorter ML, Monani UR, Burghes AH, Beattie CE (2003) Knockdown of the survival motor neuron (Smn) protein in zebrafish causes defects in motor axon outgrowth and pathfinding. J Cell Biol 162:919–931
Mentis GZ et al (2011) Early functional impairment of sensory-motor connectivity in a mouse model of spinal muscular atrophy. Neuron 69:453–467
Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H (1992) Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. J Cell Biol 119:45–54
Milbrandt J et al (1998) Persephin, a novel neurotrophic factor related to GDNF and neurturin. Neuron 20:245–253
Moisse K et al (2009a) Divergent patterns of cytosolic TDP-43 and neuronal progranulin expression following axotomy: implications for TDP-43 in the physiological response to neuronal injury. Brain Res 1249:202–211
Moisse K et al (2009b) Cytosolic TDP-43 expression following axotomy is associated with caspase 3 activation in NFL-/- mice: support for a role for TDP-43 in the physiological response to neuronal injury. Brain Res 1296:176–186
Monani UR et al (2000) The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in Smn(-/-) mice and results in a mouse with spinal muscular atrophy [In Process Citation]. Hum Mol Genet 9:333–339
Mori K et al (2013a) hnRNP A3 binds to GGGGCC repeats and is a constituent of p62-positive/TDP43-negative inclusions in the hippocampus of patients with C9orf72 mutations. Acta Neuropathol 125(3):413–423
Mori K et al (2013b) The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. Science 339(6125):1335–1338
Morlando M et al (2012) FUS stimulates microRNA biogenesis by facilitating co-transcriptional Drosha recruitment. EMBO J 31:4502–4510
Mourelatos Z, Abel L, Yong J, Kataoka N, Dreyfuss G (2001) SMN interacts with a novel family of hnRNP and spliceosomal proteins. EMBO J 20:5443–5452
Nagai M et al (2007) Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat Neurosci 10:615–622
Neumann M et al (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314:130–133
Ning K et al (2010) PTEN depletion rescues axonal growth defect and improves survival in SMN-deficient motor neurons. Hum Mol Genet 19:3159–3168
Nishihira Y et al (2009) Sporadic amyotrophic lateral sclerosis of long duration is associated with relatively mild TDP-43 pathology. Acta Neuropathol 117:45–53
Nishimura AL et al (2004) A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. Am J Hum Genet 75(5):822–831
Novak KD, Prevette D, Wang S, Gould TW, Oppenheim RW (2000) Hepatocyte growth factor/scatter factor is a neurotrophic survival factor for lumbar but not for other somatic motoneurons in the chick embryo. J Neurosci 20:326–337
Oppenheim RW (1985) Naturally occuring cell death during neural development. Trends Neurosci 8:487–493
Oppenheim RW et al (2000) Glial cell line-derived neurotrophic factor and developing mammalian motoneurons: regulation of programmed cell death among motoneuron subtypes. J Neurosci 20:5001–5011

Oppenheim RW et al (2001) Cardiotrophin-1, a muscle-derived cytokine, is required for the survival of subpopulations of developing motoneurons. J Neurosci 21:1283–1291
Oprea GE et al (2008) Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. Science 320:524–527
Orlacchio A et al (2010) SPATACSIN mutations cause autosomal recessive juvenile amyotrophic lateral sclerosis. Brain 133(Pt 2):591–598
Parkinson N et al (2006) ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). Neurology 67(6):1074–1077
Pascale A, Govoni S (2012) The complex world of post-transcriptional mechanisms: is their deregulation a common link for diseases? Focus on ELAV-like RNA-binding proteins. Cell Mol Life Sci 69:501–517
Pasinelli P et al (2004) Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. Neuron 43:19–30
Pellizzoni L (2007) Chaperoning ribonucleoprotein biogenesis in health and disease. EMBO Rep 8:340–345
Pennica D et al (1996) Cardiotrophin-1, a cytokine present in embryonic muscle, supports long-term survival of spinal motoneurons. Neuron 17:63–74
Pesiridis GS, Lee VM, Trojanowski JQ (2009) Mutations in TDP-43 link glycine-rich domain functions to amyotrophic lateral sclerosis. Hum Mol Genet 18:R156–R162
Pesiridis GS, Tripathy K, Tanik S, Trojanowski JQ, Lee VM (2011) A "two-hit" hypothesis for inclusion formation by carboxyl-terminal fragments of TDP-43 protein linked to RNA depletion and impaired microtubule-dependent transport. J Biol Chem 286:18845–18855
Plachta N et al (2007) Identification of a lectin causing the degeneration of neuronal processes using engineered embryonic stem cells. Nat Neurosci 10:712–719
Poesen K et al (2008) Novel role for vascular endothelial growth factor (VEGF) receptor-1 and its ligand VEGF-B in motor neuron degeneration. J Neurosci 28:10451–10459
Polymenidou M et al (2011) Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. Nat Neurosci 14(4):459–468
Pun S, Santos AF, Saxena S, Xu L, Caroni P (2006) Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. Nat Neurosci 9:408–419
Rayaprolu S et al (2012) Angiogenin variation and Parkinson disease. Ann Neurol 71(5):725–727; author reply 727–728
Reaume AG et al (1996) Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. Nat Genet 13:43–47
Renton AE et al (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72:257–268
Riethmacher D et al (1997) Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. Nature 389:725–730
Rosen DR et al (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62
Rossoll W et al (2002) Specific interaction of Smn, the spinal muscular atrophy determining gene product, with hnRNP-R and gry-rbp/hnRNP-Q: a role for Smn in RNA processing in motor axons? Hum Mol Genet 11:93–105
Rossoll W et al (2003) Smn, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of beta-actin mRNA in growth cones of motoneurons. J Cell Biol 163:801–812
Ruiz R, Casanas JJ, Torres-Benito L, Cano R, Tabares L (2010) Altered intracellular Ca2+ homeostasis in nerve terminals of severe spinal muscular atrophy mice. J Neurosci 30:849–857
Rutherford NJ et al (2008) Novel mutations in TARDBP (TDP-43) in patients with familial amyotrophic lateral sclerosis. PLoS Genet 4:e1000193
Sagot Y, Tan SA, Hammang JP, Aebischer P, Kato AC (1996a) GDNF slows loss of motoneurons but not axonal degeneration or premature death of pmn/pmn mice. J Neurosci 16:2335–2341

Sagot Y et al (1996b) Bcl-2 overexpression prevents motoneuron cell body loss but not axonal degeneration in a mouse model of a neurodegenerative disease. J Neurosci 11:7727–7733
Sapp PC et al (2003) Identification of two novel loci for dominantly inherited familial amyotrophic lateral sclerosis. Am J Hum Genet 73(2):397–403
Schrank B et al (1997) Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc Natl Acad Sci U S A 94:9920–9925
Schwab C, Arai T, Hasegawa M, Yu S, McGeer PL (2008) Colocalization of transactivation-responsive DNA-binding protein 43 and huntingtin in inclusions of Huntington disease. J Neuropathol Exp Neurol 67:1159–1165
Seeburger JL, Tarras S, Natter H, Springer JE (1993) Spinal cord motoneurons express p75 NGFR and p145 trkB mRNA in amyotrophic lateral sclerosis. Brain Res 621:111–115
Selvaraj BT, Frank N, Bender FL, Asan E, Sendtner M (2012) Local axonal function of STAT3 rescues axon degeneration in the pmn model of motoneuron disease. J Cell Biol 199:437–451
Sendtner M (2001) Molecular mechanisms in spinal muscular atrophy: models and perspectives. Curr Opin Neurol 14:629–634
Sendtner M (2010) Therapy development in spinal muscular atrophy. Nat Neurosci 13:795–799
Sendtner M, Kreutzberg GW, Thoenen H (1990) Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy. Nature 345:440–441
Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde YA (1992a) Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. Nature 360:757–758, 1995 Sep 28; 377(6547):340–344
Sendtner M et al (1992b) Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuronopathy. Nature 358:502–504
Sephton CF et al (2011) Identification of neuronal RNA targets of TDP-43-containing ribonucleoprotein complexes. J Biol Chem 286:1204–1215
Simon CM, Jablonka S, Ruiz R, Tabares L, Sendtner M (2010) Ciliary neurotrophic factor-induced sprouting preserves motor function in a mouse model of mild spinal muscular atrophy. Hum Mol Genet 19:973–986
Simpson EP, Henry YK, Henkel JS, Smith RG, Appel SH (2004) Increased lipid peroxidation in sera of ALS patients: a potential biomarker of disease burden. Neurology 62:1758–1765
Singh KK et al (2008) Developmental axon pruning mediated by BDNF-p75NTR-dependent axon degeneration. Nat Neurosci 11:649–658
Smith RG, Henry YK, Mattson MP, Appel SH (1998) Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. Ann Neurol 44:696–699
Sreedharan J et al (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319(5870):1668–1672
Stockli KA et al (1989) Molecular cloning, expression and regional distribution of rat ciliary neurotrophic factor. Nature 342:920–923
Strong MJ et al (2007) TDP43 is a human low molecular weight neurofilament (hNFL) mRNA-binding protein. Mol Cell Neurosci 35:320–327
Subramanian M et al (2011) G-quadruplex RNA structure as a signal for neurite mRNA targeting. EMBO Rep 12:697–704
Thoenen H, Sendtner M (2002) Neurotrophins: from enthusiastic expectations through sobering experiences to rational therapeutic approaches. Nat Neurosci 5(Suppl):1046–1050
Tollervey JR et al (2011) Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. Nat Neurosci 14:452–458
Torres-Benito L, Ruiz R, Tabares L (2012) Synaptic defects in spinal muscular atrophy animal models. Dev Neurobiol 72:126–133
Urushitani M et al (2006) Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis. Nat Neurosci 9:108–118
Van Damme P et al (2007) Astrocytes regulate GluR2 expression in motor neurons and their vulnerability to excitotoxicity. Proc Natl Acad Sci U S A 104:14825–14830

Vance C et al (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 323(5918):1208–1211
Volkening K, Leystra-Lantz C, Yang W, Jaffee H, Strong MJ (2009) Tar DNA binding protein of 43 kDa (TDP-43), 14-3-3 proteins and copper/zinc superoxide dismutase (SOD1) interact to modulate NFL mRNA stability. Implications for altered RNA processing in amyotrophic lateral sclerosis (ALS). Brain Res 1305:168–182
Wang IF, Reddy NM, Shen CK (2002) Higher order arrangement of the eukaryotic nuclear bodies. Proc Natl Acad Sci U S A 99:13583–13588
Wegorzewska I, Baloh RH (2011) TDP-43-based animal models of neurodegeneration: new insights into ALS pathology and pathophysiology. Neurodegener Dis 8:262–274
Weidner KM et al (1991) Evidence for the identity of human scatter factor and human hepatocyte growth factor. Proc Natl Acad Sci U S A 88:7001–7005
Wirth B (2000) An update of the mutation spectrum of the survival motor neuron gene (SMN1) in autosomal recessive spinal muscular atrophy (SMA). Hum Mutat 15:228–237
Wong PC et al (1995) An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. Neuron 14:1105–1116
Wood-Allum C, Shaw PJ (2010) Motor neurone disease: a practical update on diagnosis and management. Clin Med 10:252–258
Wu CH et al (2012) Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature 488(7412):499–503
Yamamoto Y et al (1997) Hepatocyte growth factor (HGF/SF) is a muscle-derived survival factor for a subpopulation of embryonic motoneurons. Development 124:2903–2913
Yamazaki T et al (2012) FUS-SMN protein interactions link the motor neuron diseases ALS and SMA. Cell Rep 2:799–806
Zinszner H, Albalat R, Ron D (1994) A novel effector domain from the RNA-binding protein TLS or EWS is required for oncogenic transformation by CHOP. Genes Dev 8:2513–2526

Neurotrophic Factors in Spinal Cord Injury

Vanessa S. Boyce and Lorne M. Mendell

Abstract

A major challenge in repairing the injured spinal cord is to assure survival of damaged cells and to encourage regrowth of severed axons. Because neurotrophins are known to affect these processes during development, many experimental approaches to improving function of the injured spinal cord have made use of these agents, particularly Brain derived neurotrophic factor (BDNF) and Neurotrophin-3 (NT-3). More recently, neurotrophins have also been shown to affect the physiology of cells and synapses in the spinal cord. The effect of neurotrophins on circuit performance adds an important dimension to their consideration as agents for repairing the injured spinal cord. In this chapter we discuss the role of neurotrophins in promoting recovery after spinal cord injury from both a structural and functional perspective.

Keywords

Spinal cord injury • Cell survival • Axon growth • NMDA receptors • Collateral sprouting • Autonomic dysreflexia • Stepping • BDNF • NT-3 • NGF • EPSP • Pain

1 Introduction

Neurotrophic factors have been implicated in the response to central nervous system injury for more than two decades. Early experiments noted that with cortical injury came the production of "neuronotrophic factors" (Nieto-Sampedro et al. 1982), thought to be responsible for the survival of neurons in vitro.

V.S. Boyce • L.M. Mendell (✉)
Department of Neurobiology and Behavior, Stony Brook University, Stony Brook, NY 11794-5230, USA
e-mail: lorne.mendell@stonybrook.edu

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_16,

These molecules were later identified by other groups as belonging to a family of neurotrophic factors, consisting of nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) (Barde et al. 1982; Müller et al. 1984). The neurotrophins act via tropomyosin-related kinase (trk) receptors which selectively bind neurotrophins (trkA: NGF; trkB: BDNF; NT-4/5; trkC: NT-3). Expression of the different trk receptors on different populations of neurons assures selectivity of neurotrophin action. Another neurotrophin receptor, p75, is activated by all neurotrophins and is not a basis for selectivity. Neurotrophins and their receptors are discussed in more detail in the chapter by Chao.

During development neurotrophins have been identified as target-derived molecules that are retrogradely (DiStefano et al. 1992) and anterogradely (Conner et al. 1997) transported via intrinsic axonal transport mechanisms. These mechanisms are used to deliver exogenously supplied neurotrophic factors to the central nervous system, after either peripheral (Fortun et al. 2009; Petruska et al. 2010) or central administration (Tuszynski et al. 1994; Blits et al. 2003; Arvanian et al. 2003; Boyce et al. 2007, 2012). Neurotrophins promote cell survival and axonal growth (see below) which make them excellent candidates for use in spinal cord injury. Although these effects indicate a strong potential for roles in spinal cord repair processes, more recent studies have indicated additional actions for neurotrophins that could contribute to enhancing the function of the damaged spinal cord. Here, we are referring to their role in altering the physiology of neurons and their synapses. Since most spinal cord injuries are anatomically incomplete, even though apparently functionally complete, any therapy that enhances cellular and synaptic function of surviving neurons is potentially useful. Recent work has demonstrated that neurotrophins elicit physiological effects in the damaged spinal cord that can improve function. However, as we shall discuss, some of the effects of neurotrophins can be detrimental, and this must be carefully considered before using them as therapy after spinal cord injury.

This chapter discusses the many ways in which neurotrophins have been used to improve functional outcomes in experimental studies of spinal cord injury. Herein, we compare the efficacy of such treatments across animal models, lesion severities, and delivery methods and attempt to distill the substantial body of work that has been done in this field. In so doing, this chapter hopes to contribute to the discussion of how best neurotrophic factor approaches can be optimized to address the problems that are caused by spinal cord injury.

2 Cell Survival and Axonal Growth

Many deficits result from spinal cord injury. Foremost among these is axonal damage resulting from the mechanical insult. Secondary damage due to neurotoxic and a plethora of inhibitory molecules causes apoptotic cell loss and axonal

degeneration (reviewed in McDonald 1999; Fitch and Silver 2008; Beattie 2004). In addition the glial scar presents a physical barrier to axonal regrowth, the resulting outcome being loss of function correlated with injury severity. It is no wonder then that neurotrophic factors became attractive agents used by many groups (Henderson et al. 1993; Xu et al. 1995; Tuszynski et al. 1996; Ye and Houle 1997; Liu et al. 1999; Brock et al. 2010) to address cell survival and axonal regeneration following a spinal cord injury (reviewed in Bregman 1998).

A major challenge for damaged neurons is survival. Although cell death is not generally an immediate consequence of axotomy, cells disconnected from their target often do not survive indefinitely. Axotomized motoneurons in neonatal rats fail to survive, a fate that can be delayed for several weeks by providing the neurotrophin BDNF, a trkB agonist (Sendtner et al. 1992; Yan et al. 1993; Koliatsos et al. 1993). TrkB agonists (BDNF or NT-4/5) also enhance survival of axotomized rubrospinal (Kobayashi et al. 1997; Bretzner et al. 2008) and corticospinal neurons (Giehl and Tetzlaff 1996; Brock et al. 2010) in the central nervous system.

Surviving axotomized neurons must grow processes in order to participate in the recovery of function. In the peripheral nervous system, BDNF has been found to encourage growth of motor axons (Novikova et al. 2002; Boyd and Gordon 2003) with low doses of BDNF facilitating regeneration and higher doses discouraging it, the latter perhaps via its action on the p75 neurotrophin receptor (Boyd and Gordon 2003). Regeneration of sensory neurons to their central targets in the spinal cord faces a barrier because of the inability of regeneration within the cord. However, it has been demonstrated that provision of neurotrophins to the growing fibers helps them overcome these barriers to enable synaptic connectivity with cells in the gray matter (Ramer et al. 2000, 2002). Fibers regenerate according to the neurotrophin that is provided, i.e., trkC-expressing muscle spindle afferents regenerate when NT-3 is provided, etc.

In the CNS there are also numerous demonstrations of the effects of neurotrophins in promoting axon growth. NT-3 in the dorsal columns promotes growth of axotomized sensory axons (Bradbury et al. 1999), and placing NT-3 in the dorsal column nuclei has been found to encourage ingrowth of axotomized sensory fibers from the dorsal columns with ultrastructural evidence for formation of synapses on relay cells (Alto et al. 2009). NT-3 has also been found to induce sprouting from damaged corticospinal tract fibers (Grill et al. 1997; von Meyenburg et al. 1998; Schnell et al. 1994; Tuszynski et al. 2003) but not from intact corticospinal fibers (Zhou et al. 2003). The mechanisms of neurotrophin action on axon growth remain incompletely described because of evidence that BDNF in some cases stimulates sprouting of damaged corticospinal axons, (Bregman et al. 1997; Vavrek et al. 2006) and in other studies NT-3 has been found to inhibit the sprouting response (Hagg et al. 2005). BDNF and NT-4/5 exert a positive growth action on damaged rubrospinal, vestbulospinal, and reticulospinal fibers (Menei et al. 1998; Jin et al. 2002).

The ability to encourage regeneration of fiber tracts using neurotrophins has stimulated more ambitious projects to improve function after spinal cord injury. These efforts have included the use of bridges to improve growth of axons across

lesions as well the use of transplanted cells to act as relays between damaged axons and functioning parts of the nervous system. Neurotrophins have played important roles in both undertakings. For example, axons growing through a Schwann cell bridge placed into the damaged spinal cord are more successful in growing into a bridge treated with NT-3 and/or BDNF (Xu et al. 1995). In addition, fibers reenter the spinal cord on the other side of the bridge in greater numbers and for greater distances when the host cord itself is treated with BDNF and/or NT-3 (Bamber et al. 2001). In a recent study Bonner et al. (2011) demonstrated that embryonic spinal neurons implanted into a dorsal column lesion site in rats received input from dorsal column axons, and under the influence of BDNF sent axons into the dorsal column nuclei. These axons, exhibited electrophysiological function, although there was no definitive evidence that they made functional synapses on cells in the dorsal column nuclei. Further studies in rats using cultured neural stem cells implanted with a cocktail of growth factors including BDNF and NT-3 into a total transection at T3 have revealed formation of a functional bridge connecting both sides of the lesion (Lu et al. 2012). This bridge was associated with behavioral improvement.

In addition to promoting growth of axons, neurotrophins also support proliferation of oligodendrocytes with the resultant myelination of nearby axons (McTigue et al. 1998; Althaus et al. 2008). This is an exceedingly important function after spinal cord injury where glial cells, including oligodendrocytes, are known to undergo apoptosis, resulting in axons that are unable to conduct action potentials normally. They are either subject to blockade, for example at branch points, or fail to conduct faithfully at normal frequencies (Tan et al. 2007). These deficits in presynaptic impulse discharge frequency can have substantial effects on the response of postsynaptic cells.

A different strategy to improve spinal cord repair making use of the ability of neurotrophins to promote axonal growth has involved attempts to generate bypasses or detours around partial spinal lesions such as hemisections. One strategy already discussed involves using implanted cell populations to serve as a bridge connecting damaged axons to their usual target. Another strategy takes advantage of the ability of neurotrophins to stimulate axonal growth or sprouting to enable damaged cells to synapse on new populations of neurons that would conduct impulses past the region of damage. Such anatomical plasticity has been documented particularly after partial spinal cord injuries (Bareyre et al. 2004; Courtine et al. 2009; Murray et al. 2010). In these instances reorganization and functional reconnection of supraspinal tracts occurred via the formation of "bypass circuitry" around the lesion utilizing long descending propriospinal fibers. In fact, cortical application of BDNF increased the formation of these new corticospinal connections onto propriospinal fibers (Vavrek et al. 2006).

Neurotrophic factors are instrumental in the remodeling of spinal cord circuitry post-injury, with axonal sprouting chief among the benefits of their administration (Senut et al. 1995). In particular sprouting of corticospinal (Schnell et al. 1994;

Sasaki et al. 2009), cholinergic (Jakeman et al. 1998), and rubrospinal (Tobias et al. 2003) fibers occurs with NT-3 or BDNF administration to the injured spinal cord. Arvanian et al. (2006) demonstrated in the neonatal rat that axons of the ventrolateral fasciculus deprived of monosynaptic connections to motoneurons by hemisection can develop polysynaptic connections to ipsilateral motoneurons if the cord is treated with NT-3. A factor enhancing NT-3 action is the NR2D regulatory subunit of the NMDA receptor. This subunit is normally downregulated in the immediate postnatal period, and this decline is responsible for Mg^{2+} block of the NMDA receptor without which NT-3 is ineffective (Arvanian et al. 2004). In the adult rat where growth of CNS axons is restricted due to the presence of inhibitory factors such as Nogo, formation of a functional detour requires neutralization of Nogo in addition to NT-3 and NR2D (Schnell et al. 2011). Another successful strategy with a similar outcome has involved the use of chondroitinase in addition to NT-3 and NR2D (García-Alías et al. 2011).

The ability for neurotrophins to influence connectivity in the damaged spinal cord has stimulated efforts to deliver them chronically. Genetically modified fibroblasts are among the cellular therapies used for this purpose (Tuszynski et al. 1994; Grill et al. 1997; Liu et al. 1999; Brock et al. 2010). In addition to serving as a "biological mini-pump," producing the neurotrophin of interest, the potential benefit of these cells is provision of a scaffold for regenerating axons. These allografts require implantation of large numbers of cells and immunosuppression of the host in order to ensure graft survival (but see Tobias et al. 2001). In order to circumvent the need for long-term immunosuppression, autologous cell therapies have been examined (Li et al. 1997; Feron et al. 2005) as well as neurotrophin secreting marrow stromal cells (Lu et al. 2005) or mesenchymal stem cells (Sasaki et al. 2009). Viral constructs also effectively deliver neurotrophins to the spinal cord by infecting cells in the host which then express the gene product and transport it to the axon terminals (Liu et al. 1997; Hermens and Verhaagen 1998; Blits et al. 2003; Hendriks et al. 2004). These are able to affect spinal function even if placed in peripheral tissue (e.g., muscle) and transported to the spinal cord (Fortun et al. 2009; Petruska et al. 2010).

None of the delivery methods discussed above is ideal. Issues of immunosuppression and transgene downregulation occur in cellular therapies. With viral approaches the cellular targets are unknown and once infected, these cells continue to elicit their effects for several months but not indefinitely (Petruska et al. 2010), and so the effective dose needed to produce behavioral and/or anatomical improvements is uncertain.

3 Cellular and Synaptic Changes Elicited by Neurotrophins

There is now substantial information indicating that administration of neurotrophins results in functional changes that in many cases appear to contribute to recovery. Much of the initial work on functional changes was centered on the role

of nerve growth factor (NGF) in inducing inflammatory pain (See Chapter by Lewin). Behavioral and electrophysiological studies confirmed that NGF is pronociceptive when delivered to skin and that it is upregulated in the skin, visceral organs, and bone during inflammatory pain (Petruska and Mendell 2009). Furthermore, antagonism of NGF reduces the pain associated with inflammation (Mantyh et al. 2011). Some of the effects of NGF are virtually immediate, within a few minutes, too soon to be caused by growth processes. These have been attributed to direct effects on the nociceptive terminal whereby activation of the trkA receptor enhances the response of noxious heat-responsive TRPV1 receptors. Other changes are delayed and have been identified with changes in gene expression in nociceptors, e.g., changes in Na^+ channel or peptide (SP, CGRP, BDNF) expression that enhance transmitter release rather than being related to growth of axons (Mantyh et al. 2011).

BDNF and NT-3 have been shown to have synaptic effects in the spinal cord and elsewhere. As is the case with the effects of NGF on the function of nociceptors, both have immediate effects on synaptic transmission. Superfusion of the isolated spinal cord with NT-3 elicits virtually immediate potentiation of the monosynaptic EPSP produced in motoneurons of neonatal rats by stimulation of the segmental (group Ia) inputs (Arvanov et al. 2000) which lasts for at least several hours (Fig. 1a). This effect on the AMPA receptor-mediated response requires active NMDA receptors (Arvanian and Mendell 2001a). The effects of BDNF are more complicated, with an initial immediate facilitation followed by a long lasting inhibition, both NMDA receptor-dependent; the latter appears to involve presynaptic inhibition (Arvanian and Mendell 2001b). Again these immediate effects are too rapid to be accounted for by a growth process, such as sprouting, and are likely the result of changes in AMPA receptor sensitivity or number. The requirement for NMDA receptor activity and the blockade of the NT-3 effect by Ca^{2+} chelation (Arvanov et al. 2000) suggest that Ca^{2+} entry via the NMDA receptor affects AMPA receptor sensitivity or number, perhaps via CAM kinase activity (Strack and Colbran 1998). These acute effects of neurotrophins observed in the isolated cord are no longer observed after P14 because NMDA receptors lose the NR2D regulatory subunit and become subject to Mg^{2+} blockade (Arvanian et al. 2004—see above).

In other experiments NT-3 has been applied chronically using either fibroblasts or adeno-associated viruses (AAVs) (Fig. 1b) and has been found to result in an increase in synaptic potentials from intact segmental inputs (Arvanian et al. 2003; Petruska et al. 2010; Boyce et al. 2012). These findings are consistent with earlier studies in adult cats demonstrating that NT-3 applied to the stump of a severed muscle nerve could either prevent or reverse the loss of synaptic efficacy from the axotomized muscle afferent fibers (Mendell et al. 1999). The mechanism (s) underlying this enhanced synaptic activity is (are) not yet defined. It could result from a growth process, e.g., collateral sprouting, as has been described after spinal injury (see above), a developmental process elicited by NT-3 (Seebach et al. 1999;

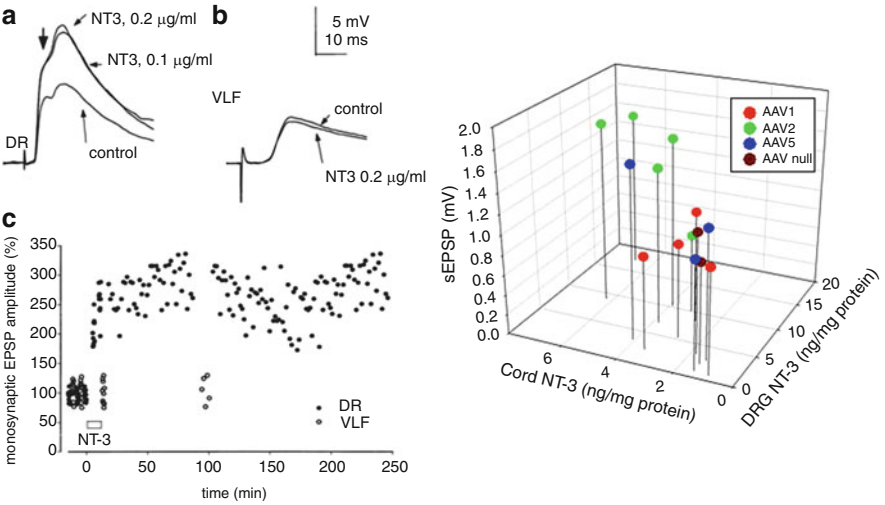

Fig. 1 *Left*: (**a**) Acute potentiation of dorsal root (DR) evoked EPSP in motoneuron recorded intracellularly in the neonatal rat cord recorded in vitro. Note that increasing the dose did not increase the magnitude of the potentiation. (**b**) In the same motoneuron the response to stimulation of the ventrolateral funiculus (VLF) white matter did not potentiate because NMDA receptors associated with this input are blocked at this postnatal developmental stage. (**c**) The graph displays the time course and magnitude of potentiation of the same motoneurons to both synaptic inputs. Note the several hour duration of the DR potentiation even though NT-3 was removed after 10 min. From Arvanov et al. 2000 with permission. *Right*: Potentiation of the monosynaptic EPSP evoked by muscle nerve stimulation (sEPSP) in the adult rat as a function of spinal cord and dorsal root ganglion levels of NT-3. NT-3 was delivered to the muscle via AAV virus of different serotypes (see legend). At high NT-3 cord concentrations, EPSP amplitude was elevated. From Petruska et al. (2010) with permission

Chen et al. 2003; Shneider et al. 2009), or it could be persistence of the acute effects involving NMDA receptors. The blockade of NMDA receptors that might have prevented NT-3 from having its NMDA receptor-dependent acute action (see above) would be expected to be reduced or even eliminated in the intact spinal cord where these experiments were done because the cells would often be depolarized due to tonic synaptic activity; this is known to reduce or even eliminate the Mg^{2+} blockade (Nowak et al. 1984). This ambiguity is important to resolve in determining the mechanism responsible for the functional effects of neurotrophins after spinal cord injury.

NT-3 does not facilitate transmission equally for all synaptic inputs to motoneurons in the in vitro spinal cord. Arvanov et al. (2000) showed that motoneurons exhibiting robust facilitation of the group Ia segmental EPSP by NT-3 displayed no potentiation of the EPSPs elicited in the same motoneuron by fibers in the ventrolateral funiculus (VLF) white matter of the spinal cord (Fig. 1a).

In later studies this was attributed to differences in the NMDA receptors activated by these two groups of fibers, specifically that in the neonatal cord, the NMDA receptor activated by VLF fibers were already blocked by Mg^{2+} at a stage where the NMDA receptors activated by group Ia fibers were not blocked. The initial view was that this reflected a different timescale for decline of the NR2D subunit associated with these two inputs: the VLF NMDA receptor already had reduced NR2D expression at birth whereas the group Ia NR2D subunit declined only in the second postnatal week. The concept that NMDA receptors associated with the different classes of inputs to the same motoneuron has been extended more recently by the demonstration that during the first postnatal week NMDA receptors associated with VLF input are less mobile than group Ia NMDA receptors (Shanthanelson et al. 2009). Furthermore, NMDA receptors associated with VLF synapses on motoneurons have proportionally lower expression of the NR2B subunit than group Ia fiber NMDA receptors (Shanthanelson and Mendell 2010). Since NMDA receptors are important participants in at least some of the synaptic actions of neurotrophins, the properties of NMDA receptors on different classes of cells is an important issue to consider in determining neurotrophin effects in the injured adult spinal cord.

In addition to affecting synaptic transmission, elevation of neurotrophin levels has been found to affect cellular properties. This has been studied most extensively for motoneurons. The most clear-cut effect is on excitability where elevation in spinal cord NT-3 levels in adult rats has been found to reduce excitability measured as an increase in threshold current (rheobase) (Petruska et al. 2010). This is associated with a decrease in input resistance of the motoneurons suggesting that the cells increase their surface area in response to NT-3, an effect that is similar to that observed in visual cortex (McAllister et al. 1997). The inverse relationship between rheobase and input resistance is expected since large cells should require more current to depolarize them to threshold. Interestingly, BDNF applied peripherally to muscle nerves has been found to reduce rheobase of the associated motoneurons that is associated with a decrease in motoneuron surface area (Gonzalez and Collins 1997) similar to that observed by McAllister et al. (1997). Similar opposite effects of NT-3 and BDN F on motoneuron rheobase (i.e., excitability) were observed in the transected spinal cord after intraspinal delivery of BDNF via AAV (Boyce et al. 2012). These opposing effects of NT-3 and BDNF on motoneuron rheobase and size were not observed in similar studies in motoneurons of neonatal rats treated with these agents although the same treatments were effective in causing opposing changes in the amplitude of the segmental monosynaptic EPSP: increased by NT-3, decreased by BDNF (Seebach et al. 1999). No studies are available on the effects of neurotrophins on defined populations of interneurons although the ability of neurotrophins to affect stepping movements (see below) suggests that such effects exist.

4 Recovery of Function in the Injured Cord

Given the extensive effects of neurotrophins on axon regeneration and sprouting, as well as its effects, both acute and chronic, on properties of cells and synapses, it would be expected that administration of neurotrophins should have effects on function. Such effects might be very useful in promoting recovery of certain behaviors that are lost after injury of the spinal cord. Important functions requiring spinal circuitry include hind limb stepping, forelimb reaching, micturition, sexual function and control of neuropathic pain. Evidence exists for modification of some of these functions by neurotrophins.

Grill et al. (1997) used fibroblasts engineered to secrete NT-3 implanted into the midthoracic cord to determine the degree of recovery from a midthoracic dorsal hemisection of the spinal cord. They observed significant elongation of labeled corticospinal axons up to about 8 mm through the gray matter, but not the white matter. These anatomical changes were accompanied by evidence of motor recovery, specifically in the number of footfalls when walking across a grid. The interpretation of such experiments in terms of the recovery of function being due to the reestablishment of corticospinal projections is complicated by the lack of electrophysiological evidence indicating functional connections of such regenerated fibers to target cells as well as the more recent evidence indicating that infusion of NT-3 enhances synaptic connectivity from segmental afferent fibers to motoneurons, at least in neonates (Arvanian et al. 2003). The improvement in performance might have been largely due to local connections within the distal cord. In a conceptually similar experiment (Liu et al. 1999) fibroblasts engineered to secrete BDNF were implanted into a partial cervical hemisection. They found regeneration of rubrospinal fibers up to four segments and remarkably this regeneration occurred through the white matter rather than being restricted to the gray matter. This was attributed to a higher intrinsic growth capacity of rubrospinal compared to corticospinal fibers that enabled them to regenerate through the hostile environment of white matter. They observed improved reaching behavior of the forelimb on the lesioned side in the BDNF-treated animals. An important control carried out by these authors was to relesion the regenerated fibers and to demonstrate that the recovered behavior was diminished substantially for up to 5 weeks after the relesion. This control experiment points to a functional effect of the regenerated fibers.

Fortun et al. (2009) demonstrated that the loss of forelimb function after a C4/C5 dorsal lesion in rats could be partially reversed after provision of NT-3. In these experiments NT-3 was delivered non invasively using AAV viruses injected into forelimb muscles. The NT-3 was transported to the spinal cord and it was concluded that this enhanced the function of projections from the corticospinal tract. This was supported by findings of decreased astrogliosis and a denser corticospinal tract projection due either to less retraction or more sprouting of this synaptic input into the zone of the injury.

The clearest example of the effect of neurotrophins on locomotor function has come in studies where the cord was transected and no regeneration was allowed. It

is well established that hind limb stepping function can be enhanced after a complete thoracic transection by locomotor training (Lovely et al. 1986; Barbeau and Rossignol 1987; de Leon et al. 1998; Leblond et al. 2003) due to preservation of locomotor modules necessary for stepping after spinalization (Boyce and Lemay 2009). Rats transected as neonates can be trained to step on a treadmill, but rats transected as adults can only be trained if epidural stimulation is provided during the training (Courtine et al. 2009). Recent experiments have demonstrated that successful training affects certain electrophysiological parameters, particularly the amplitude of the spindle-evoked monosynaptic EPSP which is increased and the amplitude of the AHP which is decreased (Petruska et al. 2007). Since neurotrophins also affect these cellular and synaptic properties (see above), it might be expected that they would be able to elicit stepping without the need for training or perhaps facilitate the effects of training. A possible role for neurotrophins in recovery of stepping after spinal cord injury is further supported by the finding that neurotrophin levels (BDNF, NT-3 and NT-4/5) are elevated in the decentralized, distal portion of the transected cord of rats subjected to step training (Ying et al. 2005; Côté et al. 2011) (Fig. 2). Another piece of evidence in favour of a role for neurotrophins is the finding that sequestering neurotrophins reduces the effect of step training in spinal rats (Ying et al. 2008).

Boyce et al. (2007) first demonstrated that provision of a combination of BDNF and NT-3 to cats with complete thoracic spinal transection lesions via engineered fibroblasts enabled them to recover hindlimb stepping ability on a treadmill without the need for training (Fig. 3). Training further improved stepping performance by increasing hind limb step length. Based on the available electrophysiological data it seems reasonable to suggest that increasing the stretch reflex might play some role in this recovery of stepping function (Pearson 2001) although the elevated stretch reflex might interfere with functional recovery of stepping by increasing spasticity (Thompson et al., 2013). More important is the need to consider the effect of neurotrophins on the interneurons responsible for the patterned activity of stepping, specifically the central pattern generator.

Another issue requiring evaluation is whether both BDNF and NT-3 are required to elicit stepping in spinal animals. Previous investigators have reported that BDNF delivered via osmotic minipump (Jakeman et al. 1998) or viral vectors (Blits et al. 2003) could provoke hindlimb movements which fell short of walking. Recent evidence (Boyce et al. 2012) suggests that spinal rats treated with either AAV-NT-3 or AAV-BDNF at the thoracic transection site can step on a treadmill, but AAV-NT-3 is less effective requiring additional input usually in the form of high intensity perineal stimulation. Treadmill stepping after BDNF treatment requires no such additional stimulation, and furthermore BDNF-treated rats can walk overground across a stationary platform supporting their own weight on their hindlimbs. This finding has potentially important translational implications; however, increased sensitivity of nociceptive pathways and the spasticity that accompany the locomotion may prevent its widespread use unless the side effects can be eliminated or at least minimized.

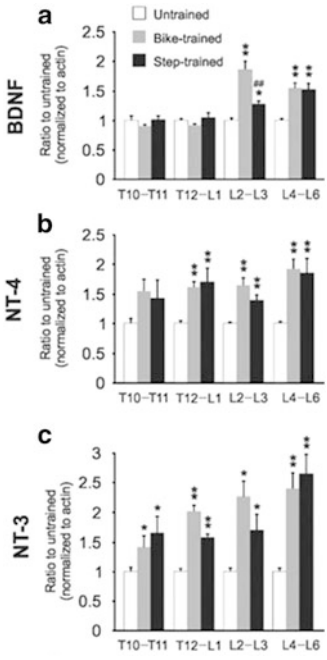

Fig. 2 Elevation of neurotrophin levels in spinal cord of adult rats caudal to transection after step or bike training. Note the increased level of BDNF, NT-4, and NT-3 especially in the lumbar cord segments. From Coté et al., J Neurotrauma 2011 with permission

The foregoing discussion has emphasized the potential effects of delivering exogenous neurotrophins to overcome deficits in animals with an injured spinal cord. In contrast there are deficits that are reversed by neutralization of endogenous neurotrophins. Autonomic dysreflexia, a condition that develops after spinal cord injury, is characterized by dangerous increases in blood pressure in response to stimulation of visceral afferents. Krenz et al. (1999) showed that this rise in blood pressure could be significantly diminished in spinal injured rats (high thoracic transection) by provision of an antibody to NGF. The mechanism of action was shown to be related to a reduction in sprouting by CGRP-expressing fibers in the dorsal horn possibly as a result of increased levels of NGF observed in DRG cell bodies after spinal injury (Brown et al. 2007). This effect of NGF is similar to that observed on CGRP-expressing trkA-expressing nociceptive afferents described above. Similar NGF-induced sympathetic hyperinnervation of the heart has been suggested to be an important factor in the increased susceptibility of patients with high thoracic spinal cord injury to ventricular arrhythmias (Lujan et al. 2009). Mitsui et al. (2005) demonstrated acceleration in the recovery of micturition in thoracically contused rats treated with BDNF- and NT-3-expressing fibroblasts. Anatomical evaluation of these rats revealed increased projections from CGRP- and TRPV1-expressing sensory fibers and 5-HT- and DβH-expressing descending fibers which were speculated to contribute to the recovery of function.

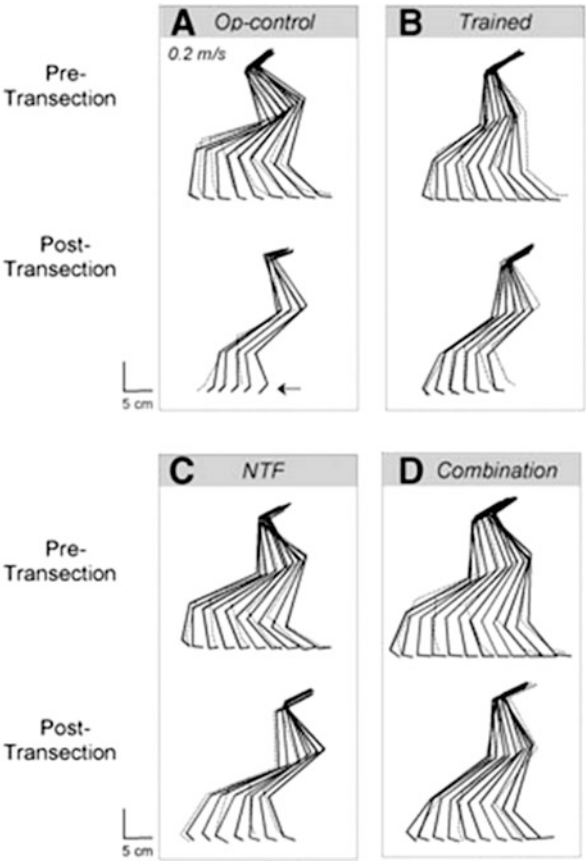

Fig. 3 Stepping carried out by hind limbs in adult cats after thoracic transection and subjected to treatment with NT-3 and BDNF via fibroblasts, treadmill step training, or both. Note the improvement in limb swing excursion (step length) and plantar stepping (as opposed to stepping on the dorsal surface of the foot) after either treatment alone and especially after the combination treatment. From Boyce et al., J Neurophysiol. 2007 (with permission)

Conclusion

It is clear that neurotrophins have potent effects on the damaged spinal cord. The challenge is to establish the actions of the individual neurotrophins and then to try to devise appropriate combinations of treatments involving different neurotrophins and other treatments such as training, plasticity enhancers such as chondroitinase (Kwok et al. 2008) and agents that promote axonal elongation in the CNS such as anti-Nogo (Starkey and Schwab 2011). The cellular location of the different trk receptors will determine which class of cells will be affected by, and will benefit from, the action of specific neurotrophin(s). Developmental studies will be important to take into account since cells in the injured cord may revert to a developmentally less advanced stage, and it has been shown in at least some cases that the same neurotrophin can have different actions during different stages of development (Zhu et al. 2004). Finally, neurotrophins can elicit different actions depending on which intracellular signaling system is activated, an area that is discussed at length in the chapter by Chao, and the evidence in simple systems suggests that different channels can be induced depending on the

time course of neurotrophin application (Toledo-Aral et al. 1995). Together, these studies suggest that there are many factors influencing the action of neurotrophins and it will not be sufficient to investigate them singly. Furthermore, the possibility for interaction or cooperativity between the different effects must be taken into account. Thus although the neurotrophins are very promising tools to reverse the effects of spinal cord injury, it is important to view them in a broad context in order to make the most effective use of their properties in repairing the damaged spinal cord.

Acknowledgment The authors' research was supported by the Christopher and Dana Reeve Foundation and a grant from the National Institutes of Health (5 R01 NS 16996).

References

Althaus HH, Klöppner S, Klopfleisch S, Schmitz M (2008) Oligodendroglial cells and neurotrophins: a polyphonic cantata in major and minor. J Mol Neurosci 35:65–79

Alto LT, Havton LA, Conner JM, Hollis ER, Blesch A, Tuszynski MH (2009) Chemotropic guidance facilitates axonal regeneration and synapse formation after spinal cord injury. Nat Neurosci 12:1106–1113

Arvanian VL, Mendell LM (2001a) Removal of NMDA receptor Mg^{2+} block extends the action of neurotrophin-3 on synaptic transmission in neonatal rat motoneurons. J Neurophysiol 86:123–129

Arvanian VL, Mendell LM (2001b) Acute modulation of synaptic transmission to motoneurons by BDNF in the neonatal rat spinal cord. Eur J Neurosci 14:1800–1808

Arvanian VL, Horner PJ, Gage FH, Mendell LM (2003) Chronic Neurotrophin-3 strengthens synaptic connections to motoneurons in the neonatal rat. J Neurosci 23:8706–8712

Arvanian VL, Bowers WJ, Petruska JC, Manuzon H, Narrow WC, Motin V, Federoff HJ, Mendell LM (2004) Viral delivery of NR2D subunits reduces Mg^{2+} block of NMDA receptor and restores NT-3-induced potentiation of AMPA/kainate responses in maturing rat motoneurons. J Neurophysiol 92:2394–2404

Arvanian VL, Bowers WJ, Anderson AJ, Horner PJ, Federoff HJ, Mendell LM (2006) Combined delivery of neurotrophin-3 and NMDA receptors 2D subunit strengthens synaptic transmission in contused and staggered double hemisected spinal cord of neonatal rat. Exp Neurol 197:347–352

Arvanov VL, Seebach BS, Mendell LM (2000) NT-3 evokes an LTP- like facilitation of AMPA/Kainate- mediated synaptic transmission in the neonatal rat spinal cord. J Neurophysiol 84:752–758

Bamber NI, Li H, Lu X, Oudega M, Aebischer P, Xu XM (2001) Neurotrophins BDNF and NT-3 promote axonal re-entry into the distal host spinal cord through Schwann cell-seeded mini-channels. Eur J Neurosci 3:257–268

Barbeau H, Rossignol S (1987) Recovery of locomotion after chronic spinalization in the adult cat. Brain Res 412:84–95

Barde YA, Edgar D, Thoenen H (1982) Purification of a new neurotrophic factor from mammalian brain. EMBO J 1:549–553

Bareyre FM, Kerschensteiner M, Raineteau O, Mettenleiter TC, Weinmann O, Schwab ME (2004) The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. Nat Neurosci 7:269–277

Beattie MS (2004) Inflammation and apoptosis: linked therapeutic targets in spinal cord injury. Trends Mol Med 10:580–583

Blits B, Oudega M, Boer GJ, Bartlett Bunge M, Verhaagen J (2003) Adeno-associated viral vector-mediated neurotrophin gene transfer in the injured adult rat spinal cord improves hind-limb function. Neuroscience 118:271–281

Bonner JF, Connors TM, Silverman WF, Kowalski DP, Lemay MA, Fischer I (2011) Grafted neural progenitors integrate and restore synaptic connectivity across the injured spinal cord. J Neurosci 31:4675–4686

Boyce VS, Lemay MA (2009) Modularity of endpoint force patterns evoked using intraspinal microstimulation in treadmill trained and/or neurotrophin-treated chronic spinal cats. J Neurophysiol 101:1309–1320

Boyce VS, Tumolo M, Fischer I, Murray M, Lemay MA (2007) Neurotrophic factors promote and enhance locomotor recovery in untrained spinalized cats. J Neurophysiol 98:1988–1996

Boyce VS, Park J, Gage FH, Mendell LM (2012) Differential effects of BDNF and NT-3 on hindlimb function in paraplegic rats. Eur J Neurosci 53:221–232

Boyd JG, Gordon T (2003) Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. Mol Neurobiol 2:277–324

Bradbury EJ, Khemani S, King VR, Priestley JV, McMahon SB (1999) NT-3 promotes growth of lesioned adult rat sensory axons ascending in the dorsal columns of the spinal cord. Eur J Neurosci 11:3873–3883

Bregman BS (1998) Regeneration in the spinal cord. Curr Opin Neurobiol 8:800–807

Bregman BS, McAtee M, Dai HN, Kuhn PL (1997) Neurotrophic factors increase axonal growth after spinal cord injury and transplantation in the adult rat. Exp Neurol 148:475–494

Bretzner F, Liu J, Currie E, Roskams AJ, Tetzlaff W (2008) Undesired effects of a combinatorial treatment for spinal cord injury–transplantation of olfactory ensheathing cells and BDNF infusion to the red nucleus. Eur J Neurosci 28:1795–1807

Brock JH, Rosenzweig ES, Blesch A, Moseanko R, Havton LA, Edgerton VR, Tuszynski MH (2010) Local and remote growth factor effects after primate spinal cord injury. J Neurosci 30:9728–9737

Brown A, Ricci MJ, Weaver LC (2007) NGF mRNA is expressed in the dorsal root ganglia after spinal cord injury in the rat. Exp Neurol 205:283–286

Chen HH, Hippenmeyer S, Arber S, Frank E (2003) Development of the monosynaptic stretch reflex circuit. Curr Opin Neurobiol 13:96–102

Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S (1997) Distribution of brain derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. J Neurosci 17:2295–2313

Côté MP, Azzam GA, Lemay MA, Zhukareva V, Houle JD (2011) Activity-dependent increase in neurotrophic factors is associated with an enhanced modulation of spinal reflexes after spinal cord injury. J Neurotrauma 28:299–309

Courtine G, Gerasimenko Y, van den Brand R, Yew A, Musienko P, Zhong H, Song B, Ao Y, Ichiyama RM, Lavrov I, Roy RR, Sofroniew MV, Edgerton VR (2009) Transformation of nonfunctional spinal circuits into functional states after the loss of brain input. Nat Neurosci 12:1333–1342

de Leon RD, Hodgson JA, Roy RR, Edgerton VR (1998) Locomotor capacity attributable to step training versus spontaneous recovery after spinalization in adult cats. J Neurophysiol 79:1329–1340

DiStefano PS, Friedman B, Radziejewski C, Alexander C, Boland P, Schick CM, Lindsay RM, Wiegand SJ (1992) The neurotrophins BDNF, NT-3, and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons. Neuron 8:983–993

Feron F, Perry C, Cochrane J, Licina P, Nowitzke A, Urquhart S, Geraghty T, Mackay-Sim A (2005) Autologous olfactory ensheathing cell transplantation in human spinal cord injury. Brain 128:2951–2960

Fitch MT, Silver J (2008) CNS injury, glial scars, and inflammation: inhibitory extracellular matrices and regeneration failure. Exp Neurol 209:294–301

Fortun J, Puzis R, Pearse DD, Gage FH, Bunge MB (2009) Muscle injection of AAV-NT3 promotes anatomical reorganization of CST axons and improves behavioral outcome following SCI. J Neurotrauma 26:941–953

García-Alías G, Petrosyan H, Schnell H, Horner PJ, Bowers WJ, Mendell LM, Fawcett JW, Arvanian VL (2011) Chondroitinase ABC combined with NT3 secretion and NR2D expression promotes axonal plasticity and functional recovery in rats with lateral hemisection of the spinal cord. J Neurosci 31:17788–17799

Giehl KM, Tetzlaff W (1996) BDNF and NT-3, but not NGF, prevent axotomy-induced death of rat corticospinal neurons in vivo. Eur J Neurosci 8:1167–1175

Gonzalez M, Collins WF III (1997) Modulation of motoneuron excitability by brain derived neurotrophic factor. J Neurophysiol 77:502–506

Grill R, Murai K, Blesch A, Gage FH, Tuszynski MH (1997) Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury. J Neurosci 17:5560–5572

Hagg T, Baker KA, Emsley JG, Tetzlaff W (2005) Prolonged local neurotrophin-3 infusion reduces ipsilateral collateral sprouting of spared corticospinal axons in adult rats. Neuroscience 130:875–887

Henderson CE, Camu W, Mettling C, Gouin A, Poulsen K, Karihaloo M, Rullamas J, Evans T, McMahon SB, Armanini MP, Berkemeier L, Phillips HS, Rosenthal A (1993) Neurotrophins promote motor neuron survival and are present in embryonic limb bud. Nature 363:266–270

Hendriks WTJ, Ruitenberg MJ, Blits B, Boer GJ, Verhaagen J (2004) Viral vector-mediated gene transfer of neurotrophins to promote regeneration of the injured spinal cord. In: Aloe L, Calza L (eds) NGF and related molecules in health and disease, vol 146, Progress in brain research. Elsevier, Amsterdam, pp 451–476

Hermens WT, Verhaagen J (1998) Viral vectors, tools for gene transfer in the nervous system. Prog Neurobiol 55:399–432

Jakeman LB, Wei P, Guan Z, Stokes BT (1998) Brain derived neurotrophic factor stimulates hindlimb stepping and sprouting of cholinergic fibers after spinal cord injury. Exp Neurol 154:170–184

Jin Y, Tessler A, Fischer I, Houle JD (2002) Transplants of fibroblasts genetically modified to express BDNF promote axonal regeneration from supraspinal neurons following chronic spinal cord injury. Exp Neurol 177:265–275

Kobayashi NR, Fan D, Giehl KM, Bedard AM, Wiegand SJ, Tetzlaff W (1997) BDNF and NT-4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-43 and T_1-tubulin mRNA expression and promote axonal regeneration. J Neurosci 17:9583–9595

Koliatsos VE, Clatterbuck RE, Winslow JW, Cayouette MH, Price DL (1993) Evidence that brain derived neurotrophic factor is a trophic factor for motor neurons in vivo. Neuron 10:359–367

Krenz NR, Meakin SO, Krassioukov AV, Weaver LC (1999) Neutralizing intraspinal nerve growth factor blocks autonomic dysreflexia caused by spinal cord injury. J Neurosci 19:7405–7414

Kwok JC, Afshari F, García-Alías G, Fawcett JW (2008) Proteoglycans in the central nervous system: plasticity, regeneration and their stimulation with chondroitinase ABC. Restor Neurol Neurosci 26:131–145

Leblond H, L'Esperance M, Orsal D, Rossignol S (2003) Treadmill locomotion in the intact and spinal mouse. J Neurosci 23:11411–11419

Li Y, Field PM, Raisman G (1997) Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. Science 277:2000–2002

Liu Y, Himes BT, Moul J, Huang W, Chow SY, Tessler A, Fischer I (1997) Application of recombinant adenovirus for in vivo gene delivery to spinal cord. Brain Res 768:19–29

Liu Y, Kim D, Himes BT, Chow SY, Schallert T, Murray M, Tessler A, Fischer I (1999) Transplants of fibroblasts genetically modified to express BDNF promote regeneration of adult rat rubrospinal axons and recovery of forelimb function. J Neurosci 19:4370–4387

Lovely RG, Gregor RJ, Roy RR, Edgerton VR (1986) Effects of training on the recovery of full-weight-bearing stepping in the adult spinal cat. Exp Neurol 92:421–435
Lu P, Jones LL, Tuszynski MH (2005) BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury. Exp Neurol 191:344–360
Lu P, Wang Y, Graham L, McHale K, Gao M, Wu D, Brock J, Blesch A, Rosenzweig ES, Havton LA, Zheng B, Conner JM, Marsala M, Tuszynski MH (2012) Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. Cell 150:1264–1273
Lujan HL, Chen Y, Dicarlo SE (2009) Paraplegia increased cardiac NGF content, sympathetic tonus, and the susceptibility to ischemia-induced ventricular tachycardia in conscious rats. Am J Physiol Heart Circ Physiol 296:H1364–H1372
Mantyh PW, Koltzenburg M, Mendell LM, Tive L, Shelton DL (2011) Antagonism of nerve growth factor-TrkA signaling and the relief of pain. Anesthesiology 115:189–204
McAllister AK, Katz LC, Lo DC (1997) Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. Neuron 18:767–778
McDonald JW (1999) Repairing the damaged spinal cord. Sci Am 281:64–73
McTigue DM, Horner PJ, Stokes BT, Gage FH (1998) Neurotrophin-3 and brain derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. J Neurosci 18:5354–5365
Mendell LM, Johnson RD, Munson JB (1999) Neurotrophin modulation of the monosynaptic reflex after peripheral nerve transection. J Neurosci 19:3162–3170
Menei P, Montero-Menei C, Whittemore SR, Bunge RP, Bunge MB (1998) Schwann cells genetically modified to secrete human BDNF promote enhanced axonal regrowth across transected adult rat spinal cord. Eur J Neurosci 10:607–621
Mitsui T, Fischer I, Shumsky JS, Murray M (2005) Transplants of fibroblasts expressing BDNF and NT-3 promote recovery of bladder and hindlimb function following spinal contusion injury in rats. Exp Neurol 194:410–431
Müller HW, Beckh S, Seifert W (1984) Neurotrophic factor for central neurons. Proc Natl Acad Sci USA 81:1248–1252
Murray KC, Nakae A, Stephens MJ, Rank M, D'Amico J, Harvey PJ, Li X, Harris RLW, Ballou EW, Anelli R, Heckman CJ, Mashimo T, Vavrek R, Sanelli L, Gorassini MA, Bennett DJ, Fouad K (2010) Recovery of motoneuron and locomotor function after spinal cord injury depends on constitutive activity in 5-HT2C receptors. Nat Med 16:694–700
Nieto-Sampedro M, Lewis ER, Cotman CW, Manthorpe M, Skaper SD, Barbin G, Longo FM, Varon S (1982) Brain injury causes a time-dependent increase in neuronotrophic activity at the lesion site. Science 217:860–861
Novikova LN, Novikov LN, Kellerth JO (2002) Differential effects of neurotrophins on neuronal survival and axonal regeneration after spinal cord injury in adult rats. J Comp Neurol 452:255–263
Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesiumgates glutamate-activated channels in mouse central neurons. Nature 307:462–465
Pearson KG (2001) Could enhanced reflex function contribute to improving locomotion after spinal cord repair? J Physiol 533:75–81
Petruska JC, Mendell LM (2009) Nerve growth factor. In: Squire LR (ed) Encyclopedia of Neuroscience, vol 6. Elsevier, Oxford, pp 71–78
Petruska JC, Ichiyama RM, Crown ED, Tansey KE, Roy RR, Edgerton VR, Mendell LM (2007) Changes in motoneuron properties and synaptic inputs related to step training following spinal cord transection in rats. J Neurosci 27:4460–4471
Petruska JC, Kitay B, Boyce VS, Kaspar BK, Pearse DD, Gage FH, Mendell LM (2010) Intramuscular AAV delivery of NT-3 alters synaptic transmission to motoneurons in adult rats. Eur J Neurosci 32:997–1005
Ramer MS, Priestley JV, McMahon SB (2000) Functional regeneration of sensory axons into the adult spinal cord. Nature 403:312–316

Ramer MS, Bishop T, Dockery P, Mobarak MS, O'Leary D, Fraher JP, Priestley JV, McMahon SB (2002) Neurotrophin-3-mediated regeneration and recovery of proprioception following dorsal rhizotomy. Mol Cell Neurosci 19:239–249

Sasaki M, Radtke C, Tan AM, Zhao P, Hamada H, Houkin K, Honmou O, Kocsis JD (2009) BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. J Neurosci 29:14932–14941

Schnell L, Schneider R, Kolbeck R, Barde Y-A, Schwab ME (1994) Neurotrophin-3 enhances sprouting of corticospinal tract during development and after adult spinal cord lesion. Nature 367:170–173

Schnell L, Hunanyan A, Bowers W, Horner P, Federoff H, Gullo M, Schwab ME, Mendell LM, Arvanian VL (2011) Combined delivery of Nogo-A antibody, neurotrophin-3 and NMDA-2D subunits establishes a functional "detour" in a hemisected spinal cord. Eur J Neurosci 34:1256–1267

Seebach BS, Arvanov V, Mendell LM (1999) Neurotrophin influence on the development of segmental reflexes in the rat. J Neurophysiol 81:2398–2405

Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde Y-A (1992) Brain derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. Nature 360:757–759

Senut MC, Tuszynski MH, Raymon HK, Suhr ST, Liou NH, Jones KR, Reichardt LF, Gage FH (1995) Regional differences in responsiveness of adult CNS axons to grafts of cells expressing human neurotrophin 3. Exp Neurol 135:36–55

Shanthanelson M, Mendell LM (2010) Differential NR2B- subunit expression at dorsal root and ventrolateral funiculus synapses on lumbar motoneurons of neonatal rat. Neuroscience 166:730–737

Shanthanelson M, Arvanian VL, Mendell LM (2009) Input- specific plasticity of NMDA- receptor mediated synaptic responses in neonatal rat motoneurons. Eur J Neurosci 29:2125–2136

Shneider NA, Mentis GZ, Schustak J, O'Donovan MJ (2009) Functionally reduced sensorimotor connections form with normal specificity despite abnormal muscle spindle development: the role of spindle-derived neurotrophin 3. J Neurosci 29:4719–4735

Starkey ML, Schwab ME (2011) Anti-Nogo-A and training: can one plus one equal three? Exp Neurol 232:81–89

Strack S, Colbran RJ (1998) Autophosphorylation-dependent targeting of calcium/calmodulin-dependent protein kinase II by the NR2B subunit of the N-methyl- D-aspartate receptor. J Biol Chem 273:20689–20692

Tan AM, Petruska JC, Mendell LM, Levine JM (2007) Sensory afferents regenerated into dorsal columns after spinal cord injury remain in a chronic pathophysiological state. Exp Neurol 206:257–268

Thompson AK, Pomerantz FR, Wolpaw JR (2013) Operant conditioning of a spinal reflex can improve locomotion after spinal cord injury in humans. J Neurosci 33:2365–2375

Tobias CA, Dhoot NO, Wheatley MA, Tessler A, Murray M, Fischer I (2001) Grafting of encapsulated BDNF-producing fibroblasts into the injured spinal cord without immune suppression in adult rats. J Neurotrauma 18:287–301

Tobias CA, Shumsky JS, Shibata M, Tuszynski MH, Fischer I, Tessler A, Murray M (2003) Delayed grafting of BDNF and NT-3 producing fibroblasts into the injured spinal cord stimulates sprouting, partially rescues axotomized red nucleus neurons from loss and atrophy, and provides limited regeneration. Exp Neurol 184:97–113

Toledo-Aral JJ, Brehm P, Halegoua S, Mandel G (1995) A single pulse of nerve growth factor triggers long-term neuronal excitability through sodium channel gene induction. Neuron 14:607–611

Tuszynski MH, Peterson DA, Ray J, Baird A, Nakahara Y, Gage FH (1994) Fibroblasts genetically modified to produce nerve growth factor induce robust neuritic ingrowth after grafting to the spinal cord. Exp Neurol 126:1–14

Tuszynski MH, Mafong E, Meyer S (1996) Central infusions of brain derived neurotrophic factor and neurotrophin-4/5, but not nerve growth factor and neurotrophin-3, prevent loss of the cholinergic phenotype in injured adult motor neurons. Neuroscience 71:761–771

Tuszynski MH, Grill R, Jones LL, Brant A, Blesch A, Low K, Lacroix S, Lu P (2003) NT-3 gene delivery elicits growth of chronically injured corticospinal axons and modestly improves functional deficits after chronic scar resection. Exp Neurol 181:47–56

Vavrek R, Girgis J, Tetzlaff W, Hiebert GW, Fouad K (2006) BDNF promotes connections of corticospinal neurons onto spared descending interneurons in spinal cord injured rats. Brain 129:1534–1545

von Meyenburg J, Brosamle C, Metz GAS, Schwab ME (1998) Regeneration and sprouting of chronically injured corticospinal tract fibers in adult rats promoted by NT-3 and the mAb IN-1, which neutralizes myelin-associated neurite growth inhibitors. Exp Neurol 154:583–594

Xu XM, Guenard V, Kleitman N, Aebischer P, Bunge MB (1995) A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. Exp Neurol 134:261–272

Yan Q, Elliott JL, Matheson C, Sun J, Zhang L, Mu X, Rex KL, Snider WD (1993) Influences of neurotrophins on mammalian motoneurons in vivo. J Neurobiol 24:1555–1577

Ye JH, Houle JD (1997) Treatment of the chronically injured spinal cord with neurotrophic factors can promote axonal regeneration from supraspinal neurons. Exp Neurol 143:70–81

Ying Z, Roy RR, Edgerton VR, Gómez-Pinilla F (2005) Exercise restores levels of neurotrophins and synaptic plasticity following spinal cord injury. Exp Neurol 193:411–419

Ying Z, Roy RR, Zhong H, Zdunowski S, Edgerton VR, Gomez-Pinilla F (2008) BDNF-exercise interactions in the recovery of symmetrical stepping after a cervical hemisection in rats. Neuroscience 155:1070–1078

Zhou L, Baumgartner BJ, Hill-Felberg SJ, McGowen LR, Shine HD (2003) Neurotrophin-3 expressed in situ induces axonal plasticity in the adult injured spinal cord. J Neurosci 23:1424–1431

Zhu W, Galoyan SM, Petruska JC, Oxford GS, Mendell LM (2004) A developmental switch in acute sensitization of small dorsal root ganglion (DRG) neurons to capsaicin or noxious heating by NGF. J Neurophysiol 92:3148–3152

Neurotrophins and Psychiatric Disorders

E. Castrén

Abstract

Increasing number of studies has during the last decade linked neurotrophic factors with the pathophysiology of neuropsychiatric disorders and with the mechanisms of action of drugs used for the treatment of these disorders. In particular, brain-derived neurotrophic factor BDNF and its receptor TrkB have been connected with the pathophysiology in mood disorders, and there is strong evidence that BDNF signaling is critically involved in the recovery from depression with both pharmacological and psychological means. Neurotrophins play a central role in neuronal plasticity and network connectivity in developing adult brain, and recent evidence links plasticity and network rewiring with mood disorders and their treatment. Therefore, neurotrophins should not be seen as happiness factors but as critical tools in the process where brain networks are optimally tuned to environment, and it is against this background that the effects of neurotrophins on neuropsychiatric disorders should be looked at.

Keywords

BDNF • TrkB • Mood disorders • Depression • Anxiety • Schizophrenia • Antidepressant drugs

1 Introduction

Neuropsychiatric disorders are complex brain disease with unknown etiology. Many of them have a clear genetic predisposition; however, genetic association studies have been largely contradictory and frustrating. However, recent genome-

E. Castrén (✉)
Neuroscience Center, University of Helsinki, PO Box 56, 00014 Helsinki, Finland

Department of Psychiatry, Columbia University, New York, NY, USA
e-mail: eero.castren@helsinki.fi

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_17,

wide association studies have begun to reveal genetic background of schizophrenia (Cichon et al. 2009), but a similar progress in mood disorders remains still to be achieved (Wray et al. 2012). While it is clear that environmental factors also influence the risk of neuropsychiatric disorders, identification of such factors has been equally difficult as has been the case with genetic factors. Stress and early life trauma have been for a long time known as predisposing factors for mood disorders (Karg et al. 2011; Caspi et al. 2003; Caspi and Moffitt 2006), and there is evidence to suggest a combination of environmental and genetic factors may explain the predisposition better than either factor alone (Caspi et al. 2003; Caspi and Moffitt 2006; Casey et al. 2009). A particular problem related to neuropsychiatric disorders is the lack of suitable animal models. Although several genetic and environmental models have been proposed, essentially all are unsatisfactory in one way or another (Krishnan and Nestler 2008; David et al. 2009).

There is increasing evidence that development and maturation of neuronal connectivity are critical component in the pathophysiology of essentially all neuropsychiatric disorders (Krishnan and Nestler 2008; Lewis et al. 2005). As neurotrophins have been implicated in brain development and in particular in the plasticity and maturation of neuronal circuits, it is understandable that neurotrophins have been popular candidate genes for psychiatric diseases. Increasing evidence has, indeed, implicated BDNF and TrkB in mood disorders and in particular in the mechanisms of action of antidepressant drug treatment.

2 Neurotrophins and Mood Disorders

The role of neurotrophins in the pathophysiology of mood disorders and in the treatment strategies to alleviate these disorders have received by far the most attention among the potential interaction of neurotrophins with neuropsychiatric disorders (Krishnan and Nestler 2008; Castrén and Rantamäki 2010). Most studies have focused on depression and antidepressant drugs, but increasing numbers of studies are now revealing a role for BDNF signaling in anxiety disorders (Casey et al. 2009). It is important to note that depression and anxiety are often comorbid in humans and that antidepressant drugs are also widely used to treat anxiety disorders. Therefore, even though there are separate genetic models and behavioral test for anxiety and depression, it is probable that the underlying mechanisms are, at least to a certain extent, shared between depression and anxiety.

2.1 Genetic Association of Neurotrophins with Mood Disorders

Several different genetic approaches have linked BDNF and TrkB to neurodevelopmental and behavioral disorders. Humans heterozygous for the loss of a BDNF or a TrkB allele show mental retardation and cognitive deficits, including impaired memory and extreme obesity at the age of 9 years (Han et al. 2008; Gray et al. 2006; Yeo et al. 2004). A recent study suggests that haplo-insufficiency of BDNF gene

may be associated with autism, attention deficiency, and bipolar disorder (Shinawi et al. 2011). It is noteworthy that haplo-insufficiency of BDNF and TrkB produces a very similar phenotype, although only very few cases have been described so far and only at young age (Gray et al. 2006; Yeo et al. 2004).

Since the description of a common polymorphism in the pro-region of human BDNF gene, Val66Met, a vast number of studies have investigated the association of this polymorphism in a variety of neurological and neuropsychiatric disorders, including mood disorders, but the results have generally been very variable and positive associations have often not been independently replicated (Frielingsdorf et al. 2010). Early studies indicated association with depression and bipolar disorder, but meta-analyses have not confirmed these findings (Zou et al. 2010; Kang et al. 2010; Liu et al. 2009; Verhagen et al. 2010; Tsai et al. 2003; Domschke et al. 2010), although a significant association to depression was reported in one meta-analysis (Verhagen et al. 2010). However, there is evidence that an association is significant in the group of depressed patients with adverse early life experiences (Kaufman et al. 2006; Gerritsen et al. 2012) or in stroke patients (Kim et al. 2007). Under laboratory conditions, met allele carriers show impairment in extinguishing conditioned fear response (Soliman et al. 2010). Interestingly, transgenic mice carrying methionine in this locus show a comparable phenotype, with enhanced anxiety and impaired extinction of fear responses (Frielingsdorf et al. 2010; Chen et al. 2006; Soliman et al. 2010).

2.2 BDNF in Brain and Serum in Depressed Patients

BDNF levels have been found to be reduced in postmortem samples of patients having suffered from depression (Karege et al. 2005b; Chen et al. 2001; Pandey et al. 2010; Dwivedi et al. 2003). Furthermore, the activity of the MAP kinase pathway, a major signaling pathway downstream of TrkB and also a pathway regulating BDNF synthesis, was recently shown to be reduced in depressed patients (Duric et al. 2010; Dwivedi et al. 2006a, 2009), while the MAP kinase phosphatase, a negative regulator of this pathway, was increased (Duric et al. 2010). The expression of MAP kinase phosphatase is increased by stress in rodents and mice lacking this enzyme are resilient to stress, indicating that MAP kinase pathway, potentially regulated by TrkB activity, plays an important role in depression and stress (Duric et al. 2010; Duman 2002).

BDNF is abundant in blood platelets, and it is released upon platelet activation. Consequently, while BDNF levels in plasma are very low, serum levels are high and variable among individuals. Several studies, including two meta-analyses, have shown that serum BDNF levels are reduced in depressed patients (Karege et al. 2005a; Shimizu et al. 2003; Matrisciano et al. 2009; Sen et al. 2008; Brunoni et al. 2008). BDNF levels in whole blood do not seem to be altered in depression suggesting that it is the release of BDNF from activated platelets that varies with mood, not the concentration of BDNF in platelets (Karege et al. 2005a). Interestingly, serum BDNF levels are normalized upon successful treatment of depression

with a variety of different treatments, including chemical antidepressants, electroconvulsive therapy, sleep deprivation therapy, and repetitive transcranial stimulation (Sen et al. 2008; Lee and Kim 2008; Matrisciano et al. 2009; Okamoto et al. 2008; Gorgulu and Caliyurt 2009; Zanardini et al. 2006; Gonul et al. 2005). It is currently unclear whether platelet BDNF is derived from megakaryocytes or taken up by circulating platelets (Fujimura et al. 2002) and what, if any, is the relationship between BDNF in platelets and neurons. Nevertheless, these data suggest a possibility that a similar kind of impediment of BDNF release from both platelets and neurons might be associated with mood disorders. In this context, it was recently shown that peripheral subcutaneous administration of BDNF to rats increased BDNF levels and signaling in brain and produced antidepressant-like effects in behavioral tests (Schmidt and Duman 2010).

2.3 Stress and BDNF Signaling

Stress predisposes to depression in humans, and chronic stress has been widely used as a model of depression in experimental animals. Stress has widespread effects on brain BDNF levels. Chronic stress reduces BDNF mRNA (Duman and Monteggia 2006; Nibuya et al. 1995; Smith et al. 1995; Haenisch et al. 2009; Russo-Neustadt et al. 2001; Alfonso et al. 2006; Duric and McCarson 2005) and BDNF protein in brain (Haenisch et al. 2009; Xu et al. 2004). Chronic corticosterone administration, another model of stress, has also been shown to be associated with reduced BDNF levels (Paizanis et al. 2010; Dwivedi et al. 2006b). Antidepressant treatment seems to prevent the effects of stress on BDNF expression (Russo-Neustadt et al. 2001; Haenisch et al. 2009; MacQueen et al. 2003; Nibuya et al. 1995; Xu et al. 2004; Bravo et al. 2009; Gersner et al. 2010), and BDNF gene transfer increases stress resilience (Taliaz et al. 2011). Environmental stress, such as perinatal exposure to methyl mercury, brings about long-lasting reduction in BDNF mRNA levels in the hippocampus and is associated with cognitive and emotional disturbances in adulthood (Onishchenko et al. 2007, 2008). Interestingly, both behavioral effects and BDNF levels can be reversed in adulthood by antidepressant drug treatment (Onishchenko et al. 2008).

2.4 Effects of BDNF on Depression-Like Behavior in Rodents

Injection of BDNF into midbrain or hippocampal regions produces an antidepressant-like behavior in rats and mice in the forced swimming test (FST) and the learned helplessness paradigm (Siuciak et al. 1997; Hoshaw et al. 2005; Shirayama et al. 2002; Sirianni et al. 2010). Furthermore, overexpression of TrkB or BDNF in brain also mimics the effects of antidepressant drugs (Koponen et al. 2005; Govindarajan et al. 2006). Moreover, local viral injection of BDNF into rat hippocampus counteracts behavioral effects of stress (Taliaz et al. 2011). Interestingly, hippocampal injection of BDNF potentiates the effects of

antidepressant administration on brain serotonin levels and on FST behavior (Deltheil et al. 2008, 2009) However, injection of BDNF into the ventral tegmental area increases depression-like behavior and inhibition of BDNF signaling in the nucleus accumbens, which is the target area of the dopaminergic pathway from the VTA, produces an antidepressant-like response (Eisch et al. 2003; Berton et al. 2006; Krishnan and Nestler 2008), suggesting that behavioral consequences of BDNF overexpression are dependent on the normal function of the affected networks. In humans, patients with amyotrophic lateral sclerosis receiving intrathecal BDNF administration in a context of a clinical trial showed dose-dependent disturbances of sleep and signs of mania (Dr. Richard D. Penn, University of Chicago, personal communication).

Even though stress reduces BDNF levels in brain, the majority of studies have not found any evidence that reduction in brain BDNF levels or TrkB signaling in transgenic mice would produce depression-like behavior (Saarelainen et al. 2003; MacQueen et al. 2001; Monteggia et al. 2004, 2007), although some studies have suggested that reduced BDNF levels might produce depression-like behavior in female mice and in rats (Monteggia et al. 2007; Taliaz et al. 2010). These findings are consistent with the lack of association between BDNF Val66Met polymorphism and depression (Liu et al. 2009; Verhagen et al. 2010; Domschke et al. 2010). However, mice carrying Val66Met mutation in their BDNF gene show anxiety-like behavior (Chen et al. 2006) and both human and mouse methionine carriers are impaired in extinguishing a conditioned fear response (Soliman et al. 2010), suggesting that BDNF signaling may play a more important role in the development of anxiety than depression.

2.5 Role of BDNF in the Antidepressant Drug Action

While the evidence for the role of neurotrophins in the pathophysiology of mood disorders remains controversial, a solid body of data accumulated during the last years has firmly established the role of BDNF and TrkB signaling in the mechanism of action of antidepressant drugs. Original observations from the Duman lab demonstrated that electroconvulsive shock treatment (ECT) as well as chronic administration of various chemical antidepressants increased BDNF mRNA levels in the rat hippocampus (Nibuya et al. 1995). Subsequent studies have largely confirmed this finding and extended the treatment method that increase BDNF mRNA and protein levels to include transcranial magnetic stimulation, vagus nerve stimulation, as well as atypical antidepressants (Duman and Monteggia 2006; Russo-Neustadt et al. 2001; Xu et al. 2004; Jacobsen and Mork 2004; Altar et al. 2003; Molteni et al. 2006; Czubak et al. 2009; Arunrut et al. 2009; Martinez-Turrillas et al. 2005; Li et al. 2007; Balu et al. 2008; Rogoz et al. 2008; Larsen et al. 2008; Dwivedi et al. 2006b; Soumier et al. 2009; Muller et al. 2000; Biggio et al. 2009). However, not all studies have observed an increase in BDNF mRNA or protein levels (Jacobsen and Mork 2004; Altar et al. 2003; Balu et al. 2008; Larsen et al. 2008; Schulte-Herbruggen et al. 2009; Cooke et al. 2009; Calabrese

et al. 2007; Coppell et al. 2003; Reagan et al. 2007). This variation may reflect differences in treatment times and in mouse strains used (Balu et al. 2009). Antidepressants have also been shown to increase BDNF protein levels not only in the hippocampus but also in various cortical regions (Jacobsen and Mork 2004; Altar et al. 2003; Balu et al. 2008; Schulte-Herbruggen et al. 2009; Cooke et al. 2009; Dwivedi et al. 2006b; Calabrese et al. 2007; Mannari et al. 2008; Maya Vetencourt et al. 2008). The increase in BNDF mRNA levels is only detectable after several days of treatment, which has been linked to the delayed appearance of the clinical antidepressant effect observed in humans. However, the regulation of BDNF mRNA has been suggested to be biphasic, with an early decrease followed by an increase in levels (Coppell et al. 2003; Madhav et al. 2001).

Phosphorylation of TrkB receptors has been used as an indirect assay to investigate the release of BDNF and its binding to TrkB receptors in brain in vivo (Aloyz et al. 1999). Using this assay, antidepressant drugs and treatments belonging to a variety of different chemical classes have been demonstrated to increase TrkB phosphorylation and signaling in rodent hippocampus and cortex in vivo (Saarelainen et al. 2003; Rantamäki et al. 2007). Interestingly, the increase in TrkB phosphorylation and signaling through phospholipase C-gamma and cyclic-AMP response element binding protein (CREB) are increased acutely within 30 min of drug administration and persist for at least 3 weeks of continuous treatment (Saarelainen et al. 2003; Rantamäki et al. 2007). Antidepressants have also been reported to induce a relocation of TrkB receptors to synaptic sites (Wyneken et al. 2006). Consistent with the observations that long-term antidepressant treatment is required for the increase in BDNF levels, but not in TrkB phosphorylation, recent studies have demonstrated that the increase in TrkB phosphorylation induced by antidepressants is independent of BDNF, perhaps mediated through G-protein coupled receptors (Rantamäki et al. 2011). Signaling pathways downstream of TrkB have also been observed to be activated by antidepressant drugs, particularly phosphorylation of CREB (Saarelainen et al. 2003; Conti et al. 2002; Rantamäki et al. 2007).

Consistent evidence from several laboratories has demonstrated that BDNF signaling through TrkB is necessary for the action of antidepressant drugs. The behavioral effects induced by antidepressants are blunted in mice with reduced levels of BDNF in brain (Saarelainen et al. 2003; Monteggia et al. 2004; Monteggia et al. 2007; Guiard et al. 2008; Deltheil et al. 2008), with inhibited TrkB signaling (Saarelainen et al. 2003; Li et al. 2008) or in mice carrying the met-allele of the human Val66Met polymorphism (Chen et al. 2006). Dentate gyrus appears to be the critical brain region in this context, as reduction of BDNF levels in the DG, but not in the CA1 region, blocks the effects of antidepressants (Adachi et al. 2008). More specifically, deletion of TrkB in the newborn neurons within the dentate gyrus is sufficient to inhibit the effects of antidepressants indicating a unique role for these newborn cells in the antidepressant response (Li et al. 2008). Extracellular serotonin levels are increased, and serotonin transporter function is impaired in BDNF heterozygous null mice (Guiard et al. 2008; Deltheil et al. 2008). Together with the

observations indicating that BDNF injection or expression produces an antidepressant-like behavioral response (see above), these data suggest that BDNF signaling through TrkB is both necessary and sufficient for the behavioral effects of antidepressant drugs at least in rodents.

2.6 Neuronal Plasticity and the Antidepressant Effect

If BDNF signaling is necessary and sufficient for the antidepressant response, what does it tell us about the mechanisms mediating these responses and about the pathophysiology of mood disorders? It is well established that BDNF signaling is a central and critical mediator of neuronal plasticity in developing an adult brain and a central tool for structural changes in neuronal network connectivity (Thoenen 2000; Poo 2001; Lu et al. 2014). It has, therefore, been suggested that neuronal plasticity might be a central mechanism through which antidepressant drugs mediate their effects (Castrén 2005, 2013; Castrén and Hen 2013; Duman and Monteggia 2006; Krystal et al. 2009).

Adult neurogenesis in the dentate gyrus is a prominent form of neuronal plasticity. Chronic treatment with antidepressant drugs with a variety of primary mechanisms of action have been shown to increase proliferation and survival of newly born neurons in the rodent hippocampus, but not in the subventricular regions that is the origin of newborn neurons migrating to the olfactory bulb (Malberg et al. 2000; Warner-Schmidt and Duman 2006; Madsen et al. 2000; Dulawa et al. 2004; Sahay and Hen 2007; Warner-Schmidt and Duman 2006; Samuels and Hen 2011), and there is evidence that antidepressant treatment increases neurogenesis also in human brain (Boldrini et al. 2009). Moreover, hippocampal neurogenesis has been shown to be necessary for the behavioral effects of antidepressant drugs in some, but not all tests (Santarelli et al. 2003; Li et al. 2008; Bergami et al. 2008; David et al. 2009).

Reduction of BDNF signaling or TrkB function does not interfere with the antidepressant-induced proliferation of hippocampal progenitor cells, but the survival of the newly born neurons is compromised in BDNF+/− and dominant-negative TrkB overexpressing mice (Sairanen et al. 2005; Bergami et al. 2008), indicating that BDNF plays a role in the maturation of these neurons or that BDNF might be a target-derived survival factor for newborn hippocampal neurons (Castrén 2004). Deletion or TrkB receptors specifically in adult-born hippocampal neurons interferes with their maturation and survival, and TrkB expression in the newborn neurons appears to be required for the behavioral effects of antidepressants (Bergami et al. 2008; Li et al. 2008).

In addition to neurogenesis, antidepressant drugs also regulate neuronal plasticity at a smaller scale. There is evidence that antidepressants increase synaptic turnover (Hajszan et al. 2005, 2009, 2010; O'Leary et al. 2009; Chen et al. 2008, 2009) and enhanced network activity in the hippocampus (Airan et al. 2007), and increase in plasticity-associated genes in hippocampus and prefrontal cortex (Sairanen et al. 2007). At least some of these effects are inhibited when TrkB signaling is compromised (O'Leary et al. 2009).

Recent experiments in visual cortex are beginning to shed light to the mechanisms through which antidepressant-induced plasticity regulates neuronal network function. Chronic fluoxetine administration has been shown to reactivate critical period-like plasticity in the rat and mouse visual cortex and lead to a functional recovery of developmentally miswired neuronal networks in adulthood (Maya Vetencourt et al. 2008; Chen et al. 2011). A similar kind of reactivation of markers of juvenile-type neurons was also reported by chronic antidepressant treatment in the dentate gyrus (Kobayashi et al. 2010) and amygdala (Karpova et al. 2011). The reactivation of developmental plasticity by antidepressants in the visual cortex requires BDNF signaling (Maya Vetencourt et al. 2008) and serotonin (Maya Vetencourt et al. 2011), and appears to be mediated by functional and structural effects on cortical interneurons (Maya Vetencourt et al. 2008; Chen et al. 2011). These findings are consistent with the network model of antidepressant drug action where antidepressants reactivate cortical and hippocampal plasticity, which then, under the environmental guidance, leads to the functional reorganization of neuronal networks (Castrén 2005, 2013; Castrén and Hen 2013).

2.7 NGF in Mood Disorders

In comparison with BDNF, much less information exists for a potential role of other neurotrophins in the regulation of mood. The very first description of behavioral role of any neurotrophin was the demonstration that intermale aggression increases NGF mRNA and protein levels in the hypothalamus (Spillantini et al. 1989), but there have been few subsequent studies to elaborate on this finding. Stress decreases NGF mRNA and protein levels in hippocampus, prefrontal cortex and amygdala (Alfonso et al. 2004, 2006; Schulte-Herbruggen et al. 2006; von Richthofen et al. 2003) and antidepressant treatment may reverse this effect (Alfonso et al. 2006). NGF levels have also been reported to be reduced in the Flinders sensitive rat line, which has been proposed as a model of depression (Angelucci et al. 2000). Furthermore, NGF injection reverses the depression-like behavior in these rats (Overstreet et al. 2010).

NGF may also play a role in the antidepressant drug action. Seizure activity and ECT increase NGF mRNA levels in the hippocampus (Gall and Isackson 1989; Sartorius et al. 2009; Angelucci et al. 2000), although changes in protein levels have been more variable (Sartorius et al. 2009; Angelucci et al. 2000). However, in contrast to BDNF and NT-3, injection of NGF into the hippocampus does not elicit an antidepressant-like effect in rats (Shirayama et al. 2002).

3 Neurotrophins in Schizophrenia

Neurotrophins, particularly BDNF and NT-3, have been linked to the pathophysiology of schizophrenia, but compared to mood disorders, results have been more variable and particularly genetic studies have often not been replicated. The pathophysiology of schizophrenia is unknown, but increasing evidence suggests

that the development of inhibitory interneurons and their participation in developing circuits plays a critical role in schizophrenia. Indeed, interneurons in the prefrontal cortex of schizophrenic subjects appear to show immature features (Lewis et al. 2005). For example, the mRNA level for the 67 kDa isoform of GABA synthesizing enzyme glutamate decarboxylase (GAD67) and the GABA transporter are reduced particularly in the parvalbumin containing interneurons in the prefrontal cortex of these patients (Lewis et al. 2005). BDNF and TrkB have been implicated in the normal maturation of GABAergic interneurons (Woo and Lu 2006), and the mRNA levels for BDNF and TrkB have been found to be reduced in the prefrontal cortex of schizophrenia patients (Hashimoto et al. 2005; Weickert et al. 2005; Bellon et al. 2011). Furthermore, there is a significant correlation between BDNF and TrkB mRNA levels and those for GAD67 in these brain areas (Hashimoto et al. 2005). BDNF levels have been found to be reduced also in serum of schizophrenic patients (Green et al. 2011). It remains unclear, however, whether these findings are causal for the behavioral abnormalities found in schizophrenia or rather a consequence of an earlier developmental defect.

BDNF levels are regulated in some animal models of schizophrenia (Roceri et al. 2002; Fumagalli et al. 2003b; Linden et al. 2000). NMDA-receptor antagonists, including ketamine and phencyclidine, produce an acute schizophrenia-like disorder and have been proposed as a model of schizophrenia (Corlett et al. 2011; Olney and Farber 1995; Javitt 2007). NMDA-receptor antagonists produce region-specific effects on the expression of BDNF mRNA in brain (Castrén et al. 1993; Väisänen et al. 1999; Marvanova et al. 2001; Linden et al. 2000), and these effects can be at least partially counteracted by antipsychotic drug treatments (Fumagalli et al. 2003a; Linden et al. 2000). However, alterations in TrkB receptor activity in brain of transgenic mice do not appear to change responses to NMDA-receptor antagonists (Väisänen et al. 2003).

In contrast to BDNF, NT-3 is not regulated by neuronal activity in brain, and the physiological and pathophysiological role of NT-3 in brain remains unclear. NT-3 displays a transient expression in brain regions affected in schizophrenia, such as limbic cortical and hippocampal regions, and disappears from most of these regions during the first weeks of postnatal life (Friedman et al. 1991). Early genetic studies have linked NT-3 with schizophrenia (Nanko et al. 1994), but many negative associations have also been published and a meta-analysis does not support an association (Lin and Tsai 2004; Bellon et al. 2011). Likewise, evidence that would associate NT-3 with mood disorders or their treatment is largely lacking.

Conclusions

Recent evidence has brought about an avalanche of studies linking BDNF signaling with mood disorders and their treatment and to somewhat lesser extent also to schizophrenia. The association of BDNF with mood has often led to a conclusion that BDNF is some sort of happiness molecule that improves mood when increased. However, recent studies with the role of BDNF and antidepressant drugs in the modulation of use-dependent neuronal network emphasize the role of BDNF as a tool in the plastic modulation of neuronal network in the

process where they are tuned to better accommodate to the environment. Therefore, BDNF and other factors involved in activity-dependent neuronal plasticity appear to play a permissive role in neuropsychiatric disorders, facilitating the environmental effect on mood regulation.

Acknowledgments I would like to thank Drs. Olivia O'Leary and Tomi Rantamäki and all the members of my lab for their help in the preparation of this manuscript. This manuscript was prepared during a sabbatical at the lab of René Hen at Columbia University. I would like to thank René and all his lab members for enjoyable scientific and social interaction. My work is supported by Sigrid Jusélius Foundation, Academy of Finland Center for Excellence Program, Senior Investigator Grant from the Academy of Finland and Schaefer Scholarship by Columbia University. I am an advisor and shareholder of Hermo Pharma.

References

Adachi M, Barrot M, Autry AE, Theobald D, Monteggia LM (2008) Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. Biol Psychiatry 63:642–649

Airan RD, Meltzer LA, Roy M, Gong Y, Chen H, Deisseroth K (2007) High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. Science 317:819–823

Alfonso J, Pollevick GD, Van Der Hart MG, Flugge G, Fuchs E, Frasch AC (2004) Identification of genes regulated by chronic psychosocial stress and antidepressant treatment in the hippocampus. Eur J Neurosci 19:659–666

Alfonso J, Frick LR, Silberman DM, Palumbo ML, Genaro AM, Frasch AC (2006) Regulation of hippocampal gene expression is conserved in two species subjected to different stressors and antidepressant treatments. Biol Psychiatry 59:244–251

Aloyz R, Fawcett JP, Kaplan DR, Murphy RA, Miller FD (1999) Activity-dependent activation of TrkB neurotrophin receptors in the adult CNS. Learn Mem 6:216

Altar CA, Whitehead RE, Chen R, Wortwein G, Madsen TM (2003) Effects of electroconvulsive seizures and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. Biol Psychiatry 54:703–709

Angelucci F, Aloe L, Vasquez PJ, Mathe AA (2000) Mapping the differences in the brain concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in an animal model of depression. Neuroreport 11:1369–1373

Arunrut T, Alejandre H, Chen M, Cha J, Russo-Neustadt A (2009) Differential behavioral and neurochemical effects of exercise, reboxetine and citalopram with the forced swim test. Life Sci 84(17–18):584–589

Balu DT, Hoshaw BA, Malberg JE, Rosenzweig-Lipson S, Schechter LE, Lucki I (2008) Differential regulation of central BDNF protein levels by antidepressant and non-antidepressant drug treatments. Brain Res 1211:37–43

Balu DT, Hodes GE, Anderson BT, Lucki I (2009) Enhanced sensitivity of the MRL/MpJ mouse to the neuroplastic and behavioral effects of chronic antidepressant treatments. Neuropsychopharmacology 34:1764–1773

Bellon A, Krebs MO, Jay TM (2011) Factoring neurotrophins into a neurite-based pathophysiological model of schizophrenia. Prog Neurobiol 94:77–90

Bergami M, Rimondini R, Santi S, Blum R, Gotz M, Canossa M (2008) Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. Proc Natl Acad Sci U S A 105:15570–15575

Berton O, McClung CA, DiLeone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ (2006) Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311:864–868

Biggio F, Gorini G, Utzeri C, Olla P, Marrosu F, Mocchetti I, Follesa P (2009) Chronic vagus nerve stimulation induces neuronal plasticity in the rat hippocampus. Int J Neuropsychopharmacol 12(9):1209–1221

Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J, Arango V (2009) Antidepressants increase neural progenitor cells in the human hippocampus. Neuropsychopharmacology 34(11):2376–2389

Bravo JA, Diaz-Veliz G, Mora S, Ulloa JL, Berthoud VM, Morales P, Arancibia S, Fiedler JL (2009) Desipramine prevents stress-induced changes in depressive-like behavior and hippocampal markers of neuroprotection. Behav Pharmacol 20:273–285

Brunoni AR, Lopes M, Fregni F (2008) A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. Int J Neuropsychopharmacol 11:1169–1180

Calabrese F, Molteni R, Maj PF, Cattaneo A, Gennarelli M, Racagni G, Riva MA (2007) Chronic duloxetine treatment induces specific changes in the expression of BDNF transcripts and in the subcellular localization of the neurotrophin protein. Neuropsychopharmacology 32 (11):2351–2359

Casey BJ, Glatt CE, Tottenham N, Soliman F, Bath K, Amso D, Altemus M, Pattwell S, Jones R, Levita L, McEwen B, Magarinos AM, Gunnar M, Thomas KM, Mezey J, Clark AG, Hempstead BL, Lee FS (2009) Brain-derived neurotrophic factor as a model system for examining gene by environment interactions across development. Neuroscience 164:108–120

Caspi A, Moffitt TE (2006) Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci 7:583–590

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301:386–389

Castrén E (2004) Neurotrophic effects of antidepressant drugs. Curr Opin Pharmacol 4:58–64

Castrén E (2005) Is mood chemistry? Nat Rev Neurosci 6:241–246

Castrén E (2013) Neuronal network plasticity and recovery from depression. JAMA Psychiatry 70:983–989

Castrén E, Hen R (2013) Neuronal plasticity and antidepressant effects. Trends Neurosci 36:259–267

Castrén E, Rantamäki T (2010) The role of BDNF and its receptors in depression and antidepressant drug action: reactivation of developmental plasticity. Dev Neurobiol 70:289–297

Castrén E, da Penha BM, Lindholm D, Thoenen H (1993) Differential effects of MK-801 on brain-derived neurotrophic factor mRNA levels in different regions of the rat brain. Exp Neurol 122:244–252

Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT (2001) Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 50:260–265

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314:140–143

Chen F, Madsen TM, Wegener G, Nyengaard JR (2008) Changes in rat hippocampal CA1 synapses following imipramine treatment. Hippocampus 18:631–639

Chen F, Madsen TM, Wegener G, Nyengaard JR (2009) Repeated electroconvulsive seizures increase the total number of synapses in adult male rat hippocampus. Eur Neuropsychopharmacol 19:329–338

Chen JL, Lin WC, Cha JW, So PT, Kubota Y, Nedivi E (2011) Structural basis for the role of inhibition in facilitating adult brain plasticity. Nat Neurosci 14(5):587–594

Cichon S, Craddock N, Daly M, Faraone SV, Gejman PV, Kelsoe J, Lehner T, Levinson DF, Moran A, Sklar P, Sullivan PF (2009) Genomewide association studies: history, rationale, and prospects for psychiatric disorders. Am J Psychiatry 166:540–556

Conti AC, Cryan JF, Dalvi A, Lucki I, Blendy JA (2002) cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. J Neurosci 22:3262–3268

Cooke JD, Grover LM, Spangler PR (2009) Venlafaxine treatment stimulates expression of brain-derived neurotrophic factor protein in frontal cortex and inhibits long-term potentiation in hippocampus. Neuroscience 162:1411–1419

Coppell AL, Pei Q, Zetterstrom TS (2003) Bi-phasic change in BDNF gene expression following antidepressant drug treatment. Neuropharmacology 44:903–910

Corlett PR, Honey GD, Krystal JH, Fletcher PC (2011) Glutamatergic model psychoses: prediction error, learning, and inference. Neuropsychopharmacology 36:294–315

Czubak A, Nowakowska E, Kus K, Burda K, Metelska J, Baer-Dubowska W, Cichocki M (2009) Influences of chronic venlafaxine, olanzapine and nicotine on the hippocampal and cortical concentrations of brain-derived neurotrophic factor (BDNF). Pharmacol Rep 61:1017–1023

David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, Drew M, Craig DA, Guiard BP, Guilloux JP, Artymyshyn RP, Gardier AM, Gerald C, Antonijevic IA, Leonardo ED, Hen R (2009) Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62:479–493

Deltheil T, Guiard BP, Cerdan J, David DJ, Tanaka KF, RepÈrant C, Guilloux JP, CoudorÈ F, Hen R, Gardier AM (2008) Behavioral and serotonergic consequences of decreasing or increasing hippocampus brain-derived neurotrophic factor protein levels in mice. Neuropharmacology 55:1006–1014

Deltheil T, Tanaka K, Reperant C, Hen R, David DJ, Gardier AM (2009) Synergistic neurochemical and behavioural effects of acute intrahippocampal injection of brain-derived neurotrophic factor and antidepressants in adult mice. Int J Neuropsychopharmacol 12(7):905–915

Domschke K, Lawford B, Laje G, Berger K, Young R, Morris P, Deckert J, Arolt V, McMahon FJ, Baune BT (2010) Brain-derived neurotrophic factor (BDNF) gene: no major impact on antidepressant treatment response. Int J Neuropsychopharmacol 13:93–101

Dulawa SC, Holick KA, Gundersen B, Hen R (2004) Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology 29:1321–1330

Duman RS (2002) Windows on the human brain and the neurobiology of psychiatric illness. Neuropsychopharmacology 26:141–142

Duman RS, Monteggia LM (2006) A neurotrophic model for stress-related mood disorders. Biol Psychiatry 59:1116–1127

Duric V, McCarson KE (2005) Hippocampal neurokinin-1 receptor and brain-derived neurotrophic factor gene expression is decreased in rat models of pain and stress. Neuroscience 133:999–1006

Duric V, Banasr M, Licznerski P, Schmidt HD, Stockmeier CA, Simen AA, Newton SS, Duman RS (2010) A negative regulator of MAP kinase causes depressive behavior. Nat Med 16:1328–1332

Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN (2003) Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. Arch Gen Psychiatry 60:804–815

Dwivedi Y, Rizavi HS, Conley RR, Pandey GN (2006a) ERK MAP kinase signaling in postmortem brain of suicide subjects: differential regulation of upstream Raf kinases Raf-1 and B-Raf. Mol Psychiatry 11:86–98

Dwivedi Y, Rizavi HS, Pandey GN (2006b) Antidepressants reverse corticosterone-mediated decrease in brain-derived neurotrophic factor expression: differential regulation of specific exons by antidepressants and corticosterone. Neuroscience 139:1017–1029

Dwivedi Y, Rizavi HS, Zhang H, Roberts RC, Conley RR, Pandey GN (2009) Aberrant extracellular signal-regulated kinase (ERK)1/2 signalling in suicide brain: role of ERK kinase 1 (MEK1). Int J Neuropsychopharmacol 12:1337–1354

Eisch AJ, Bolanos CA, De Wit J, Simonak RD, Pudiak CM, Barrot M, Verhaagen J, Nestler EJ (2003) Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. Biol Psychiatry 54:994–1005

Friedman WJ, Ernfors P, Persson H (1991) Transient and persistent expression of NT-3/HDNF mRNA in the rat brain during postnatal development. J Neurosci 11:1577–1584
Frielingsdorf H, Bath KG, Soliman F, Difede J, Casey BJ, Lee FS (2010) Variant brain-derived neurotrophic factor Val66Met endophenotypes: implications for posttraumatic stress disorder. Ann N Y Acad Sci 1208:150–157
Fujimura H, Altar CA, Chen R, Nakamura T, Nakahashi T, Kambayashi J, Sun B, Tandon NN (2002) Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Thromb Haemost 87:728–734
Fumagalli F, Molteni R, Roceri M, Bedogni F, Santero R, Fossati C, Gennarelli M, Racagni G, Riva MA (2003a) Effect of antipsychotic drugs on brain-derived neurotrophic factor expression under reduced N-methyl-D-aspartate receptor activity. J Neurosci Res 72:622–628
Fumagalli F, Racagni G, Colombo E, Riva MA (2003b) BDNF gene expression is reduced in the frontal cortex of dopamine transporter knockout mice. Mol Psychiatry 8:898–899
Gall CM, Isackson PJ (1989) Limbic seizures increase neuronal production of messenger RNA for nerve growth factor. Science 245:758–761
Gerritsen L, Tendolkar I, Franke B, Vasquez AA, Kooijman S, Buitelaar J, Fernandez G, Rijpkema M (2012) BDNF Val66Met genotype modulates the effect of childhood adversity on subgenual anterior cingulate cortex volume in healthy subjects. Mol Psychiatry 17(6):597–603
Gersner R, Toth E, Isserles M, Zangen A (2010) Site-specific antidepressant effects of repeated subconvulsive electrical stimulation: potential role of brain-derived neurotrophic factor. Biol Psychiatry 67:125–132
Gonul AS, Akdeniz F, Taneli F, Donat O, Eker C, Vahip S (2005) Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. Eur Arch Psychiatry Clin Neurosci 255:381–386
Gorgulu Y, Caliyurt O (2009) Rapid antidepressant effects of sleep deprivation therapy correlates with serum BDNF changes in major depression. Brain Res Bull 80(3):158–162
Govindarajan A, Rao BSS, Nair D, Trinh M, Mawjee N, Tonegawa S, Chattarji S (2006) Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. Proc Natl Acad Sci U S A 103:13208–13213
Gray J, Yeo GS, Cox JJ, Morton J, Adlam AL, Keogh JM, Yanovski JA, El Gharbawy A, Han JC, Tung YC, Hodges JR, Raymond FL, O'Rahilly S, Farooqi IS (2006) Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. Diabetes 55:3366–3371
Green MJ, Matheson SL, Shepherd A, Weickert CS, Carr VJ (2011) Brain-derived neurotrophic factor levels in schizophrenia: a systematic review with meta-analysis. Mol Psychiatry 16(9): 960–972
Guiard BP, David DJ, Deltheil T, Chenu F, Le Maitre E, Renoir T, Leroux-Nicollet I, Sokoloff P, Lanfumey L, Hamon M, Andrews AM, Hen R, Gardier AM (2008) Brain-derived neurotrophic factor-deficient mice exhibit a hippocampal hyperserotonergic phenotype. Int J Neuropsychopharmacol 11:79–92
Haenisch B, Bilkei-Gorzo A, Caron MG, Bonisch H (2009) Knockout of the norepinephrine transporter and pharmacologically diverse antidepressants prevent behavioral and brain neurotrophin alterations in two chronic stress models of depression. J Neurochem 111:403–416
Hajszan T, MacLusky NJ, Leranth C (2005) Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. Eur J Neurosci 21:1299–1303
Hajszan T, Dow A, Warner-Schmidt JL, Szigeti-Buck K, Sallam NL, Parducz A, Leranth C, Duman RS (2009) Remodeling of hippocampal spine synapses in the rat learned helplessness model of depression. Biol Psychiatry 65:392–400
Hajszan T, Szigeti-Buck K, Sallam NL, Bober J, Parducz A, Maclusky NJ, Leranth C, Duman RS (2010) Effects of estradiol on learned helplessness and associated remodeling of hippocampal spine synapses in female rats. Biol Psychiatry 67:168–174

Han JC, Liu Q-R, Jones M, Levinn RL, Menzie CM, Jefferson-George KS, Adler-Wailes DC, Sanford EL, Lacbawan FL, Uhl GR, Rennert OM, Yanovski JA (2008) Brain-derived neurotrophic factor and obesity in the WAGR syndrome. N Engl J Med 359:918–927

Hashimoto T, Bergen SE, Nguyen QL, Xu B, Monteggia LM, Pierri JN, Sun Z, Sampson AR, Lewis DA (2005) Relationship of brain-derived neurotrophic factor and its receptor TrkB to altered inhibitory prefrontal circuitry in schizophrenia. J Neurosci 25:372–383

Hoshaw BA, Malberg JE, Lucki I (2005) Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. Brain Res 1037:204–208

Jacobsen JP, Mork A (2004) The effect of escitalopram, desipramine, electroconvulsive seizures and lithium on brain-derived neurotrophic factor mRNA and protein expression in the rat brain and the correlation to 5-HT and 5-HIAA levels. Brain Res 1024:183–192

Javitt DC (2007) Glutamate and schizophrenia: phencyclidine, N-methyl-D-aspartate receptors, and dopamine-glutamate interactions. Int Rev Neurobiol 78:69–108

Kang R, Chang H, Wong M, Choi M, Park J, Lee H, Jung I, Joe S, Kim L, Kim S, Kim Y, Han C, Ham B, Ko Y, Lee M (2010) Brain-derived neurotrophic factor gene polymorphisms and mirtazapine responses in Koreans with major depression. J Psychopharmacol 24(12): 1755–1763

Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry JM, Bertschy G (2005a) Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. Biol Psychiatry 57: 1068–1072

Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R (2005b) Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Brain Res Mol Brain Res 136:29–37

Karg K, Burmeister M, Shedden K, Sen S (2011) The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. Arch Gen Psychiatry 68(5):444–454

Karpova NN, Pickenhagen A, Lindholm J, Tiraboschi E, Kulesskaya N, Ágústsdóttir A, Antila H, Popova D, Akamine Y, Bahi A, Sullivan R, Hen R, Drew LI, Castrén E (2011) Fear erasure in mouse requires synergy between antidepressant drug treatment and exposure therapy. Science 334:1731–1734

Kaufman J, Yang BZ, Douglas-Palumberi H, Grasso D, Lipschitz D, Houshyar S, Krystal JH, Gelernter J (2006) Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. Biol Psychiatry 59:673–680

Kim JM, Stewart R, Kim SW, Yang SJ, Shin IS, Kim YH, Yoon JS (2007) Interactions between life stressors and susceptibility genes (5-HTTLPR and BDNF) on depression in Korean elders. Biol Psychiatry 62:423–428

Kobayashi K, Ikeda Y, Sakai A, Yamasaki N, Haneda E, Miyakawa T, Suzuki H (2010) Reversal of hippocampal neuronal maturation by serotonergic antidepressants. Proc Natl Acad Sci U S A 107:8434–8439

Koponen E, Rantamaki T, Voikar V, Saarelainen T, MacDonald E, Castrén E (2005) Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. Cell Mol Neurobiol 25:973–980

Krishnan V, Nestler EJ (2008) The molecular neurobiology of depression. Nature 455:894–902

Krystal JH, Tolin DF, Sanacora G, Castner S, Williams G, Aikins D, Hoffman R, D'Souza DC (2009) Neuroplasticity as a target for the pharmacotherapy of anxiety disorders, mood disorders, and schizophrenia. Drug Discov Today 14(13–14):690–697

Larsen MH, Hay-Schmidt A, Ronn LC, Mikkelsen JD (2008) Temporal expression of brain-derived neurotrophic factor (BDNF) mRNA in the rat hippocampus after treatment with selective and mixed monoaminergic antidepressants. Eur J Pharmacol 578:114–122

Lee HY, Kim YK (2008) Plasma brain-derived neurotrophic factor as a peripheral marker for the action mechanism of antidepressants. Neuropsychobiology 57:194–199

Lewis DA, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 6:312–324

Li B, Suemaru K, Cui R, Araki H (2007) Repeated electroconvulsive stimuli have long-lasting effects on hippocampal BDNF and decrease immobility time in the rat forced swim test. Life Sci 80:1539–1543

Li Y, Luikart BW, Birnbaum S, Chen J, Kwon CH, Kernie SG, Bassel-Duby R, Parada LF (2008) TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. Neuron 59:399–412

Lin PY, Tsai G (2004) Meta-analyses of the association between genetic polymorphisms of neurotrophic factors and schizophrenia. Schizophr Res 71:353–360

Linden AM, Vaisanen J, Lakso M, Nawa H, Wong G, Castrén E (2000) Expression of neurotrophins BDNF and NT-3, and their receptors in rat brain after administration of antipsychotic and psychotrophic agents. J Mol Neurosci 14:27–37

Liu X, Xu Y, Jiang S, Cui D, Qian Y, Jiang K (2009) Family-based association study between brain-derived neurotrophic factor gene and major depressive disorder of Chinese descent. Psychiatry Res 169:169–172

Lu B, Nagappan G, Lu Y (2014) BDNF and synaptic plasticity, cognitive function, and dysfunction. In: Lewin GR, Carter BD (eds) Neurotrophic factors. Springer, Heidelberg

MacQueen GM, Ramakrishnan K, Croll SD, Siuciak JA, Yu G, Young LT, Fahnestock M (2001) Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. Behav Neurosci 115:1145–1153

MacQueen GM, Ramakrishnan K, Ratnasingan R, Chen B, Young LT (2003) Desipramine treatment reduces the long-term behavioural and neurochemical sequelae of early-life maternal separation. Int J Neuropsychopharmacol 6:391–396

Madhav TR, Pei Q, Zetterstrom TSC (2001) Serotonergic cells of the rat raphe nuclei express mRNA of tyrosine kinase B (trkB), the high-affinity receptor for brain derived neurotrophic factor (BDNF). Mol Brain Res 93:56–63

Madsen TM, Treschow A, Bengzon J, Bolwig TG, Lindvall O, Tingstrom A (2000) Increased neurogenesis in a model of electroconvulsive therapy. Biol Psychiatry 47:1043–1049

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104–9110

Mannari C, Origlia N, Scatena A, Del Debbio A, Catena M, Dell'agnello G, Barraco A, Giovannini L, Dell'osso L, Domenici L, Piccinni A (2008) BDNF level in the rat prefrontal cortex increases following chronic but not acute treatment with duloxetine, a dual acting inhibitor of noradrenaline and serotonin re-uptake. Cell Mol Neurobiol 28:457–468

Martinez-Turrillas R, Del Rio J, Frechilla D (2005) Sequential changes in BDNF mRNA expression and synaptic levels of AMPA receptor subunits in rat hippocampus after chronic antidepressant treatment. Neuropharmacology 49:1178–1188

Marvanova M, Lakso M, Pirhonen J, Nawa H, Wong G, Castrén E (2001) The neuroprotective agent memantine induces brain-derived neurotrophic factor and trkB receptor expression in rat brain. Mol Cell Neurosci 18:247–258

Matrisciano F, Bonaccorso S, Ricciardi A, Scaccianoce S, Panaccione I, Wang L, Ruberto A, Tatarelli R, Nicoletti F, Girardi P, Shelton RC (2009) Changes in BDNF serum levels in patients with major depression disorder (MDD) after 6 months treatment with sertraline, escitalopram, or venlafaxine. J Psychiatr Res 43:247–254

Maya Vetencourt JF, Sale A, Viegi A, Baroncelli L, De Pasquale R, O'Leary FF, Castrén E, Maffei L (2008) The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science 320: 385–388

Maya Vetencourt JF, Tiraboschi E, Spolidoro M, Castrén E, Maffei L (2011) Serotonin triggers a transient epigenetic mechanism that reinstates adult visual cortex plasticity in rats. Eur J Neurosci 33:49–57

Molteni R, Calabrese F, Bedogni F, Tongiorgi E, Fumagalli F, Racagni G, Riva MA (2006) Chronic treatment with fluoxetine up-regulates cellular BDNF mRNA expression in rat dopaminergic regions. Int J Neuropsychopharmacol 9:307–317

Monteggia LM, Barrot M, Powell CM, Berton O, Galanis V, Gemelli T, Meuth S, Nagy A, Greene RW, Nestler EJ (2004) Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proc Natl Acad Sci U S A 101:10827–10832

Monteggia LM, Luikart B, Barrot M, Theobold D, Malkovska I, Nef S, Parada LF, Nestler EJ (2007) Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biol Psychiatry 61:187–197

Muller MB, Toschi N, Kresse AE, Post A, Keck ME (2000) Long-term repetitive transcranial magnetic stimulation increases the expression of brain-derived neurotrophic factor and cholecystokinin mRNA, but not neuropeptide tyrosine mRNA in specific areas of rat brain. Neuropsychopharmacology 23:205–215

Nanko S, Hattori M, Kuwata S, Sasaki T, Fukuda R, Dai XY, Yamaguchi K, Shibata Y, Kazamatsuri H (1994) Neurotrophin-3 gene polymorphism associated with schizophrenia. Acta Psychiatr Scand 89:390–392

Nibuya M, Morinobu S, Duman RS (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 15: 7539–7547

O'Leary OF, Wu X, Castrén E (2009) Chronic fluoxetine treatment increases expression of synaptic proteins in the hippocampus of the ovariectomized rat: role of BDNF signalling. Psychoneuroendocrinology 34:367–381

Okamoto T, Yoshimura R, Ikenouchi-Sugita A, Hori H, Umene-Nakano W, Inoue Y, Ueda N, Nakamura J (2008) Efficacy of electroconvulsive therapy is associated with changing blood levels of homovanillic acid and brain-derived neurotrophic factor (BDNF) in refractory depressed patients: a pilot study. Prog Neuropsychopharmacol Biol Psychiatry 32:1185–1190

Olney JW, Farber NB (1995) Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry 52:998–1007

Onishchenko N, Tamm C, Vahter M, Hokfelt T, Johnson JA, Johnson DA, Ceccatelli S (2007) Developmental exposure to methylmercury alters learning and induces depression-like behavior in male mice. Toxicol Sci 97(2):428–437

Onishchenko N, Karpova N, Sabri F, Castrén E, Ceccatelli S (2008) Long-lasting depression-like behavior and epigenetic changes of BDNF gene expression induced by perinatal exposure to methylmercury. J Neurochem 106:1378–1387

Overstreet DH, Fredericks K, Knapp D, Breese G, McMichael J (2010) Nerve growth factor (NGF) has novel antidepressant-like properties in rats. Pharmacol Biochem Behav 94(4):553–560

Paizanis E, Renoir T, Lelievre V, Saurini F, Melfort M, Gabriel C, Barden N, Mocaer E, Hamon M, Lanfumey L (2010) Behavioural and neuroplastic effects of the new-generation antidepressant agomelatine compared to fluoxetine in glucocorticoid receptor-impaired mice. Int J Neuropsychopharmacol 13:759–774

Pandey GN, Dwivedi Y, Rizavi HS, Ren X, Zhang H, Pavuluri MN (2010) Brain-derived neurotrophic factor gene and protein expression in pediatric and adult depressed subjects. Prog Neuropsychopharmacol Biol Psychiatry 34:645–651

Poo MM (2001) Neurotrophins as synaptic modulators. Nat Rev Neurosci 2:24–32

Rantamäki T, Hendolin P, Kankaanpää A, Mijatovic J, Piepponen P, Domenici E, Chao MV, Männistö PT, Castrén E (2007) Pharmacologically diverse antidepressants rapidly activate brain-derived neurotrophic factor receptor TrkB and induce phospholipase-Cgamma signaling pathways in mouse brain. Neuropsychopharmacology 32:2152–2162

Rantamäki T, Vesa L, Antila H, Di Lieto A, Tammela P, Schmitt A, Lesch KP, Rios M, Castrén E (2011) Antidepressant drugs transactivate TrkB neurotrophin receptors in the adult rodent brain independently of BDNF and monoamine transporter blockade. PLoS One 6(6):e20567

Reagan LP, Hendry RM, Reznikov LR, Piroli GG, Wood GE, McEwen BS, Grillo CA (2007) Tianeptine increases brain-derived neurotrophic factor expression in the rat amygdala. Eur J Pharmacol 565:68–75

Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA (2002) Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. Mol Psychiatry 7:609–616

Rogoz Z, Skuza G, Legutko B (2008) Repeated co-treatment with fluoxetine and amantadine induces brain-derived neurotrophic factor gene expression in rats. Pharmacol Rep 60:817–826

Russo-Neustadt A, Ha T, Ramirez R, Kesslak JP (2001) Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. Behav Brain Res 120:87–95

Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E, Agerman K, Haapasalo A, Nawa H, Aloyz R, Ernfors P, Castrén E (2003) Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. J Neurosci 23:349–357

Sahay A, Hen R (2007) Adult hippocampal neurogenesis in depression. Nat Neurosci 10: 1110–1115

Sairanen M, Lucas G, Ernfors P, Castrén M, Castrén E (2005) Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. J Neurosci 25:1089–1094

Sairanen M, O'Leary OF, Knuuttila JE, Castrén E (2007) Chronic antidepressant treatment selectively increases expression of plasticity-related proteins in the hippocampus and medial prefrontal cortex of the rat. Neuroscience 144:368–374

Samuels BA, Hen R (2011) Neurogenesis and affective disorders. Eur J Neurosci 33:1152–1159

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805–809

Sartorius A, Hellweg R, Litzke J, Vogt M, Dormann C, Vollmayr B, Danker-Hopfe H, Gass P (2009) Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats. Pharmacopsychiatry 42:270–276

Schmidt HD, Duman RS (2010) Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. Neuropsychopharmacology 35:2378–2391

Schulte-Herbruggen O, Chourbaji S, Muller H, Danker-Hopfe H, Brandwein C, Gass P, Hellweg R (2006) Differential regulation of nerve growth factor and brain-derived neurotrophic factor in a mouse model of learned helplessness. Exp Neurol 202:404–409

Schulte-Herbruggen O, Fuchs E, Abumaria N, Ziegler A, Danker-Hopfe H, Hiemke C, Hellweg R (2009) Effects of escitalopram on the regulation of brain-derived neurotrophic factor and nerve growth factor protein levels in a rat model of chronic stress. J Neurosci Res 87:2551–2560

Sen S, Duman R, Sanacora G (2008) Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol Psychiatry 64:527–532

Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, Nakazato M, Watanabe H, Shinoda N, Okada S, Iyo M (2003) Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry 54:70–75

Shinawi M, Sahoo T, Maranda B, Skinner SA, Skinner C, Chinault C, Zascavage R, Peters SU, Patel A, Stevenson RE, Beaudet AL (2011) 11p14.1 microdeletions associated with ADHD, autism, developmental delay, and obesity. Am J Med Genet A 155:1272–1280

Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 22: 3251–3261

Sirianni RW, Olausson P, Chiu AS, Taylor JR, Saltzman WM (2010) The behavioral and biochemical effects of BDNF containing polymers implanted in the hippocampus of rats. Brain Res 1321:40–50

Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM (1997) Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacol Biochem Behav 56:131–137

Smith MA, Makino S, Kvetnansky R, Post RM (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 15:1768–1777

Soliman F, Glatt CE, Bath KG, Levita L, Jones RM, Pattwell SS, Jing D, Tottenham N, Amso D, Somerville LH, Voss HU, Glover G, Ballon DJ, Liston C, Teslovich T, Van Kempen T, Lee FS, Casey BJ (2010) A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. Science 327:863–866

Soumier A, Banasr M, Lortet S, Masmejean F, Bernard N, Kerkerian-Le-Goff L, Gabriel C, Millan MJ, Mocaer E, Daszuta A (2009) Mechanisms contributing to the phase-dependent regulation of neurogenesis by the novel antidepressant, agomelatine, in the adult rat hippocampus. Neuropsychopharmacology 34:2390–2403

Spillantini MG, Aloe L, Alleva E, DeSimone R, Goedert M, Levi-Montalcini R (1989) Nerve growth factor mRNA and protein increase in hypothalamus in a mouse model of aggression. Proc Natl Acad Sci U S A 86:8555–8559

Taliaz D, Stall N, Dar DE, Zangen A (2010) Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. Mol Psychiatry 15:80–92

Taliaz D, Loya A, Gersner R, Haramati S, Chen A, Zangen A (2011) Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. J Neurosci 31:4475–4483

Thoenen H (2000) Neurotrophins and activity-dependent plasticity. Prog Brain Res 128:183–191

Tsai SJ, Cheng CY, Yu YW, Chen TJ, Hong CJ (2003) Association study of a brain-derived neurotrophic-factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. Am J Med Genet B Neuropsychiatr Genet 123B:19–22

Väisänen J, Lindén AM, Lakso M, Wong G, Heinemann U, Castrén E (1999) Excitatory actions of NMDA receptor antagonists in rat entorhinal cortex and cultured entorhinal cortical neurons. Neuropsychopharmacology 21:137–146

Väisänen J, Saarelainen T, Koponen E, Castrén E (2003) Altered trkB neurotrophin receptor activation does not influence the N-methyl-D-aspartate receptor antagonist-mediated neurotoxicity in mouse posterior cingulate cortex. Neurosci Lett 350:1–4

Verhagen M, van der Meij A, van Deurzen PA, Janzing JG, Arias-Vasquez A, Buitelaar JK, Franke B (2010) Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. Mol Psychiatry 15(3):260–271

von Richthofen S, Lang UE, Hellweg R (2003) Effects of different kinds of acute stress on nerve growth factor content in rat brain. Brain Res 987:207–213

Warner-Schmidt JL, Duman RS (2006) Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. Hippocampus 16:239–249

Weickert CS, Ligons DL, Romanczyk T, Ungaro G, Hyde TM, Herman MM, Weinberger DR, Kleinman JE (2005) Reductions in neurotrophin receptor mRNAs in the prefrontal cortex of patients with schizophrenia. Mol Psychiatry 10:637–650

Woo NH, Lu B (2006) Regulation of cortical interneurons by neurotrophins: from development to cognitive disorders. Neuroscientist 12:43–56

Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR, Ripke S, Macintyre DJ, McGhee KA, Maclean AW, Smit JH, Hottenga JJ, Willemsen G, Middeldorp CM, de Geus EJ, Lewis CM, McGuffin P, Hickie IB, van den Oord EJ, Liu JZ, Macgregor S, McEvoy BP, Byrne EM, Medland SE, Statham DJ, Henders AK, Heath AC, Montgomery GW, Martin NG, Boomsma DI, Madden PA, Sullivan PF (2012) Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. Mol Psychiatry 17 (1):36–48

Wyneken U, Sandoval M, Sandoval S, Jorquera F, Gonzalez I, Vargas F, Falcon R, Monari M, Orrego F (2006) Clinically relevant doses of fluoxetine and reboxetine induce changes in the TrkB content of central excitatory synapses. Neuropsychopharmacology 31:2415–2423

Xu H, Luo C, Richardson JS, Li XM (2004) Recovery of hippocampal cell proliferation and BDNF levels, both of which are reduced by repeated restraint stress, is accelerated by chronic venlafaxine. Pharmacogenomics J 4:322–331

Yeo GS, Connie Hung CC, Rochford J, Keogh J, Gray J, Sivaramakrishnan S, O'Rahilly S, Farooqi IS (2004) A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. Nat Neurosci 7:1187–1189

Zanardini R, Gazzoli A, Ventriglia M, Perez J, Bignotti S, Rossini PM, Gennarelli M, Bocchio-Chiavetto L (2006) Effect of repetitive transcranial magnetic stimulation on serum brain derived neurotrophic factor in drug resistant depressed patients. J Affect Disord 91:83–86

Zou YF, Wang Y, Liu P, Feng XL, Wang BY, Zang TH, Yu X, Wei J, Liu ZC, Liu Y, Tao M, Li HC, Li KQ, Hu J, Li M, Zhang KR, Ye DQ, Xu XP (2010) Association of brain-derived neurotrophic factor genetic Val66Met polymorphism with severity of depression, efficacy of fluoxetine and its side effects in Chinese major depressive patients. Neuropsychobiology 61: 71–78

Brain-Derived Neurotrophic Factor and Rett Syndrome

D.M. Katz

Abstract

Rett syndrome (RTT) is a devastating neurodevelopmental disorder with autistic features caused by loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (*MECP2*), a transcriptional regulatory protein. RTT has attracted widespread attention not only because of the urgent need for treatments, but also because it has become a window into basic mechanisms underlying epigenetic regulation of neuronal genes, including BDNF. In addition, work in mouse models of the disease has demonstrated the possibility of symptom reversal upon restoration of normal gene function. This latter finding has resulted in a paradigm shift in RTT research and, indeed, in the field of neurodevelopmental disorders as a whole, and spurred the search for potential therapies for RTT and related syndromes. In this context, the discovery that expression of BDNF is dysregulated in RTT and mouse models of the disease has taken on particular importance. This chapter reviews the still evolving story of how MeCP2 might regulate expression of BDNF, the functional consequences of BDNF deficits in *Mecp2* mutant mice, and progress in developing BDNF-targeted therapies for the treatment of RTT.

Keywords

BDNF • MeCP2 • TrkB • Autism spectrum disorders • Neurodevelopmental • Brainstem

D.M. Katz (✉)
Department of Neurosciences, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106, USA
e-mail: david.katz@case.edu

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_18,

Abbreviations

The following abbreviations are used for the gene encoding methyl-CpG-binding protein 2 and its protein product:

MECP2, BDNF	Human gene
Mecp2, Bdnf	Mouse gene
MeCP2, BDNF	Protein

1 Introduction

Rett syndrome (RTT) is a complex neurodevelopmental disorder that affects approximately 1 in 10,000 live female births worldwide (Chahrour and Zoghbi 2007). RTT is characterized by apparently normal early postnatal development with neurological symptoms appearing around 6–18 months of age. The subsequent course of the disorder is variable and patients exhibit a diverse array of symptoms that generally includes loss of acquired speech, head growth deceleration, autistic features such as emotional withdrawal and diminished eye contact, motor stereotypies, early hypotonia followed by rigidity, epileptiform seizures, exaggerated responses to stress, and severe respiratory and autonomic (cardiac and gastrointestinal) dysfunction (Chahrour and Zoghbi 2007; Hagberg et al. 1983; Katz et al. 2009; Shahbazian and Zoghbi 2002; Vorsanova et al. 2004; Weese-Mayer et al. 2006, 2008). Up to a quarter of RTT patients may die prematurely of cardiorespiratory failure (Kerr et al. 1997).

The vast majority of typical RTT cases result from loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2; Amir et al. 1999; Chahrour and Zoghbi 2007), a transcriptional regulatory protein (Klose and Bird 2006). Over 250 different *MECP2* mutations have been identified in RTT patients, most of which tend to cluster either within the methyl-binding or transcription repression domains. The *MECP2* gene is X-linked, and affected females are heterozygotes and somatic mosaics for MeCP2, i.e., cells in which the mutated allele occurs on the inactive X are phenotypically normal for MeCP2 expression, whereas cells in which the mutated allele occurs on the active X are mutant. Disease phenotype is therefore affected not only by the specific *MECP2* mutation but also by the skewing of X chromosome inactivation; individuals in which inactivation is skewed towards the mutant allele are less severely affected, and vice versa. Hemizygosity in males is usually fatal, and the chances of homozygosity in females are exceedingly small, given that most disease-causing mutations arise in the paternal germ line and child-bearing by affected females is extremely rare.

The full scope of MeCP2 function in neurons remains a subject of some controversy. Although it is clear that MeCP2 binds methylated DNA and can potently silence transcription (Klose and Bird 2006), additional functions, including

transcriptional activation (Chahrour et al. 2008), regulation of RNA processing (Young et al. 2005), and control of higher order chromatin structure (Georgel et al. 2003), have been proposed. Moreover, it is unclear whether or not MeCP2 selectively regulates transcription of specific genes or, alternatively, acts globally to regulate chromatin state across the genome. A recent study by Skene et al. (2010) demonstrated that MeCP2 protein is abundantly expressed in neurons at levels comparable to histone octamers, i.e., sufficient to blanket the genome at methylated CpG dinucleotides. Therefore, these authors have suggested that the primary function of MeCP2 is to globally repress spurious transcription, e.g., of nucleotide repeats across the genome rather than to dynamically regulate expression of specific genes. However, Skene et al. (2010) showed that, in addition to its widespread binding across genome, MeCP2 also shows peaks of even higher binding at specific sites within promoter regions. Whether or not this is evidence for a more specific role in dynamic regulation of particular genes remains unclear. Nonetheless, what is clear is that expression of many genes is disrupted, either directly or indirectly, by loss-of-function mutations in *MECP2* and that the complexity of RTT is related to the diversity of affected gene targets.

2 Regulation of BDNF Expression, Trafficking, and Secretion by MeCP2

The debate concerning the role of MeCP2 in gene regulation is particularly relevant to understanding the evolution of current thinking regarding BDNF and the pathogenesis of RTT. The initial suggestion that dysregulation of BDNF expression might play a role in RTT came from in vitro evidence that BDNF is a transcriptional target of MeCP2 and repressed by MeCP2 binding to BDNF promoter regions. Specifically, Chen et al. (2003) and Martinowich et al. (2003) used chromatin immunoprecipitation to demonstrate binding of MeCP2 protein to BDNF promoter IV (referred to at the time as promoter III), one of nine BDNF promoters and one that is particularly important for activity-dependent regulation of BDNF expression. Moreover, MeCP2 binding appears to recruit a transcriptional repressor complex that includes HDAC1 and Sin3A to the BDNF locus (Martinowich et al. 2003). Chen et al. (2003) and Martinowich et al. (2003) further showed that MeCP2 binding to the BDNF gene was dynamic and subject to regulation in cultured neurons by exposure to depolarizing stimuli, such as elevated potassium chloride (KCl). Specifically, strong chemical depolarization reduces MeCP2 binding to BDNF promoter IV (Martinowich et al. 2003) in association with a change in the phosphorylation state of MeCP2 (Chen et al. 2003), reduces methylation of promoter IV (Martinowich et al. 2003), and increases BDNF expression (see also Ballas et al. 2005). Subsequently, Zhou and colleagues (Zhou et al. 2006) demonstrated that phosphorylation of MeCP2 at serine 421 is particularly important for activity-dependent increases in BDNF expression in cultured hippocampal neurons. Consistent with this repression model, Chen et al. (2003) showed that *Mecp2* null embryonic cortical neurons cultured in the presence of blockers of

neuronal activity exhibited higher levels of BDNF exon IV mRNA than wild-type cells. However, in the presence of a depolarizing concentration of KCl, wild-type and mutant cells exhibited similar levels of BDNF expression, which the authors interpreted as consistent with BDNF already being derepressed in the mutant cells to levels similar to those achieved in wild-type cells upon stimulation. More recently, evidence has emerged that BDNF expression can also be regulated by the acetylation state of MeCP2 in a manner consistent with the repression model. Specifically, mice lacking functional SIRT1, a nicotinamide-adenine dinucleotide-dependent histone deacetylase, exhibit increased MeCP2 binding to the BDNF exon IV promoter and decreased levels of BDNF mRNA and protein (Zocchi and Sassone-Corsi 2012).

The hypothesis that MeCP2 normally represses BDNF transcription predicted that loss of MeCP2 function in RTT, or mouse models of the disease, would be associated with elevated BDNF expression. However, this prediction has not been borne out, as *Mecp2* null or heterozygous mice exhibit *reduced* levels of BDNF mRNA and protein in vivo (Chang et al. 2006; Ogier et al. 2007; Wang et al. 2006). Similarly, two studies of postmortem brain samples from RTT patients have demonstrated reduced levels of BDNF mRNA (Abuhatzira et al. 2007; Deng et al. 2007). The BDNF mRNA and protein deficits observed in the brain and peripheral nervous system of *Mecp2* mutant mice are progressive (Chang et al. 2006; Ogier et al. 2007; Wang et al. 2006), being virtually undetectable at birth and declining to as much as 50 % of wild-type levels in some tissues by 5 weeks of age in male nulls (Wang et al. 2006). Moreover, the postnatal decline in BDNF levels occurs with a slower time course in heterozygous females compared to male nulls (Schmid et al. 2012). Clearly, these in vivo data are inconsistent with a model in which MeCP2 simply represses expression of BDNF.

Various hypotheses have been offered to explain how loss of MeCP2 function could lead to deficits in BDNF expression. One idea, already introduced above, is that MeCP2 activates rather than represses gene expression. In support of this hypothesis, Chahrour et al. (2008) showed that global overexpression of MeCP2 in mice is associated with increased expression of BDNF mRNA in the hypothalamus, whereas MeCP2 loss is associated with decreased BDNF. Similarly, selective deletion of *Mecp2* from Sim-1-positive neurons also causes a reduction in BDNF in the hypothalamus (Fyffe et al. 2008). The activator hypothesis is also supported by a recent report by Li et al. (2013) demonstrating global reductions in transcription and Akt/mTOR-dependent protein translation—including BDNF—in human iPSC-derived neurons in which the *Mecp2* gene was deleted using TALEN-mediated DNA editing. One caveat to these findings is that the possible contribution of decreased BDNF mRNA and/or protein stability, rather than decreased gene transcription per se, has not been ruled out. Further support for the activator model comes from studies showing that derepression of microRNA (miRNA)-mediated inhibition of MeCP2 translation in cultured neurons increases expression not only of MeCP2 but BDNF as well (Klein et al. 2007).

A recent approach to resolving the repressor *versus* activator debate is the "dual operation model" (Li and Pozzo-Miller 2013). This model is motivated by data

from one study showing that either knockdown or overexpression of MeCP2 in cultured neurons leads to increased expression of BDNF (Larimore et al. 2009), as well as evidence that MeCP2 can undergo diverse posttranslational modifications, including phosphorylation, acetylation, and ubiquitylation, leading to unique associations with either co-repressors or co-activators (Gonzales et al. 2012).

A second hypothesis that has been proposed to explain decreased BDNF expression in the absence of MeCP2 function is that MeCP2 normally represses the activity of repressors of BDNF expression, i.e., the RE1 silencing transcription factor (REST)/CoREST complex (Abuhatzira et al. 2007). This model is based on data from mice and humans demonstrating elevated levels of REST/CoREST in RTT patients and *Mecp2*-deficient mice, presumably leading to reduced BDNF expression through repressive interactions with the RE1 element in BDNF promoter regions. A third hypothesis is that reduced BDNF expression in *Mecp2* null neurons is a consequence of reduced neuronal activity (Sun and Wu 2006). This idea was based on the finding that cortical neurons from *Mecp2* null mice exhibit reduced firing rates associated with a loss of excitatory synaptic drive (Dani et al. 2005). However, we found that even after exposure to strongly depolarizing stimuli in vitro, *Mecp2* null cells express less BDNF protein than wild-type, indicating that differences in activity alone are unlikely to account for BDNF deficits in the absence of MeCP2 (Ogier et al. 2007). Thus, at present, the normal role of MeCP2 in regulating BDNF expression, as well as the mechanism (s) responsible for reduced BDNF levels in the RTT brain, remain to be clarified. One possibility is that, although loss of MeCP2 may result in derepression of BDNF gene expression, translation and/or stability of the protein may also be adversely affected, resulting in a net decrease in BDNF levels in the RTT brain. In support of this possibility, Wu et al. (2010) recently demonstrated that MeCP2 controls transcription of several microRNAs (miRNAs) that target the 3′ UTR of *Bdnf* mRNA, some of which are upregulated in the absence of MeCP2 function and negatively regulate *Bdnf* mRNA levels. Conversely, inhibition of two such miRNAs, miR-381 and miR-495, in both wild-type and *Mecp2* null neurons in vitro, increased levels of *Bdnf* mRNA and BDNF protein. Thus, Wu et al. (2010) proposed that, in the absence of MeCP2 function, the net effect of direct derepression of *Bdnf* mRNA, combined with depression of miRNAs that negatively regulate *Bdnf* mRNA, is reduced BDNF levels. This hypothesis requires further testing, as Wu et al. (2010) also identified miRNAs that target *Bdnf* mRNA and are downregulated in the absence of MeCP2 function. In particular, it will be critical to define the stoichiometry of these positive and negative influences on *Bdnf* transcription, translation, and stability in vivo in order to fully understand the role of miRNAs in BDNF protein deficits in RTT.

In addition to dysregulation of BDNF expression, loss of MeCP2 also appears to disrupt regulated secretion and transport of BDNF. Although mature sensory neurons lacking MeCP2 express lower levels of BDNF protein, they actually secrete a larger proportion of their total BDNF content than wild-type cells, at least in cell culture (Ladas et al. 2009). However, this enhanced secretion is not sufficient to completely compensate for reduced levels of BDNF expression, and

the absolute amount of BDNF released by mutant cells is nonetheless lower than wild-type. This is also seen at mossy fiber inputs onto CA3 pyramidal neurons in *Mecp2* null mice, in which activity-dependent BDNF release is reduced compared to wild type, resulting in reduced activation of TrkB and reduced signaling through TRPC3 channels (Li et al. 2012). On the other hand, in newborn *Mecp2* null neurons, which do not yet exhibit a significant deficit in BDNF expression, the absolute amount of BDNF released is actually greater than wild-type (Wang et al. 2006). These data raise the possibility that during early development, enhanced secretion of BDNF from *Mecp2* null cells could derange developmental processes that depend on tight coupling between neuronal activity and BDNF release, such as activity-dependent refinement of synaptic connections (Lein and Shatz 2000). Enhanced BDNF release appears to be just one manifestation of a more widespread dense core vesicle phenotype in *Mecp2* null mice. Studies of catecholamine release in *Mecp2* null adrenal chromaffin cells demonstrated that the readily releasable pool of dense core vesicles is significantly larger and individual vesicles are more fusigenic than in wild-type cells, resulting in hypersecretion of epinephrine (Ladas et al. 2009; Wang et al. 2006). Given that BDNF is also a dense core vesicle cargo (Decker et al. 2010; Farhadi et al. 2000; Luo et al. 2001; Salio et al. 2007; Wu et al. 2004), similar mechanisms may underlie the BDNF secretory phenotype in *Mecp2* null mice.

Recent studies indicate that BDNF signaling in *Mecp2* mutants is also impacted by deficits in axonal transport, resulting from dysregulation of huntingtin (Htt)- and huntingtin-associated protein (Hap1)-dependent vesicle trafficking (Roux et al. 2012). Specifically, the velocity of vesicular BDNF transport in cortico-striatal projection neurons is impaired by loss of MeCP2. Given the importance of cortically derived BDNF for the maintenance of striatal medium-spiny neurons (Baquet et al. 2004), these data raise the possibility that deficits in BDNF transport from the cortex contribute to striatal pathology in RTT (cf., Stearns et al. 2007).

3 Topography of BDNF Deficits in Mouse Models of RTT

The time course and distribution of BDNF deficits resulting from loss of MeCP2 have been studied in some detail in *Mecp2* null and heterozygous mice (Chang et al. 2006; Ogier et al. 2007; Wang et al. 2006; Deogracias et al. 2012). The earliest and most dramatic deficits in BDNF mRNA and protein occur in the vagal sensory nodose ganglion (NG), followed by the brainstem, cerebellum, and cortex (Chang et al. 2006; Ogier et al. 2007; Wang et al. 2006). In NG sensory neurons, for example, BDNF mRNA and protein levels fall to approximately 50 % wild-type values within 5 weeks after birth (Ogier et al. 2007), leading to synaptic dysfunction in vagal afferent inputs to the brainstem (see below). Within the brain, the effect of MeCP2 loss on BDNF levels is not uniform across cell groups. For example, although *Mecp2* null mutants exhibit marked decreases in BDNF immunostaining in the neuropil of some brainstem nuclei, such as the nucleus tractus solitarius (nTS), nucleus ambiguus, and nucleus locus coeruleus (LC), others, such as the gracile and principal sensory

trigeminal nuclei, are only mildly affected or unchanged (Kline et al. 2010). Mechanisms that underlie the differential temporal and spatial patterns of BDNF decline in the *Mecp2* mutant brain have not been defined. Recent data indicate that regional BDNF deficits in *Mecp2* null mutants are accompanied by reduced levels of TrkB phosphorylation without a change in total TrkB expression (Schmid et al. 2012).

4 BDNF Deficits in Mouse Models of RTT: Functional Consequences

With a few exceptions, relatively little is known about the specific functional consequences of reduced BDNF expression in *Mecp2* mutants and RTT patients. Morphologic and synaptic phenotypes observed in the brains of RTT patients and/or *Mecp2* null mutants, including decreased brain weight and neuronal size, reduced dendritic arborizations and impaired hippocampal long-term potentiation (reviewed in Chahrour and Zoghbi 2007), overlap with deficits seen in *Bdnf* loss-of-function mutants (Chang et al. 2006; Huang and Reichardt 2001). In addition, at least some of the behavioral features of *Mecp2* mutant mice, including irregular breathing and impaired locomotion, overlap to some degree with deficits observed in *Bdnf* mutants (Conover et al. 1995; Erickson et al. 1996). Moreover, genetic overexpression of BDNF in *Mecp2* null mutants can improve survival and locomotor function, whereas BDNF deletion hastens the onset of symptoms (Chang et al. 2006). However, few studies have examined how reduced BDNF availability in identified neural circuits is linked to specific functional deficits in RTT. What is clear is that because BDNF declines postnatally in *Mecp2* mutants, the size of neuronal populations that depend on BDNF for survival before birth is unaffected (Wang et al. 2006). Therefore, increasing attention has focused on the role of BDNF deficits in the maturation and function of the RTT brain after birth.

4.1 MeCP2 and Stimulation of Dendritic Growth by BDNF

MeCP2 plays a key role in mediating the effects of environmental stimuli, such as neuronal depolarization, on expression of genes required for neuronal maturation, including *BDNF* (Cohen et al. 2011; Ebert et al. 2013). For example, Zhou et al. (2006) demonstrated that phosphorylation of MeCP2 at serine 421 (ser421) is required for activity-dependent expression of BDNF in postnatal hippocampal neurons. BDNF, in turn, can stimulate ser421 phosphorylation of MeCP2, indicating that BDNF functions both upstream and downstream of MeCP2. MeCP2 phosphorylation at ser421 is also required for expression of mature dendritic morphologies in hippocampal neurons (Chapleau et al. 2009; Zhou et al. 2006), possibly by activating this BDNF signaling loop. In support of this possibility, overexpression of BDNF can reverse dendritic atrophy in hippocampal neurons that are null for *Mecp2* (Larimore et al. 2009).

4.2 BDNF and Synaptic Dysfunction in RTT

The potential synaptic consequences of BDNF loss have been studied in detail at primary afferent synapses between NG primary sensory neurons and second order neurons in the nTS. These synapses are the first site at which peripheral visceral sensory inputs impinge on central autonomic reflex pathways and thereby play a critical role in autonomic functions disrupted in RTT, such as respiratory, cardiovascular, and gastrointestinal homeostasis. Normally, BDNF plays a sensory gating function at these synapses by modulating postsynaptic responses to glutamate, the primary excitatory transmitter of visceral afferent neurons (Balkowiec et al. 2000). We hypothesized, therefore, that in *Mecp2* null mice, decreased BDNF expression in NG sensory neurons would be associated with a deficit in modulation of fast glutamatergic transmission at primary afferent synapses in nTS. Indeed, the amplitudes of spontaneous miniature and evoked EPSCs in nTS neurons are significantly increased in *Mecp2* null mice (Kline et al. 2010; Kron et al. 2012a), and accordingly, mutant cells are more likely than wild-type to fire action potentials in response to primary afferent stimulation (Kline et al. 2010). These changes occur without any increase in intrinsic neuronal excitability and are unaffected by blockade of inhibitory GABA currents. A prediction of these results is that autonomic reflexes mediated by primary afferent inputs to nTS would be disinhibited in the absence of MeCP2 function. This prediction has been borne out by studies demonstrating that the hypoxic ventilatory response, a reflex mediated by primary chemoafferent inputs to nTS, is markedly exaggerated in *Mecp2* null mice compared to wild-type controls (Bissonnette and Knopp 2006; Roux et al. 2008; Voituron et al. 2009). Similarly, *Mecp2* nulls exhibit a loss of habituation in the Breuer–Hering reflex, an nTS-mediated behavior that plays an essential role in regulating the post-inspiratory phase of the respiratory cycle (Stettner et al. 2007). More generally, these findings suggest that reduced sensory gating in nTS contributes to cardiorespiratory instability in RTT and that nTS is a site at which restoration of normal BDNF signaling could help to reestablish normal homeostatic controls. Indeed, exaggerated synaptic responses to primary afferent input in nTS are reversed by application of exogenous BDNF to brainstem slices in vitro (Kline et al. 2010). Moreover, respiratory function in vivo is improved by treatments that enhance BDNF/TrkB signaling in *Mecp2* mutants (see below).

4.3 BDNF and Hypothalamic Dysfunction in RTT

Feeding behavior and energy homeostasis are strongly influenced by BDNF/TrkB signaling in the hypothalamus (Noble et al. 2011; Rios et al. 2001). Specifically, increased levels of BDNF are associated with cessation of feeding and increased energy expenditure. Although the specific circuitry underlying the role of BDNF in feeding has not been completely defined, BDNF has been identified as a downstream effector of melanocortin-4 receptor (MC4R) signaling in the ventromedial hypothalamus (Noble et al. 2011; Xu et al. 2003), a key site for regulating feeding

and satiety. Fyffe et al. (2008) demonstrated that loss of *Mecp2* by Cre-mediated deletion specifically within Sim-1 expressing neurons in the hypothalamus results in reduced BDNF levels in *Mecp2* null neurons in the paraventricular nucleus, also a site of MC4R expression (Nicholson et al. 2007), as well as hyperphagia and obesity. Although the relevance of the obesity phenotype to RTT is unclear, these data provide further evidence that MeCP2 is required for maintaining normal levels of BDNF expression and metabolic homeostasis.

5 BDNF-Targeted Therapies for RTT

Recent studies in conditional *Mecp2* null mice have demonstrated that reactivation of the *Mecp2* gene, even in severely symptomatic animals, can rescue neurologic function to a remarkable degree (Guy et al. 2007). These findings indicate that deficits caused by loss of MeCP2 function are not due to irreversible changes in brain structure or function. In addition, as noted above, genetic overexpression of the BDNF gene in *Mecp2* null mice improves somatomotor function and prolongs life span (Chang et al. 2006), and exogenous BDNF can reverse synaptic deficits caused by MeCP2 deficiency (Kline et al. 2010). Together, these findings raise the possibility of rescuing neurologic function in *Mecp2* null mice and, eventually, RTT patients, by pharmacologic therapies that enhance BDNF/TrkB signaling. BDNF itself does not have good drug-like characteristics, i.e., limited half-life and poor blood–brain barrier penetration, thus motivating the search for alternative approaches to increasing BDNF/TrkB signaling in RTT. As discussed below, these approaches include enhancing expression of endogenous BDNF, increasing BDNF trafficking, and directly activating the TrkB receptor.

5.1 Increasing Expression or Delivery of Endogenous BDNF

In the first test of a BDNF-targeted therapeutic strategy, Ogier et al. (2007) examined whether or not pharmacologic elevation of endogenous BDNF expression with ampakine drugs could improve respiratory function in *Mecp2* null mice. Ampakines are benzamide derivatives that facilitate the activity of glutamatergic AMPA receptors and thereby increase expression of activity-dependent genes, including BDNF (Lynch and Gall 2006). Repeated administration of ampakines in rats and mice increases expression of BDNF mRNA and protein in the forebrain for several days (Lauterborn et al. 2003; Rex et al. 2006) and augments BDNF-dependent synaptic function (Ingvar et al. 1997; Porrino et al. 2005; Rex et al. 2006). Indeed, treatment of *Mecp2* null mutants with the ampakine CX546 for 3 days significantly increases BDNF levels in NG sensory neurons and reverses the respiratory tachypnea that is a prominent feature of breathing dysfunction in RTT (Ogier et al. 2007). Although additional studies are required to elucidate the mechanism of ampakine action in this model, these data are consistent with the hypothesis that BDNF deficits contribute to the respiratory phenotype of *Mecp2* null mice and that

BDNF signaling may be a pharmacological target for improving respiratory function in RTT. More recently, Deogracias et al. (2012) showed that fingolimod, a sphingosine-1 phosphate receptor agonist used to treat multiple sclerosis, increases BDNF in cultured neurons and protects against NMDA-induced neuronal death in a BDNF-dependent manner. In vivo, treatment of *Mecp2* mutant mice partially reversed BDNF deficits and also increased striatal volume, an index of BDNF signaling. Treated mice also showed improvement in locomotor behavior, a clinically relevant outcome measure for RTT patients. Finally, it is well known that BDNF expression in the rodent forebrain can be increased by environmental enrichment and exercise (cf., Cotman and Berchtold 2002). Indeed, rearing *Mecp2* mutant mice in an enriched environment, particularly at early stages of postnatal development, leads to improvements in motor and spatial learning, coordination, and anxiety, as well as hippocampal circuit function, that correlate well with increases in BDNF expression (Kondo et al. 2008; Lonetti et al. 2010).

Another potential strategy for enhancing BDNF/TrkB signaling in RTT is to increase the bioavailability of endogenous BDNF by promoting increased axonal transport and/or secretion. Recently, Roux et al. (2012) showed that cysteamine, a drug that increases vesicular trafficking of BDNF (Borrell-Pages et al. 2006), extends life span and improves motor function in *Mecp2* mutant mice.

5.2 Targeting the BDNF Receptor, TrkB

One potential limitation of pharmacologic approaches that globally increase BDNF is that BDNF activates receptors other than TrkB, including p75. The properties of BDNF binding to p75 as well as functioning as a full agonist at TrkB could lead to unwanted pleiotropic effects of elevated BDNF levels. An alternative approach is to directly activate TrkB; potential strategies include TrkB activating antibodies (Qian et al. 2006) and small molecules that function as direct TrkB ligands (Jang et al. 2010; Massa et al. 2010; Xie and Longo 2000). Our laboratory has recently examined the ability of a small molecule, non-peptide BDNF loop 2 domain mimetic, LM22A-4, which functions as a direct and specific partial agonist of TrkB, but not p75 (Massa et al. 2010), to increase TrkB activation and improve breathing in *Mecp2* mutant mice. LM22A-4 was developed by Longo, Massa, and colleagues by in silico screening for mimetics of BDNF loop domains that selectively activate TrkB and downstream signaling partners in vitro and in vivo (Han et al. 2012; Massa et al. 2010; Schmid et al. 2012). Recent studies in our laboratory have shown that LM22A-4 (1) reduces synaptic hyperexcitability in the brainstem respiratory network in brain slice preparations (Kron et al. 2012b), (2) reverses deficits in TrkB activation in the brainstem (Schmid et al. 2012), and (3) significantly improves respiratory function (Schmid et al. 2012), including the elimination of apneic breathing (Kron et al. 2012b), following systemic administration to symptomatic *Mecp2* null and heterozygous mice. Together, these data provide direct evidence linking TrkB signaling to respiratory dysfunction in mouse models

of RTT and further highlight the therapeutic potential of strategies aimed at enhancing BDNF/TrkB signaling for the treatment of RTT patients.

6 Summary

BDNF is only one of many genes whose expression is dysregulated in RTT (Chahrour et al. 2008). Nonetheless, given the multiplicity of roles played by BDNF signaling in brain maturation and neural circuit function across the life span, it is not surprising that deficits in BDNF protein levels have now been linked, either directly or indirectly, to diverse neurologic deficits in RTT, including reduced dendritic growth, breathing dysfunction, and impaired locomotion. Certainly, much more work is required to understand how BDNF deficits may contribute to the expression of specific RTT endophenotypes. It is encouraging, however, that the possibility of treating RTT using BDNF/TrkB-targeted therapies has already been established in principle in mouse models of the disease.

Acknowledgments The author thanks Min Lang and James Cody Howell for help in preparing and reviewing this manuscript and gratefully acknowledges funding support from the National Institutes of Health/National Institute of Neurological Diseases and Stroke, Rett Syndrome Research Foundation, and the International Rett Syndrome Foundation.

References

Abuhatzira L, Makedonski K, Kaufman Y, Razin A, Shemer R (2007) MeCP2 deficiency in the brain decreases BDNF levels by REST/CoREST-mediated repression and increases TRKB production. Epigenetics 2:214–222

Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 23:185–188

Balkowiec A, Kunze DL, Katz DM (2000) Brain-derived neurotrophic factor acutely inhibits AMPA-mediated currents in developing sensory relay neurons. J Neurosci 20:1904–1911

Ballas N, Grunseich C, Lu DD, Speh JC, Mandel G (2005) REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. Cell 121:645–657

Baquet ZC, Gorski JA, Jones KR (2004) Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. J Neurosci 24:4250–4258

Bissonnette JM, Knopp SJ (2006) Separate respiratory phenotypes in methyl-CpG-binding protein 2 (Mecp2) deficient mice. Pediatr Res 59:513–518

Borrell-Pages M, Canals JM, Cordelieres FP, Parker JA, Pineda JR, Grange G, Bryson EA, Guillermier M, Hirsch E, Hantraye P, Cheetham ME, Neri C, Alberch J, Brouillet E, Saudou F, Humbert S (2006) Cystamine and cysteamine increase brain levels of BDNF in Huntington disease via HSJ1b and transglutaminase. J Clin Invest 116:1410–1424

Chahrour M, Zoghbi HY (2007) The story of Rett syndrome: from clinic to neurobiology. Neuron 56:422–437

Chahrour M, Jung SY, Shaw C, Zhou X, Wong ST, Qin J, Zoghbi HY (2008) MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320: 1224–1229

Chang Q, Khare G, Dani V, Nelson S, Jaenisch R (2006) The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. Neuron 49:341–348

Chapleau CA, Calfa GD, Lane MC, Albertson AJ, Larimore JL, Kudo S, Armstrong DL, Percy AK, Pozzo-Miller L (2009) Dendritic spine pathologies in hippocampal pyramidal neurons from Rett syndrome brain and after expression of Rett-associated MECP2 mutations. Neurobiol Dis 35:219–233

Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, Jaenisch R, Greenberg ME (2003) Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science 302:885–889

Cohen S, Gabel HW, Hemberg M, Hutchinson AN, Sadacca LA, Ebert DH, Harmin DA, Greenberg RS, Verdine VK, Zhou Z, Wetsel WC, West AE, Greenberg ME (2011) Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. Neuron 72:72–85

Conover JC, Erickson JT, Katz DM, Bianchi LM, Poueymirou WT, McClain J, Pan L, Helgren M, Ip NY, Boland P et al (1995) Neuronal deficits, not involving motor neurons, in mice lacking BDNF and/or NT4. Nature 375:235–238

Cotman CW, Berchtold NC (2002) Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci 25:295–301

Dani VS, Chang Q, Maffei A, Turrigiano GG, Jaenisch R, Nelson SB (2005) Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. Proc Natl Acad Sci U S A 102:12560–12565

Decker H, Lo KY, Unger SM, Ferreira ST, Silverman MA (2010) Amyloid-beta peptide oligomers disrupt axonal transport through an NMDA receptor-dependent mechanism that is mediated by glycogen synthase kinase 3beta in primary cultured hippocampal neurons. J Neurosci 30: 9166–9171

Deng V, Matagne V, Banine F, Frerking M, Ohliger P, Budden S, Pevsner J, Dissen GA, Sherman LS, Ojeda SR (2007) FXYD1 is an MeCP2 target gene overexpressed in the brains of Rett syndrome patients and Mecp2-null mice. Hum Mol Genet 16:640–650

Deogracias R, Yazdani M, Dekkers MP, Guy J, Ionescu MC, Vogt KE, Barde YA (2012) Fingolimod, a sphingosine-1 phosphate receptor modulator, increases BDNF levels and improves symptoms of a mouse model of Rett syndrome. Proc Natl Acad Sci U S A 109: 14230–14235

Ebert DH, Gabel HW, Robinson ND, Kastan NR, Hu LS, Cohen S, Navarro AJ, Lyst MJ, Ekiert R, Bird AP, Greenberg ME (2013) Activity-dependent phosphorylation of MeCP2 threonine 308 regulates interaction with NCoR. Nature 499:341–345

Erickson JT, Conover JC, Borday V, Champagnat J, Barbacid M, Yancopoulos G, Katz DM (1996) Mice lacking brain-derived neurotrophic factor exhibit visceral sensory neuron losses distinct from mice lacking NT4 and display a severe developmental deficit in control of breathing. J Neurosci 16:5361–5371

Farhadi HF, Mowla SJ, Petrecca K, Morris SJ, Seidah NG, Murphy RA (2000) Neurotrophin-3 sorts to the constitutive secretory pathway of hippocampal neurons and is diverted to the regulated secretory pathway by coexpression with brain-derived neurotrophic factor. J Neurosci 20:4059–4068

Fyffe SL, Neul JL, Samaco RC, Chao HT, Ben-Shachar S, Moretti P, McGill BE, Goulding EH, Sullivan E, Tecott LH, Zoghbi HY (2008) Deletion of Mecp2 in Sim1-expressing neurons reveals a critical role for MeCP2 in feeding behavior, aggression, and the response to stress. Neuron 59:947–958

Georgel PT, Horowitz-Scherer RA, Adkins N, Woodcock CL, Wade PA, Hansen JC (2003) Chromatin compaction by human MeCP2. Assembly of novel secondary chromatin structures in the absence of DNA methylation. J Biol Chem 278:32181–32188

Gonzales ML, Adams S, Dunaway KW, LaSalle JM (2012) Phosphorylation of distinct sites in MeCP2 modifies cofactor associations and the dynamics of transcriptional regulation. Mol Cell Biol 32:2894–2903

Guy J, Gan J, Selfridge J, Cobb S, Bird A (2007) Reversal of neurological defects in a mouse model of Rett syndrome. Science 315:1143–1147

Hagberg B, Aicardi J, Dias K, Ramos O (1983) A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases. Ann Neurol 14:471–479

Han J, Pollak J, Yang T, Siddiqui MR, Doyle KP, Taravosh-Lahn K, Cekanaviciute E, Han A, Goodman JZ, Jones B, Jing D, Massa SM, Longo FM, Buckwalter MS (2012) Delayed administration of a small molecule tropomyosin-related kinase B ligand promotes recovery after hypoxic-ischemic stroke. Stroke 43:1918–1924

Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24:677–736

Ingvar M, Ambros-Ingerson J, Davis M, Granger R, Kessler M, Rogers GA, Schehr RS, Lynch G (1997) Enhancement by an ampakine of memory encoding in humans. Exp Neurol 146: 553–559

Jang SW, Liu X, Yepes M, Shepherd KR, Miller GW, Liu Y, Wilson WD, Xiao G, Blanchi B, Sun YE, Ye K (2010) A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. Proc Natl Acad Sci U S A 107:2687–2692

Katz DM, Dutschmann M, Ramirez JM, Hilaire G (2009) Breathing disorders in Rett syndrome: progressive neurochemical dysfunction in the respiratory network after birth. Respir Physiol Neurobiol 168:101–108

Kerr AM, Armstrong DD, Prescott RJ, Doyle D, Kearney DL (1997) Rett syndrome: analysis of deaths in the British survey. Eur Child Adolesc Psychiatry 6(Suppl 1):71–74

Klein ME, Lioy DT, Ma L, Impey S, Mandel G, Goodman RH (2007) Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. Nat Neurosci 10:1513–1514

Kline DD, Ogier M, Kunze DL, Katz DM (2010) Exogenous brain-derived neurotrophic factor rescues synaptic dysfunction in Mecp2-null mice. J Neurosci 30:5303–5310

Klose RJ, Bird AP (2006) Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci 31:89–97

Kondo M, Gray LJ, Pelka GJ, Christodoulou J, Tam PP, Hannan AJ (2008) Environmental enrichment ameliorates a motor coordination deficit in a mouse model of Rett syndrome – Mecp2 gene dosage effects and BDNF expression. Eur J Neurosci 27:3342–3350

Kron M, Howell CJ, Adams IT, Ransbottom M, Christian D, Ogier M, Katz DM (2012a) Brain activity mapping in Mecp2 mutant mice reveals functional deficits in forebrain circuits, including key nodes in the default mode network, that are reversed with ketamine treatment. J Neurosci 32:13860–13872

Kron M, Adams IT, Longo FM, Katz DM (2012b) A novel TrkB agonist eliminates apneic breathing and decreases synaptic hyperexcitability in brainstem respiratory nuclei in *Mecp2* mutant mice. In: Society for Neuroscience Abstracts, 246.14

Ladas T, Chan SA, Ogier M, Smith C, Katz DM (2009) Enhanced dense core granule function and adrenal hypersecretion in a mouse model of Rett syndrome. Eur J Neurosci 30:602–610

Larimore JL, Chapleau CA, Kudo S, Theibert A, Percy AK, Pozzo-Miller L (2009) Bdnf overexpression in hippocampal neurons prevents dendritic atrophy caused by Rett-associated MECP2 mutations. Neurobiol Dis 34:199–211

Lauterborn JC, Truong GS, Baudry M, Bi X, Lynch G, Gall CM (2003) Chronic elevation of brain-derived neurotrophic factor by ampakines. J Pharmacol Exp Ther 307:297–305

Lein ES, Shatz CJ (2000) Rapid regulation of brain-derived neurotrophic factor mRNA within eye-specific circuits during ocular dominance column formation. J Neurosci 20:1470–1483

Li W, Pozzo-Miller L (2013) BDNF deregulation in Rett syndrome. Neuropharmacology. doi:pii: S0028-3908(13)00120-2.10.1016 [Epub ahead of print]

Li W, Calfa G, Larimore J, Pozzo-Miller L (2012) Activity-dependent BDNF release and TRPC signaling is impaired in hippocampal neurons of Mecp2 mutant mice. Proc Natl Acad Sci U S A 109:17087–17092

Li Y, Wang H, Muffat J, Cheng AW, Orlando DA, Loven J, Kwok SM, Feldman DA, Bateup HS, Gao Q, Hockemeyer D, Mitalipova M, Lewis CA, Vander Heiden MG, Sur M, Young RA, Jaenisch R (2013) Global transcriptional and translational repression in human-embryonic-stem-cell-derived Rett syndrome neurons. Cell Stem Cell 13:446–458

Lonetti G, Angelucci A, Morando L, Boggio EM, Giustetto M, Pizzorusso T (2010) Early environmental enrichment moderates the behavioral and synaptic phenotype of MeCP2 null mice. Biol Psychiatry 67:657–665

Luo XG, Rush RA, Zhou XF (2001) Ultrastructural localization of brain-derived neurotrophic factor in rat primary sensory neurons. Neurosci Res 39:377–384

Lynch G, Gall CM (2006) Ampakines and the threefold path to cognitive enhancement. Trends Neurosci 29:554–562

Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, Fan G, Sun YE (2003) DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science 302: 890–893

Massa SM, Yang T, Xie Y, Shi J, Bilgen M, Joyce JN, Nehama D, Rajadas J, Longo FM (2010) Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. J Clin Invest 120:1774–1785

Nicholson JR, Peter JC, Lecourt AC, Barde YA, Hofbauer KG (2007) Melanocortin-4 receptor activation stimulates hypothalamic brain-derived neurotrophic factor release to regulate food intake, body temperature and cardiovascular function. J Neuroendocrinol 19:974–982

Noble EE, Billington C, Kotz CM, Wang C (2011) The lighter side of BDNF. Am J Physiol Regul Integr Comp Physiol 300(5):R1053–R1069

Ogier M, Wang H, Hong E, Wang Q, Greenberg ME, Katz DM (2007) Brain-derived neurotrophic factor expression and respiratory function improve after ampakine treatment in a mouse model of Rett syndrome. J Neurosci 27:10912–10917

Porrino LJ, Daunais JB, Rogers GA, Hampson RE, Deadwyler SA (2005) Facilitation of task performance and removal of the effects of sleep deprivation by an ampakine (CX717) in nonhuman primates. PLoS Biol 3:e299

Qian MD, Zhang J, Tan XY, Wood A, Gill D, Cho S (2006) Novel agonist monoclonal antibodies activate TrkB receptors and demonstrate potent neurotrophic activities. J Neurosci 26: 9394–9403

Rex CS, Lauterborn JC, Lin CY, Kramar EA, Rogers GA, Gall CM, Lynch G (2006) Restoration of long-term potentiation in middle-aged hippocampus after induction of brain-derived neurotrophic factor. J Neurophysiol 96:677–685

Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, Lechan RM, Jaenisch R (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol Endocrinol 15:1748–1757

Roux JC, Dura E, Villard L (2008) Tyrosine hydroxylase deficit in the chemoafferent and the sympathoadrenergic pathways of the Mecp2 deficient mouse. Neurosci Lett 447:82–86

Roux JC, Zala D, Panayotis N, Borges-Correia A, Saudou F, Villard L (2012) Modification of Mecp2 dosage alters axonal transport through the Huntingtin/Hap1 pathway. Neurobiol Dis 45:786–795

Salio C, Averill S, Priestley JV, Merighi A (2007) Costorage of BDNF and neuropeptides within individual dense-core vesicles in central and peripheral neurons. Dev Neurobiol 67:326–338

Schmid DA, Yang T, Ogier M, Adams I, Mirakhur Y, Wang Q, Massa SM, Longo FM, Katz DM (2012) A TrkB small molecule partial agonist rescues TrkB phosphorylation deficits and improves respiratory function in a mouse model of Rett syndrome. J Neurosci 32:1803–1810

Shahbazian MD, Zoghbi HY (2002) Rett syndrome and MeCP2: linking epigenetics and neuronal function. Am J Hum Genet 71:1259–1272

Skene PJ, Illingworth RS, Webb S, Kerr AR, James KD, Turner DJ, Andrews R, Bird AP (2010) Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state. Mol Cell 37:457–468

Stearns NA, Schaevitz LR, Bowling H, Nag N, Berger UV, Berger-Sweeney J (2007) Behavioral and anatomical abnormalities in Mecp2 mutant mice: a model for Rett syndrome. Neuroscience 146:907–921

Stettner GM, Huppke P, Brendel C, Richter DW, Gartner J, Dutschmann M (2007) Breathing dysfunctions associated with impaired control of postinspiratory activity in Mecp2-/y knockout mice. J Physiol 579:863–876

Sun YE, Wu H (2006) The ups and downs of BDNF in Rett syndrome. Neuron 49:321–323

Voituron N, Zanella S, Menuet C, Dutschmann M, Hilaire G (2009) Early breathing defects after moderate hypoxia or hypercapnia in a mouse model of Rett syndrome. Respir Physiol Neurobiol 168:109–118

Vorsanova SG, Iourov IY, Yurov YB (2004) Neurological, genetic and epigenetic features of Rett syndrome. J Pediatr Neurol 2:179–190

Wang H, Chan SA, Ogier M, Hellard D, Wang Q, Smith C, Katz DM (2006) Dysregulation of brain-derived neurotrophic factor expression and neurosecretory function in Mecp2 null mice. J Neurosci 26:10911–10915

Weese-Mayer DE, Lieske SP, Boothby CM, Kenny AS, Bennett HL, Silvestri JM, Ramirez JM (2006) Autonomic nervous system dysregulation: breathing and heart rate perturbation during wakefulness in young girls with Rett syndrome. Pediatr Res 60:443–449

Weese-Mayer DE, Lieske SP, Boothby CM, Kenny AS, Bennett HL, Ramirez JM (2008) Autonomic dysregulation in young girls with Rett Syndrome during nighttime in-home recordings. Pediatr Pulmonol 43:1045–1060

Wu YJ, Kruttgen A, Moller JC, Shine D, Chan JR, Shooter EM, Cosgaya JM (2004) Nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 are sorted to dense-core vesicles and released via the regulated pathway in primary rat cortical neurons. J Neurosci Res 75: 825–834

Wu H, Tao J, Chen PJ, Shahab A, Ge W, Hart RP, Ruan X, Ruan Y, Sun YE (2010) Genome-wide analysis reveals methyl-CpG-binding protein 2-dependent regulation of microRNAs in a mouse model of Rett syndrome. Proc Natl Acad Sci U S A 107:18161–18166

Xie Y, Longo FM (2000) Neurotrophin small-molecule mimetics. Prog Brain Res 128:333–347

Xu B, Goulding EH, Zang K, Cepoi D, Cone RD, Jones KR, Tecott LH, Reichardt LF (2003) Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 6:736–742

Young JI, Hong EP, Castle JC, Crespo-Barreto J, Bowman AB, Rose MF, Kang D, Richman R, Johnson JM, Berget S, Zoghbi HY (2005) Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. Proc Natl Acad Sci U S A 102:17551–17558

Zhou Z, Hong EJ, Cohen S, Zhao WN, Ho HY, Schmidt L, Chen WG, Lin Y, Savner E, Griffith EC, Hu L, Steen JA, Weitz CJ, Greenberg ME (2006) Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. Neuron 52:255–269

Zocchi L, Sassone-Corsi P (2012) SIRT1-mediated deacetylation of MeCP2 contributes to BDNF expression. Epigenetics 7:695–700

Modulation of Neurotrophin Signaling by Monoclonal Antibodies

A. Rosenthal and J.C. Lin

Abstract

The neurotrophin family is comprised of the structurally related secreted proteins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophine-4 (NT-4). They bind and activate the tyrosine kinase receptors Trk A, B, and C in a ligand-specific manner and additionally bind a shared p75NTR receptor. The neurotrophins were originally defined by their ability to support the survival and maturation of embryonic neurons. However, they also control important physiological functions of the adult nervous system including learning and memory, sensation, and energy homeostasis. For example, NGF/trkA signaling is critical for normal and pathological sensation of pain. Likewise, the BDNF/trkB pathway controls feeding and metabolism, and its dysfunction leads to severe obesity. Antibodies can modulate neurotrophin signaling. Thus, NGF blocking agents can attenuate pain in several animal models, and a recombinant humanized NGF blocking antibody (Tanezumab) has shown promising results in human clinical trials for osteoarthritic pain. On the other hand trkB agonist antibodies can modulate food intake and body weight in rodents and nonhuman primates. The power of monoclonal antibodies to modulate neurotrophin signaling promises to turn the rich biological insights into novel human medicines.

Keywords

Pain • NGF antagonists • Bone pain • Arthritis pain • Chronic low back pain • Interstitial cystitis • Tanezumab

A. Rosenthal (✉)
Alector Inc., 953 Indiana St., San Francisco, CA 94107
e-mail: Ar@alector.com

J.C. Lin
Rinat, Pfizer Inc., 230 E Grand Ave, South San Francisco, CA 94080
e-mail: john.lin@pfizer.com

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_19,

1 Introduction: Neurotrophins and Their Receptors

Nerve growth factor (NGF) was discovered and isolated by Rita Levi-Montalcini and Stanley Cohen in the 1950s as a target tissue-derived factor that supports the survival and neurite outgrowth of the developing sympathetic and sensory neurons. This was the first molecular demonstration in support of the "neurotrophic hypothesis" postulated by Viktor Hamburger (Cohen and Levi-Montalcini 1956; Cohen et al. 1954; Levi-Montalcini 1964; Levi-Montalcini and Hamburger 1951). About 30 years later, a second factor was purified based on its ability to promote the survival of primary sensory neurons. This factor was structurally related to NGF and designated brain-derived neurotrophic factor (BDNF) (Barde et al. 1982). The discovery of BDNF allowed for the identification of two additional family members, neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4, NT-5, or NT-4/5), on the basis of the DNA sequence homology between NGF and BDNF (Barde et al. 1982; Berkemeier et al. 1991; Hohn et al. 1990; Maisonpierre et al. 1990; Rosenthal et al. 1990, 1991).

BDNF is highly expressed in the developing and adult central nervous system (CNS) and peripheral nervous system (PNS), including the cerebral cortex, hippocampus, parts of basal ganglia, cerebellum, and spinal cord (Barde et al. 1987). On the other hand, NGF, NT-3, and NT-4 display a more restricted pattern of expression in the embryo and in the adult (Davies et al. 1993; Henderson et al. 1993; Ibanez et al. 1993; Kalcheim et al. 1992; Pinco et al. 1993). BDNF and NT4 share very similar profiles of biological activity. Both support the growth and survival of the sensory neurons of the trigeminal, nodose–petrosal, and dorsal root ganglia (Berkemeier et al. 1991; Davies et al. 1986, 1993; Kalcheim et al. 1987; Lindsay et al. 1985). On the other hand, NGF and NT-3 support the survival of sensory neurons of the trigeminal and dorsal root ganglia (Johnson and Yip 1985; Levi-Montalcini and Aloe 1985; Rosenthal et al. 1990). In addition, BDNF and NT4 also support the survival of motoneuron (Henderson et al. 1993; Koliatsos et al. 1994; Sendtner et al. 1992), dopaminergic neurons in the substantia nigra (Hyman et al. 1991; Hynes et al. 1994), cholinergic neurons in the basal forebrain (Knusel et al. 1992), all of which are of potential medical importance as they relate to motoneuron disease, Parkinson's disease, and Alzheimer's disease.

The neurotrophins exert their biological functions largely through binding to a family of "high affinity" tyrosine kinase receptors, trkA, B, and C, which are expressed by the target cells. The interaction between neurotrophins and trk receptors are highly ligand specific, resulting in tyrosine phosphorylation of the respective trk receptor and the recruitment of downstream signaling molecule (Chao et al. 2006; Ip et al. 1993; Patapoutian and Reichardt 2001). For instance, trkA is the primary receptor for NGF (Cordon-Cardo et al. 1991; Klein et al. 1991a). On the other hand, BDNF and NT4 mainly bind and signal through trkB, while NT3 is the only neurotrophic factor capable of binding trkC (Barbacid 1994; Klein et al. 1991b, 1992; Lamballe et al. 1991).

The in vivo significance of the pairwise neurtrophin-trk receptor interactions has been corroborated by genetic deletion of each of these genes through homologous

recombination in mice (Conover and Yancopoulos 1997; Huang and Reichardt 2001). Specifically, NGF and trkA deletion in mice both led to the severe reduction in the number of sympathetic and sensory neurons in the dorsal root ganglia (Crowley et al. 1994; Smeyne et al. 1994). Deletion of BDNF, NT4, and trkB in mice all led to the reduciton in the number of neurons of nodose, petrosal, and geniculate ganglia but not in the sympathetic ganglia (Conover et al. 1995; Erickson et al. 1996; Liu et al. 1995). The 50–60 % loss of in the nodose ganglia of BDNF−/− and in NT4−/− mice indicates that both factors are required for the full complement of neurons. The greater than 90 % loss of nodose ganglia in both the BDNF−/−; NT4−/− double knock-out and the trkB−/− mice suggests that both factors act in concert through the common trkB receptor to support the survival of these neurons in vivo.

All of the neurotrophins can bind a common receptor called p75NTR ("low affinity" receptor) in addition to their respective Trk receptors (Chao 1994). p75NTR signaling appears to depend on the specific cellular and developmental contexts and could either synergize with or antagonize the Trk signals (Cosgaya et al. 2002; Sharma et al. 2010). The fact that p75NTR interacts with many other neuronal receptors and soluble factors, such as the Nogo receptor, LINGO-1, Troy, plexinA4, ephrin-A, and amlyoid beta (Schecterson and Bothwell 2010), further enriches as well as complicates the precise interpretation of each specific function of p75NTR. In part because of these complexities, the value of p75NTR as a drug target had not been clearly demonstrated yet. Moreover, the pleiotropic effects of p75NTR due to its many ligands and binding proteins may lead to side effects of drugs targeting this receptor. As a result, p75NTR will not be discussed any further in this chapter.

The roles of neurotrophins and their specific Trk receptors during embryonic development of the nervous system have been studied extensively using neuronal cultures and gene ablation in mice (Conover and Yancopoulos 1997). Although the physiological functions of neurotrophins and their receptors during adult life are more relevant for drug discovery, it was difficult to assess these functions using traditional knockout mice. These mice almost always exhibited severe developmental deficits and were often associated with prenatal or early postnatal death. The scientific rationale and insight into new, antibody drugs that target neurotrophins or their receptors are largely derived from conditional knockouts and pharmacological interventions in the adult organisms.

2 Blocking Antibodies to NGF/trkA Pathway for Pain Relief

Developing sensory neurons transiently depend on NGF for survival during early development. For instance, rat sensory neurons require NGF for survival only until around postnatal day 2. Despite that, trkA expression in the nociceptive neurons persists beyond the dependence phase and is sustained throughout life (Gorin and Johnson 1980; Johnson et al. 1980; Yip et al. 1984). Multiple experiments indicate that, in the adult, NGF and TrkA acquire a new role as functional modulators of

neurons, particularly in pathological states. For example, injection of exogenous NGF to adult animals causes profound sensitization of nociceptive neurons leading to mechanical and thermal allodynia, i.e., pain from a stimulus that does not normally lead to the sensation of pain. Injection of NGF also leads to mechanical and thermal hyperalgesia, i.e., an extreme painful reaction to an otherwise innocuous or only mildly painful stimulus (Lewin and Mendell 1993; Lewin et al. 1993). Similar observation had been reported following injection of NGF in humans (Dyck et al. 1997; Svensson et al. 2003).

NGF mRNA and protein are frequently upregulated in sites of inflammation and in injured tissues. For instance, elevated levels of NGF are found in the synovial fluid from human subjects with rheumatoid arthritis and osteoarthitis as well as in the synovial fluid of animal models of inflammatory arthritis (Aloe et al. 1992a–c, 1993). NGF is also elevated in inflamed bladders, acute and chronic pancreatitis, and in conjunction with pancreatic cancer invasion (Friess et al. 1999; Lowe et al. 1997; Toma et al. 2000; Zhu et al. 1999). Likewise, NGF levels increase significantly after a surgical plantar or muscle incision in rats (Banik et al. 2005; Wu et al. 2009).

NGF decreases the activation threshold of sensory neurons in part by up-regulating pain-related neurotransmitters, receptors, and ion channels—including substance P, calcitonin gene-related peptide (Lindsay and Harmar 1989), the heat-gated TRPV1 channel (Winston et al. 2001), and action potential controlling sodium channels (Friedel et al. 1997). NGF also increases the cell surface level and functional level of the TRPV channels (Zhang et al. 2005) and sodium and calcium channels (Luther and Birren 2009). These gene expression and functional changes in the sensory neurons may underlie the enhanced pain sensitivity mediated by NGF.

The functional significance of the elevated NGF level in various pathological states was revealed through NGF blocking studies using either soluble trkA receptor or anti-NGF antibody. These NGF blocking agents led to a significant reduction of pain hypersensitivity in multiple pain models. These include models of inflammation-induced cutaneous or visceral pain (Bennett et al. 1998; Dmitrieva et al. 1997; McMahon et al. 1995), arthritic pain (Shelton et al. 2005), metastatic cancer-induced bone pain and pancreatic cancer pain (Halvorson et al. 2005; Sevcik et al. 2005), long bone fracture pain (Jimenez-Andrade et al. 2007; Koewler et al. 2007), surgical incision pain, and neuropathic pain conditions (Banik et al. 2005; Ro et al. 1999; Wild et al. 2007; Zahn et al. 2004).

Several characteristics of the pain relief brought about by NGF blockade are noteworthy. First, the pain relief can be achieved independent of any underlying disease modification. For example, while thermal and tactile sensitivity were fully normalized by anti-NGF in a rat model of collagen-induced autoimmune arthritis, the underlying inflammation and progression of bone and cartilage destruction were not affected by the treatment (Shelton et al. 2005). Likewise, anti-NGF antibodies elicit profound reduction in both spontaneous and induced pain as measured by reduction in guarding and flinching in a cancer pain model. However, the treatment has no effect on the growth of the prostate tumor grafts or on bone destruction

(Halvorson et al. 2005; Sevcik et al. 2005). Second, although anti-NGF can reverse allodynia and hyperalgesia, it had no effect on normal thermal and tactile sensitivity (Ghilardi et al. 2011; Sevcik et al. 2005). NGF antibody treatment was not associated with any change in the density of the peptidergic nociceptive fibers or sympathetic nerve endings in the skin of normal animals or with any impairment in the acute activation of the peptidergic nociceptors in healthy state (Ghilardi et al. 2011; Jimenez-Andrade et al. 2007; Sevcik et al. 2005). Thus, NGF does not seem to be required for the structural integrity of adult sensory neurons in non-pathological states. These pain mitigation effects of NGF antagonists are unique. In contrast to the nonsteroidal anti-inflammatory drugs or the tumor necrosis factor-α (TNF-α) antagonists, NGF antagonists do not affect the inflammatory process. Unlike opioids, they do not block normal pain sensation, hence not analgesic, and are not associated with drug tolerance (Wild et al. 2007).

Recently, Pat Mantyh and his colleagues discovered that the peptidergic, TrkA+ sensory nerve and the sympathetic nerve endings undergo abnormal sprouting and form neuroma-like structures as the bone cancers progress (Jimenez-Andrade et al. 2010; Mantyh et al. 2010). Furthermore, both the pathological nerve sprouting and cancer pain were driven by NGF because administration of anti-NGF antibodies or a pan-Trk kinase inhibitor completely abolished these pathological remodeling of nerve endings as well as cancer pain (Ghilardi et al. 2010; Jimenez-Andrade et al. 2010). These authors suggested that NGF derived from either tumor cells or tumor-associated stromal cells can lead to the pathological remodeling of nerve endings and cancer pain. It remains to be seen if the pathological remodeling of sensory and sympathetic nerve endings is also found in all the other known NGF-responsive pain states and indeed if it constitutes the underlying, unifying cellular mechanism of NGF-driven pathological pain state. It is interesting to note again that NGF blockade does not affect normal pain sensation and consistent with that does not appear to affect modulation of normal nerve endings.

Given the anticipated beneficial effects of NGF antagonists, Rinat Neuroscience Corp. and Pfizer Inc. developed Tanezumab, a fully humanized, high affinity anti-NGF monoclonal antibody. This antibody has been studied for safety and efficacy in clinical trials of multiple pain conditions. In a phase 2 trial of patients with moderate to severe pain due to osteoarthritis of the knee, Tanezumab given at 10–200 μg/kg intravenously once every 8 weeks has shown highly statistically significant efficacy. Specifically, a 45–62 % reduction of joint pain and 29–47 % improvement of function compared to placebo were achieved (Lane et al. 2010). The therapeutic effects of anti-NGF exhibited a clear trend of dose-dependent response. The most common adverse events included headache, muscle ache pain in the extremities, upper respiratory infections, and abnormal peripheral sensation/paresthesia (tingling, numbness, burning sensations, or increased sensitivity to touch). The abnormal peripheral sensation was found in 14 % of patients receiving Tanezumab and in 4 % of those receiving placebo and were mild in the majority of the patients. These sensory symptoms were predominantly transient and were not associated with neurological deficits (Lane et al. 2010; Wood 2010).

Encouraging clinical results with Tanezumab have also been reported in phase II trials with chronic low back pain (CLBP) and interstitial cystitis (IC) chronic inflammation of the urinary bladder (poster presentation at the American Academy of Pain Medicine's 26th Annual Meeting, 2010). In the CLBP study, patients randomly received a single intravenous infusion of either Tanezumab at 200 μg/kg placebo or the nonsteroidal anti-inflammatory drug naproxen, twice daily. In the IC study patients receive either a single infusion of Tanezumab at 200 μg/kg or placebo and recorded their pain severity daily before and up to 6 weeks after treatment using numeric scales. Patients treated with Tanezumab consistently reported significantly greater reductions in their pain when compared to patients treated with placebo. Moreover, for the CLBP patients, Tanezumab was more effective than naproxen (poster presentation at the American Academy of Pain Medicine's 26th Annual Meeting, 2010). Studies with Tanezumab in cancer pain are on going.

Surprisingly, however, the FDA recently raised concerns about a small number of osteoarthritis patients that were treated with Tanezumab whose osteoarthritis worsened, necessitating joint replacement. As a result, Pfizer halted enrollment and treatment of osteoarthritis patients with Tanezumab pending further evaluation of the data. Given the size of the Tanezumab clinical trials, which comprised over 9,000 patients and the fact that only 16 individuals with already advanced arthritis were affected, these reported events have to be statistically evaluated whether they are treatment dependent or not (Garber 2011; Wood 2010).

The positive clinical efficacy data indicate that NGF antagonists could become a novel class of powerful pain medicine. Nevertheless, better understanding of any potential adverse effects will be required before any new class of human therapeutics can become a reality. Since anti-NGF antibodies and other NGF and/or TrkA antagonists are now being pursued by multiple pharmaceutical companies, the therapeutic potential and limitations of targeting NGF are likely to be clarified within a few years.

A therapeutic antibody that antagonizes the neurotrophin signaling pathway such as Tanezumab has several pharmaceutical attributes distinct from the traditional small molecule pain medicines. One major difference is the target specificity offered by Tanezumab (Shelton et al. 2005) as opposed to kinase inhibitors, for example, the pan-Trk inhibitor (Ghilardi et al. 2011) which recognizes multiple targets. The high degree of target specificity offers a better safety profile compared to small molecules. Another major difference is the long plasma half-life of Tanezumab which allows applications once every 8 weeks compared to most small molecule pain drugs which often require daily application. This unique pharmacokinetic property may offset the inconvenience of intravenous or subcutaneous injections that are required to deliver antibody-based drugs. Finally, antibodies have limited access to the central nervous system (CNS) under noninflammatory conditions (DeMattos et al. 2001). While this property limits the use of antibodies for CNS disorders, it is allows safer treatment of peripheral indications, with minimal potential CNS side effects.

3 TrkB Agonist Antibody for Modulating Metabolic and Eating Disorders

Multiple genetic and pharmacological studies revealed that the BDNF/TrKB signaling system plays a key role on energy homeostasis. For example, mouse models of partial or brain-specific conditional knockout of BDNF as well as those of trkB exhibited profound hyperphagia and obesity (Duan et al. 2003; Kernie et al. 2000; Lyons et al. 1999; Rios et al. 2001; Xu et al. 2003). Likewise, regionally selective deletion of BDNF in the VMH using a virally mediated approach in the adult mouse led to hyperphagia (Unger et al. 2007). Conversely, exogenous administration of the TrkB ligands BDNF or NT-4, either systemically or centrally, led to reduction of food intake, body weight, and amelioration of various metabolic derangements associated with obesity and diabetes in a variety of rodent disease models, including the monogenic mouse models (*ob*/*ob*, *db*/*db*, A^y), high fat diet-induced obesity, and polygenic obese-diabetic mice (Nakagawa et al. 2000; Ono et al. 1997, 2000; Tonra et al. 1999; Tsao et al. 2008). Moreover, trkB-specific agonist antibodies, administered either centrally or peripherally, can also mediate beneficial metabolic effects in rodents similar to those of BDNF or NT-4 (Tsao et al. 2008), indicating that trkB is a key receptor mediating BDNF and NT-4's effects. BDNF is expressed in the ventral–medial hypothalamus (VMH) of mice, and its expression is regulated by food intake and the melanocortin pathway. (Bariohay et al. 2009; Nicholson et al. 2007; Xu et al. 2003). BDNF in turn may mediate its anorexigenic effect in part through feedback regulation on the VMH and the mesolimbic dopaminergic pathways (Cordeira et al. 2010).

In addition to their central effects on food intake, BDNF and trkB also control aspects of metabolism in the periphery. For example, BDNF enhances the hepatic insulin signaling (Tsuchida et al. 2001) and modulate glucagon secretion in the mouse pancreatic alpha cells (Hanyu et al. 2003). Mice with liver-specific ablation of BDNF exhibited normal food intake and body weight when fed with normal chow or high fat diet. However, these mice were protected from high fat diet induced dyslipidemia and hyperglycemia (Teillon et al. 2010).

The importance of BDNF, NT4, and trkB system in human metabolic disorders was underscored by genome-wide association studies of obesity and other genetic studies of human eating disorders. BDNF polymorphisms were found to be significantly associated with obesity in diverse ethnic populations by independent groups of investigators (Ng et al. 2010; Thorleifsson et al. 2009). Likewise, human individuals with either loss-of-function trkB mutation or BDNF deficiency have been associated with early onset hyperphagia and morbid obesity (Gray et al. 2007; Yeo et al. 2004). Haplo-insufficiency of the BDNF gene due to chromosomal deletion in patients with the *W*ilms' tumor, *a*niridia, *g*enitourinary anomalies, and mental *r*etardation (WAGR) syndrome is also associated with lower serum levels of BDNF and with childhood-onset obesity (Han et al. 2008). Furthermore, patients of Prader–Willi syndrome, which suffer excessive weight gain, display reduced plasma BDNF levels compared to those in the mildly obese or lean control subjects (Han et al. 2010), suggesting the decrease in BDNF may cause the hyperphagia in

these patients. It is therefore conceivable that trkB-specific agonists may be therapeutically useful in curbing the hyperphagia and obesity in human patients.

Consistent with the mouse and human genetic data, intra-cerebro-ventricular injections of BDNF or NT4 can reduce food intake in a dose-dependent manner in rhesus monkeys (Lin et al. 2008). However, when recombinant NT4 or a trkB-specific agonist antibody was given peripherally to several species of nonhuman primates, including obese baboons, lean cynomolgus, and rhesus monkeys, highly significant weight gain and appetite enhancement were observed (Lin et al. 2008). These results highlight the additional regulatory complexity in the central versus peripheral trkB system and call for further investigation into the trkB pathway and mechanism in primates. If trkB agonist antibody delivered peripherally can induce weight gain in humans, as in nonhuman primates, such an antibody may be useful in treating patients with severe anorexia or cachexia. Conversely, if the trkB agonist antibody elicits weight loss in humans, it could be considered for further evaluation as a potential therapy for obesity and hyperphagia disorders such as WAGR and Prader–Willi syndrome.

4 TrkB and TrkC Agonist Antibodies for Treating Nerve Degeneration and Neuropathy

In addition to the roles in regulating food intake and energy homeostasis, BDNF and TrkB signaling system is also implicated in neuronal degeneration or dysfunction (Zuccato and Cattaneo 2009), such as Alzheimer's disease (Blurton-Jones et al. 2009; Massa et al. 2010; Nagahara et al. 2009), Parkinson's disease (Baydyuk et al. 2011; Sun et al. 2005), Huntington's disease (Kells et al. 2004; Xie et al. 2010), motoneuron disease (Moro et al. 2006), and Rett's syndrome (Chang et al. 2006; Kline et al. 2010). For example, direct intracerebroventricular delivery of virally expressed BDNF or embryonic stem cell-derived neurons expressing BDNF can reverse cognitive impairment and neural degeneration in nonhuman primate and mouse models of Alzheimer's disease (Blurton-Jones et al. 2009; Nagahara et al. 2009). It is conceivable that TrkB agonist antibody may also be applied to achieve therapeutic effects in these degenerative diseases of the CNS to mimic the beneficial effect of BDNF. However, significant technical challenge remains regarding how to safely and conveniently deliver TrkB agonist antibodies to the target neuronal population in the CNS of these patients. Given the socioeconomic burden and the human suffering brought about by these debilitating diseases, we are hopeful that technical solutions will soon emerge to overcome this significant hurdle of neurotrophin/antibody drug development. Emerging technologies include the usage of antibodies to the insulin receptor (Boado et al. 2010) or transferring receptor (Zhou et al. 2011), which mediates transcytosis into the brain, as Trojan horses to deliver therapeutic proteins, antibodies, or other drugs to CNS targets in both mouse and primate animal models.

For the degenerative diseases of the peripheral nervous system (PNS), monoclonal antibodies may hold more immediate therapeutic potential since there is less

issue with antibody drug delivery to the target tissues. One example is Charcot–Marie–Tooth disease type 1A (CMT1A), which is a hereditary form of progressive demyelinating disease restricted to the peripheral nerves. The Trembler mice (Tr^J) with a mutation in the peripheral myelin protein-22 gene is a mouse model of this disorder. Administration of NT-3 has been shown to enhance the nerve regeneration upon crush injury and improve remyelination in this model. NT-3 administration was also effective in a disease model where Schwann cells derived from CMT1A patients were engrafted into immune-deficient nude mice (Sahenk et al. 2005).

Since NT3 acts primarily through TrkC and also weakly through TrkA and TrkB, the therapeutic potential of TrkC and TrkB agonist antibodies, either alone or in combination, was evaluated in the Tr^J mouse model. TrkB and TrkC agonist antibodies in combination significantly improved the electrophysiological measures, motor function performance, as well as nerve regeneration in this animal model of CMT1A (Sahenk et al. 2010). If Trk agonist antibodies are shown to be effective in human CMT1A patients, additional disorders associated with peripheral neuropathy such as diabetic neuropathy or chemotherapy-induced neuropathy should be considered for therapeutic evaluation.

Significant amount of future work will be needed to develop Trk agonist antibodies into useful medicine in either CNS or PNS neural degeneration. Nevertheless, it is important to note that such agonist antibodies hold promise in terms of both target selectivity and pharmaceutical properties compared to the corresponding naturally occurring agonists. First, agonist antibodies have exceptional long plasma half-life compared to natural ligand molecules (14 days vs. a few minutes or hours in human plasma) and thus are more likely to achieve efficacious levels and are more convenient to use in treating chronic degenerative diseases (ALS CNTF Treatment Study (ACTS) Phase I-II Study Group 1995; Nguyen et al. 2000; Sahenk et al. 2010). Second, several naturally occurring growth factors or agonists such as erythropoietin (EPO), thrombopoietin (TPO), and glial cell line-derived neurotrophic factor (GDNF) have been associated with the development of antidrug antibodies (ADA) in human subjects and in nonhuman primate models (Casadevall et al. 2005; Chong and Ho 2005; Gao et al. 2004; Hovland et al. 2007; Lang et al. 2006). Such ADA reactions against the recombinant protein drugs often spread to target the respective endogenous ligands due to the shared sequences and epitopes, thus resulting in life-threatening consequences. For example, ADA to the EPO protein could lead to aplastic anemia following spreading of auto immune response from the injected EPO to the endogenous EPO protein. Likewise, spreading of ADA from injected TPO to the endogenous TPO lead to thrombocytopenia. Such immunological reaction is unlikely to occur with the agonist antibodies since there is virtually no sequence similarity between the antibody and the naturally occurring growth factor.

Concluding Remarks

Here, we review the scientific rationale and the recent progress of targeting neurotrophins using monoclonal antibodies as the therapeutic agents. We give examples of therapeutic antibodies capable of blocking or activating the

neurotrophin system via the NGF/TrkA and BDNF/TrkB pathways, respectively. The clinical success of monoclonal antibodies in the areas of oncology and inflammation has now been extended to the nervous system as shown by the impressive efficacy of anti-NGF antibodies such as Tanezumab. The promise of using therapeutic antibodies to target neurotrophins will hopefully be brought to medical realty and be further expanded to the CNS indications once we understand the safety profiles and the appropriate delivery methods in a variety of clinical settings.

References

Aloe L, Tuveri MA, Carcassi U, Levi-Montalcini R (1992a) Nerve growth factor in the synovial fluid of patients with chronic arthritis. Arthritis Rheum 35:351–355

Aloe L, Tuveri MA, Levi-Montalcini R (1992b) Nerve growth factor and distribution of mast cells in the synovium of adult rats. Clin Exp Rheumatol 10:203–204

Aloe L, Tuveri MA, Levi-Montalcini R (1992c) Studies on carrageenan-induced arthritis in adult rats: presence of nerve growth factor and role of sympathetic innervation. Rheumatol Int 12:213–216

Aloe L, Probert L, Kollias G, Bracci-Laudiero L, Spillantini MG, Levi-Montalcini R (1993) The synovium of transgenic arthritic mice expressing human tumor necrosis factor contains a high level of nerve growth factor. Growth Factors 9:149–155

ALS CNTF Treatment Study (ACTS) Phase I-II Study Group (1995) The pharmacokinetics of subcutaneaously administered recombinant human ciliary neurotrophic factor (rHCNTF) in patients with amyotrophic lateral sclerosis: relation to parameters of the acute-phase response. Clin Neuropharmacol 18:500–514

Banik RK, Subieta AR, Wu C, Brennan TJ (2005) Increased nerve growth factor after rat plantar incision contributes to guarding behavior and heat hyperalgesia. Pain 117:68–76

Barbacid M (1994) The Trk family of neurotrophin receptors. J Neurobiol 25:1386–1403

Barde YA, Edgar D, Thoenen H (1982) Purification of a new neurotrophic factor from mammalian brain. EMBO J 1:549–553

Barde YA, Davies AM, Johnson JE, Lindsay RM, Thoenen H (1987) Brain derived neurotrophic factor. Prog Brain Res 71:185–189

Bariohay B, Roux J, Tardivel C, Trouslard J, Jean A, Lebrun B (2009) Brain-derived neurotrophic factor/tropomyosin-related kinase receptor type B signaling is a downstream effector of the brainstem melanocortin system in food intake control. Endocrinology 150:2646–2653

Baydyuk M, Nguyen MT, Xu B (2011) Chronic deprivation of TrkB signaling leads to selective late-onset nigrostriatal dopaminergic degeneration. Exp Neurol 228:118–125

Bennett DL, Koltzenburg M, Priestley JV, Shelton DL, McMahon SB (1998) Endogenous nerve growth factor regulates the sensitivity of nociceptors in the adult rat. Eur J Neurosci 10:1282–1291

Berkemeier LR, Winslow JW, Kaplan DR, Nikolics K, Goeddel DV, Rosenthal A (1991) Neurotrophin-5: a novel neurotrophic factor that activates trk and trkB. Neuron 7:857–866

Blurton-Jones M, Kitazawa M, Martinez-Coria H, Castello NA, Muller FJ et al (2009) Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease. Proc Natl Acad Sci U S A 106:13594–13599

Boado RJ, Hui EK, Lu JZ, Pardridge WM (2010) Drug targeting of erythropoietin across the primate blood-brain barrier with an IgG molecular Trojan horse. J Pharmacol Exp Ther 333:961–969

Casadevall N, Eckardt KU, Rossert J (2005) Epoetin-induced autoimmune pure red cell aplasia. J Am Soc Nephrol 16(Suppl 1):S67–S69

Chang Q, Khare G, Dani V, Nelson S, Jaenisch R (2006) The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. Neuron 49:341–348
Chao MV (1994) The p75 neurotrophin receptor. J Neurobiol 25:1373–1385
Chao MV, Rajagopal R, Lee FS (2006) Neurotrophin signalling in health and disease. Clin Sci (Lond) 110:167–173
Chong BH, Ho SJ (2005) Autoimmune thrombocytopenia. J Thromb Haemost 3:1763–1772
Cohen S, Levi-Montalcini R (1956) A nerve growth-stimulating factor isolated from snake venom. Proc Natl Acad Sci U S A 42:571–574
Cohen S, Levi-Montalcini R, Hamburger V (1954) A nerve growth-stimulating factor isolated from sarcom as 37 and 180. Proc Natl Acad Sci U S A 40:1014–1018
Conover JC, Yancopoulos GD (1997) Neurotrophin regulation of the developing nervous system: analyses of knockout mice. Rev Neurosci 8:13–27
Conover JC, Erickson JT, Katz DM, Bianchi LM, Poueymirou WT et al (1995) Neuronal deficits, not involving motor neurons, in mice lacking BDNF and/or NT4. Nature 375:235–238
Cordeira JW, Frank L, Sena-Esteves M, Pothos EN, Rios M (2010) Brain-derived neurotrophic factor regulates hedonic feeding by acting on the mesolimbic dopamine system. J Neurosci 30:2533–2541
Cordon-Cardo C, Tapley P, Jing SQ, Nanduri V, O'Rourke E et al (1991) The trk tyrosine protein kinase mediates the mitogenic properties of nerve growth factor and neurotrophin-3. Cell 66:173–183
Cosgaya JM, Chan JR, Shooter EM (2002) The neurotrophin receptor p75NTR as a positive modulator of myelination. Science 298:1245–1248
Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S et al (1994) Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 76:1001–1011
Davies AM, Thoenen H, Barde YA (1986) The response of chick sensory neurons to brain-derived neurotrophic factor. J Neurosci 6:1897–1904
Davies AM, Horton A, Burton LE, Schmelzer C, Vandlen R, Rosenthal A (1993) Neurotrophin-4/5 is a mammalian-specific survival factor for distinct populations of sensory neurons. J Neurosci 13:4961–4967
DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM (2001) Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 98:8850–8855
Dmitrieva N, Shelton D, Rice AS, McMahon SB (1997) The role of nerve growth factor in a model of visceral inflammation. Neuroscience 78:449–459
Duan W, Guo Z, Jiang H, Ware M, Mattson MP (2003) Reversal of behavioral and metabolic abnormalities, and insulin resistance syndrome, by dietary restriction in mice deficient in brain-derived neurotrophic factor. Endocrinology 144:2446–2453
Dyck PJ, Peroutka S, Rask C, Burton E, Baker MK et al (1997) Intradermal recombinant human nerve growth factor induces pressure allodynia and lowered heat-pain threshold in humans. Neurology 48:501–505
Erickson JT, Conover JC, Borday V, Champagnat J, Barbacid M et al (1996) Mice lacking brain-derived neurotrophic factor exhibit visceral sensory neuron losses distinct from mice lacking NT4 and display a severe developmental deficit in control of breathing. J Neurosci 16:5361–5371
Friedel RH, Schnurch H, Stubbusch J, Barde YA (1997) Identification of genes differentially expressed by nerve growth factor- and neurotrophin-3-dependent sensory neurons. Proc Natl Acad Sci U S A 94:12670–12675
Friess H, Zhu ZW, di Mola FF, Kulli C, Graber HU et al (1999) Nerve growth factor and its high-affinity receptor in chronic pancreatitis. Ann Surg 230:615–624
Gao G, Lebherz C, Weiner DJ, Grant R, Calcedo R et al (2004) Erythropoietin gene therapy leads to autoimmune anemia in macaques. Blood 103:3300–3302
Garber K (2011) Fate of novel painkiller mAbs hangs in balance. Nat Biotechnol 29:173–174

Ghilardi JR, Freeman KT, Jimenez-Andrade JM, Mantyh WG, Bloom AP et al (2010) Administration of a tropomyosin receptor kinase inhibitor attenuates sarcoma-induced nerve sprouting, neuroma formation and bone cancer pain. Mol Pain 6:87

Ghilardi JR, Freeman KT, Jimenez-Andrade JM, Mantyh WG, Bloom AP et al (2011) Sustained blockade of neurotrophin receptors TrkA, TrkB and TrkC reduces non-malignant skeletal pain but not the maintenance of sensory and sympathetic nerve fibers. Bone 48(2):389–398

Gorin PD, Johnson EM Jr (1980) Effects of long-term nerve growth factor deprivation on the nervous system of the adult rat: an experimental autoimmune approach. Brain Res 198:27–42

Gray J, Yeo G, Hung C, Keogh J, Clayton P et al (2007) Functional characterization of human NTRK2 mutations identified in patients with severe early-onset obesity. Int J Obes (Lond) 31:359–364

Halvorson KG, Kubota K, Sevcik MA, Lindsay TH, Sotillo JE et al (2005) A blocking antibody to nerve growth factor attenuates skeletal pain induced by prostate tumor cells growing in bone. Cancer Res 65:9426–9435

Han JC, Liu QR, Jones M, Levinn RL, Menzie CM et al (2008) Brain-derived neurotrophic factor and obesity in the WAGR syndrome. N Engl J Med 359:918–927

Han JC, Muehlbauer MJ, Cui HN, Newgard CB, Haqq AM (2010) Lower brain-derived neurotrophic factor in patients with Prader-Willi syndrome compared to obese and lean control subjects. J Clin Endocrinol Metab 95:3532–3536

Hanyu O, Yamatani K, Ikarashi T, Soda S, Maruyama S et al (2003) Brain-derived neurotrophic factor modulates glucagon secretion from pancreatic alpha cells: its contribution to glucose metabolism. Diabetes Obes Metab 5:27–37

Henderson CE, Camu W, Mettling C, Gouin A, Poulsen K et al (1993) Neurotrophins promote motor neuron survival and are present in embryonic limb bud. Nature 363:266–270

Hohn A, Leibrock J, Bailey K, Barde YA (1990) Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. Nature 344:339–341

Hovland DN Jr, Boyd RB, Butt MT, Engelhardt JA, Moxness MS et al (2007) Six-month continuous intraputamenal infusion toxicity study of recombinant methionyl human glial cell line-derived neurotrophic factor (r-metHuGDNF) in rhesus monkeys. Toxicol Pathol 35:676–692

Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24:677–736

Hyman C, Hofer M, Barde YA, Juhasz M, Yancopoulos GD et al (1991) BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 350:230–232

Hynes MA, Poulsen K, Armanini M, Berkemeier L, Phillips H, Rosenthal A (1994) Neurotrophin-4/5 is a survival factor for embryonic midbrain dopaminergic neurons in enriched cultures. J Neurosci Res 37:144–154

Ibanez CF, Ernfors P, Timmusk T, Ip NY, Arenas E et al (1993) Neurotrophin-4 is a target-derived neurotrophic factor for neurons of the trigeminal ganglion. Development 117:1345–1353

Ip NY, Stitt TN, Tapley P, Klein R, Glass DJ et al (1993) Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and nonneuronal cells. Neuron 10:137–149

Jimenez-Andrade JM, Martin CD, Koewler NJ, Freeman KT, Sullivan LJ et al (2007) Nerve growth factor sequestering therapy attenuates non-malignant skeletal pain following fracture. Pain 133:183–196

Jimenez-Andrade JM, Bloom AP, Stake JI, Mantyh WG, Taylor RN et al (2010) Pathological sprouting of adult nociceptors in chronic prostate cancer-induced bone pain. J Neurosci 30:14649–14656

Johnson EM Jr, Yip HK (1985) Central nervous system and peripheral nerve growth factor provide trophic support critical to mature sensory neuronal survival. Nature 314:751–752

Johnson EM Jr, Gorin PD, Brandeis LD, Pearson J (1980) Dorsal root ganglion neurons are destroyed by exposure in utero to maternal antibody to nerve growth factor. Science 210:916–918

Kalcheim C, Barde YA, Thoenen H, Le Douarin NM (1987) In vivo effect of brain-derived neurotrophic factor on the survival of developing dorsal root ganglion cells. EMBO J 6:2871–2873

Kalcheim C, Carmeli C, Rosenthal A (1992) Neurotrophin 3 is a mitogen for cultured neural crest cells. Proc Natl Acad Sci U S A 89:1661–1665

Kells AP, Fong DM, Dragunow M, During MJ, Young D, Connor B (2004) AAV-mediated gene delivery of BDNF or GDNF is neuroprotective in a model of Huntington disease. Mol Ther 9:682–688

Kernie SG, Liebl DJ, Parada LF (2000) BDNF regulates eating behavior and locomotor activity in mice. EMBO J 19:1290–1300

Klein R, Jing SQ, Nanduri V, O'Rourke E, Barbacid M (1991a) The trk proto-oncogene encodes a receptor for nerve growth factor. Cell 65:189–197

Klein R, Nanduri V, Jing SA, Lamballe F, Tapley P et al (1991b) The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. Cell 66:395–403

Klein R, Lamballe F, Bryant S, Barbacid M (1992) The trkB tyrosine protein kinase is a receptor for neurotrophin-4. Neuron 8:947–956

Kline DD, Ogier M, Kunze DL, Katz DM (2010) Exogenous brain-derived neurotrophic factor rescues synaptic dysfunction in Mecp2-null mice. J Neurosci 30:5303–5310

Knusel B, Beck KD, Winslow JW, Rosenthal A, Burton LE et al (1992) Brain-derived neurotrophic factor administration protects basal forebrain cholinergic but not nigral dopaminergic neurons from degenerative changes after axotomy in the adult rat brain. J Neurosci 12:4391–4402

Koewler NJ, Freeman KT, Buus RJ, Herrera MB, Jimenez-Andrade JM et al (2007) Effects of a monoclonal antibody raised against nerve growth factor on skeletal pain and bone healing after fracture of the C57BL/6J mouse femur. J Bone Miner Res 22:1732–1742

Koliatsos VE, Cayouette MH, Berkemeier LR, Clatterbuck RE, Price DL, Rosenthal A (1994) Neurotrophin 4/5 is a trophic factor for mammalian facial motor neurons. Proc Natl Acad Sci U S A 91:3304–3308

Lamballe F, Klein R, Barbacid M (1991) trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. Cell 66:967–979

Lane NE, Schnitzer TJ, Birbara CA, Mokhtarani M, Shelton DL et al (2010) Tanezumab for the treatment of pain from osteoarthritis of the knee. N Engl J Med 363:1521–1531

Lang AE, Gill S, Patel NK, Lozano A, Nutt JG et al (2006) Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. Ann Neurol 59:459–466

Levi-Montalcini R (1964) Growth control of nerve cells by a protein factor and its antiserum: discovery of this factor may provide new leads to understanding of some neurogenetic processes. Science 143:105–110

Levi-Montalcini R, Aloe L (1985) Differentiating effects of murine nerve growth factor in the peripheral and central nervous systems of Xenopus laevis tadpoles. Proc Natl Acad Sci U S A 82:7111–7115

Levi-Montalcini R, Hamburger V (1951) Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. J Exp Zool 116:321–361

Lewin GR, Mendell LM (1993) Nerve growth factor and nociception. Trends Neurosci 16:353–359

Lewin GR, Ritter AM, Mendell LM (1993) Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. J Neurosci 13:2136–2148

Lin JC, Tsao D, Barras P, Bastarrachea RA, Boyd B et al (2008) Appetite enhancement and weight gain by peripheral administration of TrkB agonists in non-human primates. PLoS One 3:e1900

Lindsay RM, Harmar AJ (1989) Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. Nature 337:362–364

Lindsay RM, Barde YA, Davies AM, Rohrer H (1985) Differences and similarities in the neurotrophic growth factor requirements of sensory neurons derived from neural crest and neural placode. J Cell Sci Suppl 3:115–129

Liu X, Ernfors P, Wu H, Jaenisch R (1995) Sensory but not motor neuron deficits in mice lacking NT4 and BDNF. Nature 375:238–241

Lowe EM, Anand P, Terenghi G, Williams-Chestnut RE, Sinicropi DV, Osborne JL (1997) Increased nerve growth factor levels in the urinary bladder of women with idiopathic sensory urgency and interstitial cystitis. Br J Urol 79:572–577

Luther JA, Birren SJ (2009) p75 and TrkA signaling regulates sympathetic neuronal firing patterns via differential modulation of voltage-gated currents. J Neurosci 29:5411–5424

Lyons WE, Mamounas LA, Ricaurte GA, Coppola V, Reid SW et al (1999) Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. Proc Natl Acad Sci U S A 96:15239–15244

Maisonpierre PC, Belluscio L, Squinto S, Ip NY, Furth ME et al (1990) Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. Science 247:1446–1451

Mantyh WG, Jimenez-Andrade JM, Stake JI, Bloom AP, Kaczmarska MJ et al (2010) Blockade of nerve sprouting and neuroma formation markedly attenuates the development of late stage cancer pain. Neuroscience 171:588–598

Massa SM, Yang T, Xie Y, Shi J, Bilgen M et al (2010) Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. J Clin Invest 120:1774–1785

McMahon SB, Bennett DL, Priestley JV, Shelton DL (1995) The biological effects of endogenous nerve growth factor on adult sensory neurons revealed by a trkA-IgG fusion molecule. Nat Med 1:774–780

Moro K, Shiotani A, Watabe K, Takeda Y, Saito K et al (2006) Adenoviral gene transfer of BDNF and GDNF synergistically prevent motoneuron loss in the nucleus ambiguus. Brain Res 1076:1–8

Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE et al (2009) Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. Nat Med 15:331–337

Nakagawa T, Tsuchida A, Itakura Y, Nonomura T, Ono M et al (2000) Brain-derived neurotrophic factor regulates glucose metabolism by modulating energy balance in diabetic mice. Diabetes 49:436–444

Ng MC, Tam CH, So WY, Ho JS, Chan AW et al (2010) Implication of genetic variants near NEGR1, SEC16B, TMEM18, ETV5/DGKG, GNPDA2, LIN7C/BDNF, MTCH2, BCDIN3D/FAIM2, SH2B1, FTO, MC4R, and KCTD15 with obesity and type 2 diabetes in 7705 Chinese. J Clin Endocrinol Metab 95:2418–2425

Nguyen CB, Harris L, Szonyi E, Baughman SA, Hale VG et al (2000) Tissue disposition and pharmacokinetics of recombinant human nerve growth factor after acute and chronic subcutaneous administration in monkeys. Drug Metab Dispos 28:598–607

Nicholson JR, Peter JC, Lecourt AC, Barde YA, Hofbauer KG (2007) Melanocortin-4 receptor activation stimulates hypothalamic brain-derived neurotrophic factor release to regulate food intake, body temperature and cardiovascular function. J Neuroendocrinol 19:974–982

Ono M, Ichihara J, Nonomura T, Itakura Y, Taiji M et al (1997) Brain-derived neurotrophic factor reduces blood glucose level in obese diabetic mice but not in normal mice. Biochem Biophys Res Commun 238:633–637

Ono M, Itakura Y, Nonomura T, Nakagawa T, Nakayama C et al (2000) Intermittent administration of brain-derived neurotrophic factor ameliorates glucose metabolism in obese diabetic mice. Metabolism 49:129–133

Patapoutian A, Reichardt LF (2001) Trk receptors: mediators of neurotrophin action. Curr Opin Neurobiol 11:272–280

Pinco O, Carmeli C, Rosenthal A, Kalcheim C (1993) Neurotrophin-3 affects proliferation and differentiation of distinct neural crest cells and is present in the early neural tube of avian embryos. J Neurobiol 24:1626–1641

Rios M, Fan G, Fekete C, Kelly J, Bates B et al (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol Endocrinol 15:1748–1757

Ro LS, Chen ST, Tang LM, Jacobs JM (1999) Effect of NGF and anti-NGF on neuropathic pain in rats following chronic constriction injury of the sciatic nerve. Pain 79:265–274

Rosenthal A, Goeddel DV, Nguyen T, Lewis M, Shih A et al (1990) Primary structure and biological activity of a novel human neurotrophic factor. Neuron 4:767–773

Rosenthal A, Goeddel DV, Nguyen T, Martin E, Burton LE et al (1991) Primary structure and biological activity of human brain-derived neurotrophic factor. Endocrinology 129:1289–1294

Sahenk Z, Nagaraja HN, McCracken BS, King WM, Freimer ML et al (2005) NT-3 promotes nerve regeneration and sensory improvement in CMT1A mouse models and in patients. Neurology 65:681–689

Sahenk Z, Galloway G, Edwards C, Malik V, Kaspar BK et al (2010) TrkB and TrkC agonist antibodies improve function, electrophysiologic and pathologic features in Trembler J mice. Exp Neurol 224:495–506

Schecterson LC, Bothwell M (2010) Neurotrophin receptors: old friends with new partners. Dev Neurobiol 70:332–338

Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde YA (1992) Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. Nature 360:757–759

Sevcik MA, Ghilardi JR, Peters CM, Lindsay TH, Halvorson KG et al (2005) Anti-NGF therapy profoundly reduces bone cancer pain and the accompanying increase in markers of peripheral and central sensitization. Pain 115:128–141

Sharma N, Deppmann CD, Harrington AW, St Hillaire C, Chen ZY et al (2010) Long-distance control of synapse assembly by target-derived NGF. Neuron 67:422–434

Shelton DL, Zeller J, Ho WH, Pons J, Rosenthal A (2005) Nerve growth factor mediates hyperalgesia and cachexia in auto-immune arthritis. Pain 116:8–16

Smeyne RJ, Klein R, Schnapp A, Long LK, Bryant S et al (1994) Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature 368:246–249

Sun M, Kong L, Wang X, Lu XG, Gao Q, Geller AI (2005) Comparison of the capability of GDNF, BDNF, or both, to protect nigrostriatal neurons in a rat model of Parkinson's disease. Brain Res 1052:119–129

Svensson P, Cairns BE, Wang K, Arendt-Nielsen L (2003) Injection of nerve growth factor into human masseter muscle evokes long-lasting mechanical allodynia and hyperalgesia. Pain 104:241–247

Teillon S, Calderon GA, Rios M (2010) Diminished diet-induced hyperglycemia and dyslipidemia and enhanced expression of PPARalpha and FGF21 in mice with hepatic ablation of brain-derived neurotropic factor. J Endocrinol 205:37–47

Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P et al (2009) Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet 41:18–24

Toma H, Winston J, Micci MA, Shenoy M, Pasricha PJ (2000) Nerve growth factor expression is up-regulated in the rat model of L-arginine-induced acute pancreatitis. Gastroenterology 119:1373–1381

Tonra JR, Ono M, Liu X, Garcia K, Jackson C et al (1999) Brain-derived neurotrophic factor improves blood glucose control and alleviates fasting hyperglycemia in C57BLKS-Lepr(db)/lepr(db) mice. Diabetes 48:588–594

Tsao D, Thomsen HK, Chou J, Stratton J, Hagen M et al (2008) TrkB agonists ameliorate obesity and associated metabolic conditions in mice. Endocrinology 149:1038–1048

Tsuchida A, Nakagawa T, Itakura Y, Ichihara J, Ogawa W et al (2001) The effects of brain-derived neurotrophic factor on insulin signal transduction in the liver of diabetic mice. Diabetologia 44:555–566

Unger TJ, Calderon GA, Bradley LC, Sena-Esteves M, Rios M (2007) Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. J Neurosci 27:14265–14274

Wild KD, Bian D, Zhu D, Davis J, Bannon AW et al (2007) Antibodies to nerve growth factor reverse established tactile allodynia in rodent models of neuropathic pain without tolerance. J Pharmacol Exp Ther 322:282–287

Winston J, Toma H, Shenoy M, Pasricha PJ (2001) Nerve growth factor regulates VR-1 mRNA levels in cultures of adult dorsal root ganglion neurons. Pain 89:181–186

Wood JN (2010) Nerve growth factor and pain. N Engl J Med 363:1572–1573

Wu C, Erickson MA, Xu J, Wild KD, Brennan TJ (2009) Expression profile of nerve growth factor after muscle incision in the rat. Anesthesiology 110:140–149

Xie Y, Hayden MR, Xu B (2010) BDNF overexpression in the forebrain rescues Huntington's disease phenotypes in YAC128 mice. J Neurosci 30:14708–14718

Xu B, Goulding EH, Zang K, Cepoi D, Cone RD et al (2003) Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 6:736–742

Yeo GS, Connie Hung CC, Rochford J, Keogh J, Gray J et al (2004) A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. Nat Neurosci 7:1187–1189

Yip HK, Rich KM, Lampe PA, Johnson EM Jr (1984) The effects of nerve growth factor and its antiserum on the postnatal development and survival after injury of sensory neurons in rat dorsal root ganglia. J Neurosci 4:2986–2992

Zahn PK, Subieta A, Park SS, Brennan TJ (2004) Effect of blockade of nerve growth factor and tumor necrosis factor on pain behaviors after plantar incision. J Pain 5:157–163

Zhang X, Huang J, McNaughton PA (2005) NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J 24:4211–4223

Zhou QH, Fu A, Boado RJ, Hui EK, Lu JZ, Pardridge WM (2011) Receptor-mediated abeta amyloid antibody targeting to Alzheimer's disease mouse brain. Mol Pharm 8:280–285

Zhu Z, Friess H, diMola FF, Zimmermann A, Graber HU et al (1999) Nerve growth factor expression correlates with perineural invasion and pain in human pancreatic cancer. J Clin Oncol 17:2419–2428

Zuccato C, Cattaneo E (2009) Brain-derived neurotrophic factor in neurodegenerative diseases. Nat Rev Neurol 5:311–322

Index

G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5,