The Reciprocal Effects of Oxidative Stress and Glutamate Neurotransmission

Sylvain Grignon and Jessica Deslauriers

Abbreviations

4-HNE	4-hydroxynonenal
AMPA	α-amino-5-methyl-3-hydroxy-4-isoxazole propionic acid
ATP	Adenosine triphosphate
cGCL	Catalytic subunit of glutamate cysteine ligase
CNS	Central nervous system
COX2	Cyclooxygenase-2
DNA	Deoxyribonucleic acid
Drp1	Dynamin-related protein 1
DTNB	5, 5′-dithio-bis[2-nitrobenzoic acid]
DTT	Dithiothreitol
EAAC	Excitatory amino acid carriers
Egr-1	Early growth response protein
EPSC	Excitatory post synaptic currents
ERK	Extracellular signal-regulated kinase
H_2O_2	Hydrogen peroxide
iNOS	Nitric oxide synthase, inducible form

S. Grignon, M.D., Ph.D. (✉)
Departments of Psychiatry and Physiology & Biophysics, University of Sherbrooke, Sherbrooke, QC, Canada

Département de Psychiatrie, Centre Hospitalier Universitaire de Sherbrooke, 580 rue Bowen sud, J1G 2E8 Sherbrooke, QC, Canada
e-mail: Sylvain.Grignon@usherbrooke.ca

J. Deslauriers, Ph.D.
Departments of Psychiatry and Physiology & Biophysics, University of Sherbrooke, Sherbrooke, QC, Canada

A. Dietrich-Muszalska et al. (eds.), *Studies on Psychiatric Disorders*,
Oxidative Stress in Applied Basic Research and Clinical Practice,
DOI 10.1007/978-1-4939-0440-2_11

KA	Kainate
MPTP	1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine
NAC	N-acetylcysteine
NFκB	Nuclear factor-kappaB
NMDA	N-methyl-D-aspartate
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOX	Nicotinamide adenine dinucleotide phosphate oxidase
NOX2	NADPH oxidase
NRF1	Nuclear respiratory factor 1
Nrf2	NF-E2-related factor
$O2^{-}$	Superoxide anion
ONOO	Peroxynitrite
OPA1	Optical atrophy protein 1
PKG	Protein kinase G
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SIN-1	3-morpholinosydnonimine
Sp1	Specificity protein 1
t-BHQ	*tert*-Butylhydroquinone
t-bOOH	*tert*-Butylhydroperoxide

1 Introduction

Abnormalities of glutamate neurotransmission are the focus of intense research for the neurobiological approach of neuropsychiatric conditions. A large body of research has demonstrated the implication of glutamate in pathological phenomena such as neurodegeneration, excitotoxicity, and apoptosis as well as its role in neuronal trophicity, synaptic plasticity, and long-term potentiation, to name a few. As specifically regards psychiatric conditions, glutamatergic mechanisms have also received ample attention. The investigation of psychotic states induced by phencyclidine or its analogues unraveled their common property of antagonism at the N-methyl-D-aspartate (NMDA) receptors and provided the initial argument for the glutamatergic hypothesis of schizophrenia, which has since received empirical support from animal, genetic, neuroimaging, and interventional studies. Conversely, the NMDA receptor antagonist ketamine has attracted interest as a therapeutic (as opposed to psychotomimetic) agent in the field of resistant depression (Zarate et al. 2006), and glutamatergic aspects of mood disorders pathophysiology are also intensely studied (Sanacora et al. 2012).

Beside classical aspects of excitotoxicity, calcium mobilization, and programmed cell death, reactive oxygen species (ROS) or reactive nitrogen species (RNS) production upon glutamate receptors stimulation has also attracted early attention (Coyle and Puttfarcken 1993). A distinct line of investigation, the impact of oxidative stress on glutamate neurotransmission, has also produced significant advances.

One subsequent topic, which is also beginning to be addressed experimentally, is the emergence of vicious circles between ambient oxidative stress, gain of glutamatergic function, and subsequent increase in oxidative stress.

2 A Brief Overview of Glutamatergic Neurotransmission

Glutamate is the main excitatory transmitter in the central nervous system (CNS). Glutamate is synthesized in neurons from glutamine under the action of the enzyme phosphate-activated glutaminase (brain/kidney phosphate-activated glutaminase product of the GLS1 gene) and from α-ketoglutarate by mitochondrial aspartate aminotransferase. Within astrocytes, glutamine synthetase converts glutamate to glutamine. The newly formed glutamine is released from astrocytes and taken up by glutamatergic neurons, where new glutamate is synthetized.

2.1 Ionotropic Receptors

Glutamate acts through two families of receptors, namely, ionotropic and metabotropic receptors. Ionotropic receptors have been defined by their preferential ligands. **AMPA** (α-amino-5-methyl-3-hydroxy-4-isoxazole propionic acid) **receptors** are usually heterotetramers (although homotetramers have been documented) of AMPA-R subunits GluR1-4 (or GluA1-4) and some bias towards the inclusion of GluA2 dimers. Functional properties such as calcium permeability, current kinetics, and pharmacology are strongly influenced by subunits composition, alternative splicing, and accessory subunits (Shepherd and Huganir 2007). AMPA receptor kinetics provide the basis for the fast, high-frequency component of excitatory postsynaptic currents (EPSC) in the CNS (Geiger et al. 1997); the control of the trafficking and membrane density of AMPA receptors is also a central mechanism in synaptic plasticity and homeostatic adjustments of EPSC strength (Shepherd and Huganir 2007). The **kainate receptors** have been nominated after their defining preferential agonist, the seaweed toxin kainic acid. They are heterotetramers formed by subunits GluR5-7 (GluK1-3) and KA1-2 (GluK4-5). GluR5-7 subunits undergo substantial editing and alternative splicing. KA1-2 subunits bear high affinity sites for kainite binding, but are unable to form homotetramers in recombinant systems, at odds with the Glu5-7 subunits. They are more sparsely distributed in the CNS than other glutamate receptor types, and their electrophysiological contribution must be "unmasked" from the larger contribution of AMPA currents. Nevertheless, they are involved in many important functions such as synaptogenesis, control of neuronal excitability (including rhythmic activity), neurosecretion (through their presynaptic component), and some forms of synaptic plasticity (Pinheiro and Mulle 2006; Jane et al. 2009). Kainate receptors also impact the properties of critical CNS networks and could play a role in the pathophysiology or treatment of epilepsy (Vincent and Mulle 2009). The **N-Methyl-D-aspartate (NMDA) receptor** has

attracted by far the most attention in the field of excitotoxicity, and in diverse aspects of normal glutamatergic neurotransmission, the most notable being its implication in long-term potentiation and other forms of neuronal plasticity. NMDA receptors are heterotetramers comprising two obligate NR1 subunits, two NR2 subunits (NR2A-D) and an accessory NR3 subunit. At normal membrane polarization, the receptor is blocked by magnesium, and a mild depolarization is necessary to relieve this block, with a half effect at −20 mV. NR1 subunits bear binding sites for the obligate coagonist glycine. Besides, NMDA receptors are endowed with a rich complement of modulatory sites enabling redox, zinc, neurosteroid, and polyamine modulatory effects. The impact of subunit composition (NR2A vs. NR2B) and cellular localization (synaptic vs. extrasynaptic) on the function and neurotoxic effects of NMDA receptors is the focus of intense research (Kohr 2006).

2.2 Metabotropic Receptors

Metabotropic receptors are G protein-coupled receptors. The group I receptors, including mGluR1 and 5, are widespread in neurons (type 1) and/or astrocytes (type 5) and predominantly postsynaptic. They couple to Gαq/11 to induce phosphoinositide breakdown and also signal through β-arrestin and extracellular signal-regulated kinase (ERK) activation (Emery et al. 2010). They enhance NMDA-mediated responses and increase neuronal excitability. Group II receptors (mGluR2-3) are pre- and postsynaptic and typically couple to Gi/o. They decrease neurotransmitter release and neuronal excitability. Group III receptors (mGluR4, 6–8) are located presynaptically (active zone) and also decrease transmitter release through Gi/o coupling.

2.3 Reuptake

Upon release, glutamate can be taken up by two neuronal excitatory amino acid carriers, EAAC1 (or EAAT3), whose quantitative contribution to the overall glutamate uptake appears quantitatively modest (Holmseth et al. 2012), but functionally important in some pathological contexts (Nafia et al. 2008; Ross et al. 2011). The main uptake process, however, is contributed by astrocytes ensheathing the synaptic process, mostly through excitatory amino acid transporter 2 (EAAT2 or GLT1) and EAAT1 (or GLAST) (Kanai and Hediger 2004).

2.4 Neuroenergetics

The disposition and metabolism of glutamate in astrocytes is a complex and compartmented process positioned at the interface of metabolic (e.g., tricarboxylic acid cycle, purine nucleotide cycle, glutathione synthesis), structural (incorporation into

proteins), or neurochemical (glutamine synthesis) functions and has been reviewed in detail (McKenna 2007). Glutamatergic and GABAergic neurons have been estimated to make up to 80–90 % of the CNS neuron complement, with glutamatergic neurons constituting the large majority. The fast turnover, energy-dependent uptake, and glutamine/glutamate recycling have been estimated to make up to 60–80 % of brain energetic consumption (Rothman et al. 2003), with significant glial contribution (including astrocytic involvement in glutamatergic "tripartite synapses"), and involve cooperation between neuronal and glial metabolic processes (notably the tricarboxylic acid cycle) and the glutamate/glutamine cycle (Serres et al. 2008).

3 The Impact of Oxidative Status on Glutamatergic Neurotransmission

3.1 Glutamate Dynamics

3.1.1 Glutamate Release

The release of glutamate due to the depletion of adenosine triphosphate (ATP), resulting from the transmembrane sodium gradient and inversion of membrane glutamate transport systems, is a defining feature of the excitotoxic component of ischemic phenomena and will not be discussed here.

Classical studies explored the effect of oxidative status modification on synaptosomal [^{3}H]aspartate release and showed that 0.01 % hydrogen peroxide (H_2O_2) increased depolarization-induced calcium-dependant release above 200 % of their basal values (Gilman et al. 1994). This effect could not be replicated at substantially lower (100 μM) H_2O_2 concentration, although in this preparation peroxide synergized with a sodium load achieved by veratridine, again in a fashion more relevant to ischemia/reperfusion events (Tretter and Adam-Vizi 2002). However, in addition to ischemia/reperfusion phenomena, the influence of inflammatory and oxidative/nitrative status effects has been studied in recent work. Bal-Price and Brown (2001) used a coculture model of cerebellar granule neurons and activated glia (lipopolysaccharide and interferon-γ stimulation) to demonstrate a massive neuronal death caused by neuronal glutamate release (as evidenced by MK-801 prevention) proceeding from nitric oxide (NO)-induced impairment of mitochondrial respiration (as evidenced by the preventive effect of two distinct inducible nitric oxide synthase (iNOS) inhibitors). These effects were mimicked by the NO donor NOC-18, which induced a doubling of extracellular glutamate concentrations in primary neuronal cultures, and an increase from virtually undetectable levels to ≈9 μM in neuronal/glial cocultures, as well as a decrease in ATP levels more pronounced in pure neuronal culture than in cocultures (Bal-Price and Brown 2001). Mitochondrial involvement in glutamate release induced by oxidative phenomena has been confirmed by the use of sodium cyanide (Dong et al. 2012), which inhibits mitochondrial cytochrome c oxidase (Leavesley et al. 2008). In cortical synaptosomes, cyanide elicited a strong glutamate release, which was completely reversed by the free radical scavengers melatonin and

manganese (III) 5,10,15,20-tetrakis (4-benzoic acid) porphyrin, as well as the H_2O_2 scavenger EUK134. Indeed, this effect was replicated by H_2O_2, albeit at the considerable concentration of 600 μM. The NMDA receptors antagonist AP5 ((2*R*)-amino-5-phosphonovaleric acid; (2*R*)-amino-5-phosphonopentanoate) prevented the effect of H_2O_2 on glutamate release, but was only partially efficient vis-à-vis the effect of cyanide, suggesting a complementary mechanism in this case. Additional results suggested that ATP synthesis inhibition was a likely mechanism to account for this discrepancy: cyanide decreased ATP synthase by more than 50 %, while the ATP synthase inhibitor 3,3′-diindolylmethane increased glutamate release. The authors suggest a role for lipid peroxidation products (also increased by cyanide) as a potential mechanism for the loss of ATP synthase activity, which seems plausible given the particular sensitivity of this enzyme to, for instance, 4-hydroxynonenal (4-HNE) modification (Perluigi et al. 2009).

Another source of reactive oxygen species (ROS) whose activity has recently been linked unequivocally to increased glutamate release is the superoxide-generating enzyme NADPH oxidase (NOX2). Acute ketamine administration in rodents is known to elicit behavioral abnormalities reminiscent of schizophrenia, to increase oxidative stress as well as glutamate release in cortical areas. Some of these effects have been convincingly linked to NOX2 activation and are indeed prevented by its inhibitor apocynin (Behrens et al. 2007). Sorce et al. have compared the effects of ketamine in wild-type and NOX2 knockout mice (NOX2-KO): compared to controls, NOX-KO mice were protected against the behavioral and neurochemical effects of ketamine, notably the hallmark increase in glutamate release (Sorce et al. 2010). Therefore, superoxide production by NOX2 is shown to be a necessary step in the behavioral and neurochemical effects of ketamine.

Overall, it appears that neuronal glutamate release can be increased in an oxidative environment by distinct pathways, namely, disruption of mitochondrial respiration, superoxide production by NOX2, and ATP synthase inhibition, the latter potentially due to lipid peroxidation products.

3.1.2 Glutamate Reuptake

Conversely, there is ample evidence that glutamate uptake processes are the target of redox modulation. Early work using different systems showed that glutamate uptake could be significantly inhibited by H_2O_2 (at concentrations as low as 100 μM) or by enzymatic ROS-producing systems such as glucose oxidase or xanthine oxidase (Piani et al. 1993; Volterra et al. 1994a). This effect could be fully reversed by the reducing agent dithiothreitol (DTT), suggesting that redox-sensitive sulfhydryl groups were involved in the phenomenon (Volterra et al. 1994b). Further mechanistic insights were provided by the incubation of astrocytic cultures with the lipid peroxidation product 4-HNE, which resulted in a dose-dependent inhibition of glutamate uptake. Again, this effect could be reversed by DTT (as well as glutathione). It was associated with the formation of adducts between 4-HNE and the glial glutamate transporter GLT-1, as well as dimerization (up to four times) of the latter.

Evidence of post-translational modifications associated with oxidative stress and uptake impairment has also been obtained for the neuronal transporter EAAC1 (or EAAT3) in the 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine (MPTP) Parkinson's model. In this case, the authors demonstrated an increase in protein tyrosine nitration. Since cysteine is also a permeant of this transporter and a precursor of glutathione synthesis, a parallel decrease in cellular glutathione content was observed, further compromising cellular redox status (Aoyama et al. 2008).

While post-translational oxidative modifications are generally associated with loss of function, a more complex picture emerges from studies of transcriptional control of the same molecules. In the primary astrocytic culture model, it was shown that mRNA and protein levels for all three transporters EAAT1, EAAT2, and EAAT3 were left unchanged by peroxide or tert-butyl hydroperoxide (t-bOOH), although the same conditions led to a decrease in glutamate uptake (Miralles et al. 2001). EAAT1 levels were decreased in a rat model of thiamine deficiency and partially rescued by the antioxidant N-acetyl-cysteine (NAC) (Hazell et al. 2010). They were also downregulated by arsenic exposure, although in this case, direct evidence of oxidative stress was lacking (Castro-Coronel et al. 2011). EAAT2 was also downregulated and rescued by NAC in the thiamine deficiency model (Hazell et al. 2010); exposure of astrocyte cocultures to excitotoxic (S)-5-fluorowillardiine (and oxidative [3-morpholinosydnonimine (SIN-1)]) resulted in a biphasic response in EAAT2 levels, with an increase at 24 h followed by a decrease at 48 h (Wallis et al. 2012). Mechanistically, EAAT2 transcription seems to be under the dependence of the transcription factor nuclear factor-kappaB (NFκB), whose sensitivity to the redox status is well known (Janssen-Heininger et al. 2000). Increases of EAAT2 transcription levels have been documented after ceftriaxone incubation (which induces NFκB activation) as well as in response to tumor necrosis factor alpha (TNFα), although in this case a repressing influence was also suspected (Sitcheran et al. 2005; Lee et al. 2008). The results are less equivocal for EAAT3. Upon exposure to L-sulforaphane and *tert*-butylhydroquinone (t-BHQ), there was a strong upregulation of EAAT3 in C6 glioma culture. This effect could be replicated in vivo and could be ascribed to the activation of the transcription pathway NF-E2-related factor 2 (Nrf2)/antioxidant response element; this response was lost in Nrf2-KO mice and could be mimicked by overexpression of Nrf2 (Escartin et al. 2011).

3.2 Redox Status and NMDA Modulation

Among the many modulating influences that have been described on the NMDA receptor, the redox site has attracted early recognition and interest. In the initial description of the phenomenon, Aizenman et al. (1989) showed that currents elicited in cultured rat cortical neurons by a combination of NMDA (10–100 μM) and glycine (1 μM) were significantly enhanced by pretreatment with the reducing agent DTT, up to 250 % above basal traces. Conversely, the oxidizing agent 5,

5′-dithio-bis[2-nitrobenzoic acid] (DTNB) induced a decrease in the signal (−22 %), which could always be restored by DTT.

Further work addressed the electrophysiological substrate of this response. Using CHO cells culture, it was shown that DTT induced an increase in opening frequencies for all three recombinant subunit combinations tested (NR1/NR2A, NR1/NR2B, or NR1/NR2C) with an increase in open dwell time only for the NR1/NR2A combination (Brimecombe et al. 1997). The redox sensitivity of NMDA receptors could be partially ascribed to two NR1 cysteines (Cys744 and 798) (Sullivan et al. 1994), whose mutation abolished the redox sensitivity of NR1/NR2C combination, and decreased that of NR2A/NR2B pairs. The coexpression of NR2A, however, rescued the redox sensitivity of mutated (C744A, C798A) NR1. This suggests that NR2A subunits also bear redox sites, which could also explain the different kinetic response (increased open dwell time) (Brimecombe et al. 1999).

Apart from the mechanistic interest of these results, recent work has also highlighted their potential pathophysiological implications. For instance, in the pilocarpine/hippocampal culture model of temporal lobe epilepsy, Di Maio et al. (2011) have shown that protracted exposure of hippocampal neurons to pilocarpine induced cellular thiol oxidation, intracellular calcium increase (ascribed to NMDA receptor activation), and resistance to further glutamate application. One plausible explanation for the latter result was that NMDA receptors were rendered resistant to glutamate because of cysteine oxidation. In support of this hypothesis, NMDA currents could be restored partially by the antioxidant NAC and more completely by the reducing agent tris(2-carboxyethyl)phosphine. These results complement earlier work using a different model of epileptiform activity elicited in hippocampal slices by low magnesium concentrations. Under these conditions, epileptiform activity appeared to be suppressed by the oxidant DNTB and restored by DTT (Sanchez et al. 2000). Therefore, in the context of epilepsy described above, it appears likely that acute reducing modulation enhances ictal activity, which in turn induces protracted oxidative phenomena and subsequent suppression of NMDA currents.

A different approach using the lipid peroxidation product 4-HNE (1 μM, a concentration in the lower range of those achieved by in vitro oxidative conditions) showed a biphasic effect with an initial stimulation of NMDA currents, which resolved within 3 h and was replaced by a protracted decrease. This effect paralleled an increase in the phosphorylation levels of NR1 and NR2A subunits and a decrease in ATP levels, which were thought to underlie, respectively, the increase and decrease in NMDA currents: okadaic acid, a phosphatase inhibitor, increased NR1 and NR2A phosphorylation and accordingly enhanced NMDA function, while the mitochondrial toxin rotenone, which depletes cellular ATP levels, induced a decrease in NMDA currents. Interestingly, the authors could not demonstrate the formation of adducts between 4-HNE and NR1 or NR2A subunits, while such adducts existed for the AMPA receptor subunits GluR1-4, although the AMPA current was left unchanged under the same conditions (Lu et al. 2001).

Another well-known modulatory site—beside the redox site—of the NMDA receptor is the binding site for the obligate coagonists glycine or D-serine. The formation of the latter product depends on the enzyme serine racemase, which has

been suggested in association studies and preclinical models to contribute to schizophrenia pathophysiology (Labrie et al. 2009; Ma et al. 2012; Morita et al. 2007). Recently, it has been shown that inflammatory, oxidative, and nitrative conditions increased the formation of covalent dimers of the enzyme, which was associated with decreased activity. Indeed, the nitric oxide donor SIN-1 induced a dose-dependent decrease in serine racemase function, at concentrations actually lower than those necessary to achieve cross-linking (Wang and Barger 2012), which suggests intramolecular events distinct from dimerization per se.

3.3 NMDA Receptor Subunits Levels

The cellular localization and transcriptional regulation of glutamate receptors have shown varying response patterns to oxidative status across receptors, across subunits, and across experimental conditions.

Available evidence suggests a robust upregulation of the NR1 subunit in response to pro-oxidative conditions. Ischemia/reperfusion paradigms lead to an early increase in NR1 and NR2A/B expression (Won et al. 2001).

In a different context, it has been shown, in cultured cortical neurons, that neurotoxicity induced by neurotrophin-4/5 involved upregulation of NR1 and, more prominently, NR2A (Choi et al. 2004). This response seemed to be under the dependence of the redox-sensitive transcription factor early growth response protein 1 (Egr-1) (Gao et al. 2009). Although, in this case, normalization of redox status by inhibitors of NOS or NOX2 did not prevent NR2A upregulation, no data were presented on an eventual normalization of NR1 by the same agents. Hypoxic conditions designed to mimic the effects of high altitude for 3,7, or 14 days induced unequivocal evidence of increased oxidative markers and a transcriptional activation and upregulation of the NR1 subunit, while the GluR2 subunit tended to decrease (Hota et al. 2008). Even a shorter (4 h) hypoxic treatment induced a strong increase in NR1 immunoreactivity in *nucleus tractus solitarius* neurons, which was partially prevented by α-tocopherol and ascorbic acid. These two antioxidants were also able to decrease NR1 levels during normal development, suggesting that some tonic level of oxidative conditions contributes to basal expression of NR1 (Wu et al. 2011). Later work by the same group demonstrated that the upregulation of NR1 was under the dependency of the transcription factor specificity protein 1 (Sp1) and could be prevented by the antioxidant acetyl-l-carnitine (Hota et al. 2010).

Exposure of hippocampal neurons to pilocarpine for 24 h upregulated NR1 and NR2B subunits by some 40 %, an effect that could be reversed by the antioxidant NAC and the NOX inhibitors apocynin and 6-aminonicotinamide (Di Maio et al. 2011). However, other work has rather documented a downregulation of NR2 subunits by oxidative conditions: an 8 weeks exposure to the diabetes mimic streptozotocin induced a decrease in hippocampal NR2A and NR2B levels, in parallel with a well-known disruption of oxidative status (Piotrowski 2003). Polyunsaturated fatty acids normalized malondialdehyde levels and partially restored NR2A/B levels

(Delibas et al. 2004). As well, repeated injections of the psychotomimetic NMDA antagonist ketamine induced a decrease in NR2A expression in the prefrontal and cingulate cortices of wild-type but not NOX2-KO mice, while the effects of ketamine on NR2B appeared independent of NOX2 (Sorce et al. 2010).

Caracciolo et al. (2011) have provided an extensive characterization of glutamate receptor subunit response to injections of kainate in wild-type or cyclooxygenase-2 (COX2) knockout mice. Indirect markers of oxidative/inflammatory status such as NFκB and iNOS levels were increased in the KA/COX2-KO group. As regards NMDA receptor subunits (NR1, NR2A-D, NR3A/B), there was a general decrease, for the same group, in the cortical and hippocampal transcript levels, with the exception of increased hippocampal NR3A mRNA. As for AMPA and KA receptors (GluR1-7 and KA1/2), there was also a general trend for decreased transcripts levels in the hippocampus, while cortical expression was generally unaffected. Social isolation increases oxidative stress and has been used to mimic some features of Alzheimer's disease (among other conditions) (Hsiao et al. 2012); in this case, social isolation was associated with a decrease in the density of GluR1 and GluR2 subunits associated with the cell membrane, while the overall cellular complement of the same subunits was unchanged, and NAC was able to reverse these changes. Conversely, in the work cited earlier (Hota et al. 2010), the GluR2 subunit level was also increased by hypoxia but resisted normalization by acetyl-l-carnitine.

Overall, there appears to be consistent evidence that NR1 expression increases in response to oxidative conditions, which could be driven by at least three types of redox-sensitive transcription factors, Sp1, Egr-1, and nuclear respiratory factor 1 (NRF1) (Dhar and Wong-Riley 2009). Results for other subunits are scarcer and less consistent.

4 Glutamate Modulates Cellular Redox Status

4.1 Demonstration

The implication of ROS in the cellular effects of glutamate was initially studied in the context of excitotoxic phenomena. Coyle and Puttfarcken (1993) put forward formal criteria for such involvement.

The demonstration of ROS production after NMDA engagement was initially obtained in cultured cerebellar granule cells: NMDA increased levels of the superoxide radical $\cdot O_2^-$ in a rapid (10 min) and transient (resolution within 40 min) manner; this response was duly abolished by the NMDA receptor antagonist MK-801, was partially calcium dependent, and was not mimicked, in this system, by KA (Lafon-Cazal et al. 1993). Similarly, NMDA increased ROS levels (as monitored by dichlorofluorescein fluorescence) in cultured cortical neurons (Reynolds and Hastings 1995). Evidence of increased O_2^- production (as evidenced by dihydroethidium fluorescence) after NMDA, KA, and AMPA administration was obtained in cultured hippocampal neurons and ex vivo slices (Bindokas et al. 1996).

The demonstration of significant oxidative damage to cellular components, another criterion for ROS involvement in glutamate effects, has been obtained for different macromolecules. Evidence of increased lipid peroxidation has been by far the most documented, notably through malondialdehyde and 4-HNE levels; all three glutamate receptor types appear to give rise to increased lipid peroxidation (Agostinho et al. 1996; Bae et al. 2002; Bruce and Baudry 1995), although some systems have yielded conflicting results (Yang et al. 2003). Similarly, all three types of receptors have been shown to induce some degree of protein carbonylation, a widely used index of protein oxidative modification (Mueller-Burke et al. 2008; Gluck et al. 2000; Tateno et al. 2004). Lastly, glutamate damage to deoxyribonucleic acid (DNA) was also demonstrated in early work (Didier et al. 1996) and could play role in excitotoxic cell death, but appears to elicit efficient repair mechanisms under milder conditions (Yang et al. 2010).

The last criterion of ROS involvement in glutamate action is the prevention/reversal of (some) toxic effects of glutamate by antioxidants or ROS scavengers. Such examples abound both in vitro and in vivo and indeed constitute the test generally used to ascertain the role of ROS in biological phenomena, but have generally—and notably—not translated well in clinical applications (Isaac et al. 2008; Muir 2006).

4.2 Effectors of Glutamate Redox Modulation

Although the place of glutamate as an inducer of oxidative stress has been overwhelmingly confirmed, the precise cellular origin of ROS (or at least the relative contribution of different cellular sources) is still a matter of debate, mostly on methodological grounds, which will not be addressed here (Brennan et al. 2009; Alekseenko et al. 2012).

4.2.1 Mitochondrial Involvement

It has been suggested that up to 50 % of CNS ROS originate from mitochondria, and more precisely from the reverse electron transport (Kudin et al. 2008), and early as well as more recent work has tried to unravel the interaction of glutamate (either as a neurotransmitter or as a metabolic substrate) with the complex mitochondrial dynamics.

One well-established mechanism relates to calcium homeostasis, in line with the robust calcium dependency of glutamate oxidative phenomena. Excessive cytoplasmic calcium concentrations are progressively transferred (through the calcium uniporter) to the mitochondrial matrix, exceeding the homeostatic possibilities of mitochondrial calcium cycling and reaching a threshold of calcium overload. The latter, associated with other triggering signals such as increases in inorganic phosphate concentration, ATP depletion, and oxidative stress, provokes the opening of the permeability transition pore with subsequent diffusion of large molecules and disruption

of the tricarboxylic acid cycle (Crompton 1999). Glutamate-dependent ROS production as such has been suggested to depend on dissipation of mitochondrial membrane potential (Scanlon and Reynolds 1998), uncoupling of respiration from ATP production (Panov et al. 2009), increased respiration (even at maximal uncoupling; Kumari et al. 2012), specific enzymatic sources such as the α-ketoglutarate dehydrogenase complex (Chinopoulos and Adam-Vizi 2006), or lifting of oxaloacetate inhibition of complex II succinate dehydrogenase (Panov et al. 2009). Glutamate has also been shown to profoundly affect the dynamics of mitochondria, notably by promoting mitochondrial fragmentation and autophagy, by upregulating mitochondrial fission markers and promoters dynamin-related protein 1 (Drp1) and Fis1 (Kumari et al. 2012; Grohm et al. 2010).

4.2.2 NOX2 Activation

Nicotinamide adenine dinucleotide phosphate oxidases (abbreviated to NOX) are a family of transmembrane which catalyze the reduction of molecular oxygen O_2 to the superoxide anion O_2^- and have been initially described as giving rise to the "phagocytic oxidative burst." The most widely studied form, in general and in the context of glutamate toxicity, is NOX2 (previously called $gp91^{phox}$). NOX2 is in obligatory and stabilizing interaction with $p22^{phox}$; upon phosphorylation and subsequent confirmation changes, a third subunit, the "organizer" $p47^{phox}$, associates with the membrane-bound NOX2/$p22^{phox}$ complex and recruits to it a number of cytoplasmic factors, among which the "activator" p67phox, $p40^{phox}$, and the GTPase Rac. The active NOX2 complex catalyzes the reduction of NADPH, the result of which is a transmembrane electron transfer with subsequent release of superoxide O_2^- in the luminal or extracellular space. NOX2 is an inducible enzyme, and its promoter bears binding sites for multiple redox-sensitive transcription factors. The cellular functions of NOX2 can be probed by a number of pharmacological inhibitors such as diphenylene iodonium, whose specificity is weak, or apocynin, a prodrug which must be activated by peroxidases and prevents the translocation of cytoplasmic components (Bedard and Krause 2007). More recently, knockout NOX2 models have provided clear results to some its functions, including in the context of glutamate-induced oxidative phenomena. Cortical neurons loaded with the redox-sensitive probe dihydroethidium showed a strong increase in fluorescence levels upon NMDA application, which was restored to control levels by MK-801, apocynin, or an inhibitor of NADPH production. Oxidative phenomena (4-HNE-positive neurons) induced by NMDA were also prevented by NOX2 inhibition. To more completely assess the respective contribution of mitochondria versus NOX2, the authors used the fact that mitochondria can use pyruvate to sustain ATP levels and ROS production, while NOX2 superoxide production is dependent on glucose: providing neurons with pyruvate (at the exclusion of glucose), in the presence of NMDA, strongly decreased the number of ethidium-positive neurons, while the level of superoxide production was unaffected by providing only glucose (at the exclusion of pyruvate); on the basis of the metabolic requirements of the two

pathways, it was thus concluded that in this system, superoxide production induced by NMDA relied mostly on NOX2. Moreover, in vivo, NMDA-induced neuronal death was prevented in $p47^{phox-/-}$ cells. Hippocampus neuronal degeneration induced by NMDA was also prevented by some 60 % in $p47^{phox-/-}$ mice, as was 4-HNE induction. Subtype-specific peptide inhibitors of protein kinase C moreover suggested that NMDA activation of NOX2 relied on PKCζ activation (Brennan et al. 2009). Similarly, Girouard et al. showed that ROS increases elicited by NMDA (as assessed by dihydroethidium fluorescence) were attenuated in NOX2-KO mice. Pharmacological assessments in cultured neurons suggested a signaling pathway between NMDA and NOX2, consisting of NO increase (mediated by the neuronal nitric oxide synthase nNOS), guanylate cyclase activation, and subsequent activation of protein kinase G (PKG) by cyclic guanosine monophosphate (cGMP) (Girouard et al. 2009). These effects have been extended, beyond cortical regions, to the striatum. After striatal glutamate injection, apocynin or NOX2 knockout decreased cell death, ROS production, and protein nitration by ≈50–60 %, a significant but partial rescue. A direct assay of NADPH oxidase activity confirmed the stimulating effect of glutamate and its prevention in NOX2-KO and apocynin-treated animals. Pharmacological analysis also showed a significant stimulating effect of non-NMDA ionotropic receptors and metabotropic M1 (and possibly M5) receptors on NADPH oxidase activity (Guemez-Gamboa et al. 2011).

4.2.3 Nitrative Phenomena

The implication of increased NO production, beside ROS, has been proposed early (Lafon-Cazal et al. 1993) to account for some of the detrimental or, for that matter, neuroplastic effects of glutamate and has received ample experimental confirmation (Ishikawa et al. 1999). NO derivatives can induce S-nitrosylation (originating from NO^- singlets) and react (NO^- triplets) with superoxide anion $O2^-$ to form peroxynitrite ONOO. There appears to be a significant interplay with the mitochondrial aspects of ROS production and consequences (Crompton 1999; Almeida and Bolanos 2001) with possible feedforward amplifying phenomena. Interestingly, it has also been shown that NO-dependent events could link NMDA receptor engagement to NOX2 activation; therefore, beside its own interaction with ROS, NO is also linked mechanistically to the two main glutamatergic oxidant sources.

4.2.4 Cystine Uptake

Cystine is transported into neurons and glia by the X_{AG} system (cysteine permeable glutamate transporter), but also, in immature neurons, oligodendrocytes and some cell lines by the X_C^- cystine glutamate exchanger. Intracellular cystine can be converted back to cysteine and incorporated in the endogenous antioxidant glutathione. Cystine and glutamate are the two preferred substrates of this system, which normally extrudes glutamate. Increasing extracellular glutamate concentrations (which

competes with cystine for cellular entry) or decreasing extracellular cystine levels have been shown to decrease glutathione levels and induce toxicity (Murphy et al. 1989), through an original mechanism of oxidative stress that does not involve excessive ROS production but decreased antioxidant mechanisms. Interestingly, the same system is also involved in the protective effects of preconditioning: mixed neuron/glia cell culture exposed to oxygen glucose deprivation, or mice exposed to 15 min carotid artery ligature, reacted by upregulating X_{CT} (a subunit of the X_C^- transport system) and the catalytic subunit of glutamate cysteine ligase (cGCL), involved in the synthesis of glutathione. This neuroprotective glial coordinated response is under the dependence of the transcription factor NRF2 (Bell et al. 2011a, b).

4.3 Antioxidant Effects of Glutamate Stimulation

Most of the data regarding glutamate toxicity tried to mimic "catastrophic" events such as ischemia/reperfusion, apoptotic cell death, or generally neurodegenerative events, at the risk of overlooking milder effects (Yang et al. 2010) or bona fide neuroprotective actions of glutamate, which are often related to synaptic (vs. extrasynaptic) NMDA receptor engagement (Hardingham and Bading 2010). These have been recently reviewed and include activation of the protective AKT/GSK-3β pathway, suppression of "prodeath" FOXO transcriptional activity, and engagement of cAMP-response element binding protein, among others (Hardingham 2009). These neuroprotective mechanisms enhanced by synaptic activity also included (1) transcriptional suppression of the thioredoxin-interacting protein (which itself inhibits thioredoxin and is therefore pro-oxidative in a FOXO dependent fashion) and (2) transcriptional activation of sestrin2 and sulfiredoxin, which can reduce oxidized forms of sulfiredoxin back to their active form (Papadia et al. 2008).

5 Concluding Remarks

The investigation of the redox modulatory effects of glutamate neurotransmission enters its fourth decade and remains a very active field.

5.1 Empirical Confirmation of the "Vicious Circle" Model

The idea that glutamatergic transmission could give rise to positive feedforward (e.g., increased NMDA subunit expression) or feedback (e.g., decreased uptake) phenomena was proposed relatively early to account for the anti-homeostatic, "catastrophic" behavior of this system (Coyle and Puttfarcken 1993). In the more restricted field of glutamate-induced oxidative stress, mitochondrial disruption and NOX2 activation are attractive partners to engage in such feedbacks, but this

conceptual framework has received scant empirical confirmation in spite of its attractiveness. Recent work has indeed explored the interaction between mitochondrial dynamics and NMDA transmission (Nguyen et al. 2011). The optical atrophy protein 1 (OPA1) is an obligate step of mitochondrial fusion and is mutated (deletion) in a frequent form of optic neuropathy, associated with the loss of retinal ganglion cells through a likely excitotoxic process partially rescued by the NMDA antagonist memantine. In mice heterozygous for the mutated OAP1, mitochondria were, as expected, both shorter and more numerous, indicating that fission was favored over fusion. Antioxidant status was compromised by decreased expression of superoxide dismutase; most notably, there was significant upregulation of NMDA subunits NR1, NR2A, and NR2B, a likely consequence of the *primum movens*, disrupted mitochondrial dynamics, but also a potential amplifying factor through increased oxidative stress and upregulation of fission promoters dynamin-related protein 1 (Drp1) and Fis1 (Kumari et al. 2012; Grohm et al. 2010), as stated above.

5.2 Relevance to Psychiatric Disorders Pathophysiology

While the results detailed in this chapter have been used to gain a better understanding of neurological conditions such as stroke, epilepsy, or neurodegenerative disorders, their relevance for the pathophysiology of psychiatric disorders is also becoming obvious. The most salient example, the phencyclidine model of schizophrenia and its relation to GAD67 downregulation, where NOX2 implication has been formally demonstrated by M. Behrens' work, will be discussed in chapter "The Impact of Oxidative Stress on GAD67 Levels and Parvalbumin-Positive Neurons" in the present volume. In a different perspective, NOX2 has recently been implied as well in the effects of social isolation, a chronic stressor mimicking, in rodents, some aspects of diverse conditions such as depression, anxiety, suicidality, or schizophrenia, among others. The authors have taken advantage of a spontaneous mutation in rats (threonine/methionine substitution at position 153) of the NADPH oxidase organizer subunit $p47^{phox}$, which reduces oxidative burst capacity by 40 %. During social isolation for at least 4 weeks, animals developed increased locomotor activity, loss of discriminating capacities in the novel object recognition test, increased glutamate levels, and loss of physiological NR2A increase. There was also a time-dependent decrease in GABAergic markers GAD67 and parvalbumin and an upregulation of NOX2 and $p47^{phox}$ that was restricted to pyramidal neurons. Accordingly, oxidative markers also increased in a time-dependent fashion. Interestingly, the loss of function mutation of $p47^{phox}$ was protective against behavioral abnormalities (locomotor activity), NR2A, and parvalbumin decrease. In another set of experiments, the authors went on to show a preventive effect of apocynin administered from weeks 4 to 7, vis-à-vis the development of behavioral abnormalities induced by social isolation.

Therefore, normalization of redox status was instrumental in attenuating some of the neurochemical and behavioral abnormalities induced by social isolation, which

shows that interrupting the feedback mechanisms between glutamatergic neurotransmission and oxidative stress could be of paramount interest in the understanding and treatment of psychiatric disorders.

References

Agostinho P, Duarte CB, Oliveira CR (1996) Activity of ionotropic glutamate receptors in retinal cells: effect of ascorbate/Fe(2+)-induced oxidative stress. J Neurochem 67:1153–1163

Aizenman E, Lipton SA, Loring RH (1989) Selective modulation of NMDA responses by reduction and oxidation. Neuron 2:1257–1263

Alekseenko AV, Lemeshchenko VV, Pekun TG, Waseem TV, Fedorovich SV (2012) Glutamate-induced free radical formation in rat brain synaptosomes is not dependent on intrasynaptosomal mitochondria membrane potential. Neurosci Lett 513:238–242

Almeida A, Bolanos JP (2001) A transient inhibition of mitochondrial ATP synthesis by nitric oxide synthase activation triggered apoptosis in primary cortical neurons. J Neurochem 77:676–690

Aoyama K, Matsumura N, Watabe M, Nakaki T (2008) Oxidative stress on EAAC1 is involved in MPTP-induced glutathione depletion and motor dysfunction. Eur J Neurosci 27:20–30

Bae JH, Mun KC, Park WK, Lee SR, Suh SI, Baek WK, Yim MB, Kwon TK, Song DK (2002) EGCG attenuates AMPA-induced intracellular calcium increase in hippocampal neurons. Biochem Biophys Res Commun 290:1506–1512

Bal-Price A, Brown GC (2001) Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. J Neurosci 21:6480–6491

Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87:245–313

Behrens MM, Ali SS, Dao DN, Lucero J, Shekhtman G, Quick KL, Dugan LL (2007) Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. Science 318:1645–1647

Bell KF, Fowler JH, Al-Mubarak B, Horsburgh K, Hardingham GE (2011a) Activation of Nrf2-regulated glutathione pathway genes by ischemic preconditioning. Oxid Med Cell Longev 2011:689524

Bell KF, Al-Mubarak B, Fowler JH, Baxter PS, Gupta K, Tsujita T, Chowdhry S, Patani R, Chandran S, Horsburgh K, Hayes JD, Hardingham GE (2011b) Mild oxidative stress activates Nrf2 in astrocytes, which contributes to neuroprotective ischemic preconditioning. Proc Natl Acad Sci U S A 108:E1–2; author reply E3-4

Bindokas VP, Jordan J, Lee CC, Miller RJ (1996) Superoxide production in rat hippocampal neurons: selective imaging with hydroethidine. J Neurosci 16:1324–1336

Brennan AM, Suh SW, Won SJ, Narasimhan P, Kauppinen TM, Lee H, Edling Y, Chan PH, Swanson RA (2009) NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. Nat Neurosci 12:857–863

Brimecombe JC, Boeckman FA, Aizenman E (1997) Functional consequences of NR2 subunit composition in single recombinant N-methyl-D-aspartate receptors. Proc Natl Acad Sci U S A 94:11019–11024

Brimecombe JC, Potthoff WK, Aizenman E (1999) A critical role of the N-methyl-D-aspartate (NMDA) receptor subunit (NR) 2A in the expression of redox sensitivity of NR1/NR2A recombinant NMDA receptors. J Pharmacol Exp Ther 291:785–792

Bruce AJ, Baudry M (1995) Oxygen free radicals in rat limbic structures after kainate-induced seizures. Free Radic Biol Med 18:993–1002

Caracciolo L, Barbon A, Palumbo S, Mora C, Toscano CD, Bosetti F, Barlati S (2011) Altered mRNA editing and expression of ionotropic glutamate receptors after kainic acid exposure in cyclooxygenase-2 deficient mice. PLoS One 6:e19398

Castro-Coronel Y, Del Razo LM, Huerta M, Hernandez-Lopez A, Ortega A, Lopez-Bayghen E (2011) Arsenite exposure downregulates EAAT1/GLAST transporter expression in glial cells. Toxicol Sci 122:539–550

Chinopoulos C, Adam-Vizi V (2006) Calcium, mitochondria and oxidative stress in neuronal pathology. Novel aspects of an enduring theme. FEBS J 273:433–450

Choi SY, Hwang JJ, Koh JY (2004) NR2A induction and NMDA receptor-dependent neuronal death by neurotrophin-4/5 in cortical cell culture. J Neurochem 88:708–716

Coyle JT, Puttfarcken P (1993) Oxidative stress, glutamate, and neurodegenerative disorders. Science 262:689–695

Crompton M (1999) The mitochondrial permeability transition pore and its role in cell death. Biochem J 341(Pt 2):233–249

Delibas N, Altuntas I, Sutcu R, Yonden Z, Koylu H (2004) Effects of dietary long chain PUFAs on hippocampal lipid peroxidation and NMDA receptor subunits A and B concentration in streptozotocin-diabetic rats. Int J Neurosci 114:1353–1364

Dhar SS, Wong-Riley MT (2009) Coupling of energy metabolism and synaptic transmission at the transcriptional level: role of nuclear respiratory factor 1 in regulating both cytochrome c oxidase and NMDA glutamate receptor subunit genes. J Neurosci 29:483–492

Di Maio R, Mastroberardino PG, Hu X, Montero L, Greenamyre JT (2011) Pilocapine alters NMDA receptor expression and function in hippocampal neurons: NADPH oxidase and ERK1/2 mechanisms. Neurobiol Dis 42:482–495

Didier M, Bursztajn S, Adamec E, Passani L, Nixon RA, Coyle JT, Wei JY, Berman SA (1996) DNA strand breaks induced by sustained glutamate excitotoxicity in primary neuronal cultures. J Neurosci 16:2238–2250

Dong Y, Zhang W, Lai B, Luan WJ, Zhu YH, Zhao BQ, Zheng P (2012) Two free radical pathways mediate chemical hypoxia-induced glutamate release in synaptosomes from the prefrontal cortex. Biochim Biophys Acta 1823:493–504

Emery AC, Pshenichkin S, Takoudjou GR, Grajkowska E, Wolfe BB, Wroblewski JT (2010) The protective signaling of metabotropic glutamate receptor 1 Is mediated by sustained, beta-arrestin-1-dependent ERK phosphorylation. J Biol Chem 285:26041–26048

Escartin C, Won SJ, Malgorn C, Auregan G, Berman AE, Chen PC, Deglon N, Johnson JA, Suh SW, Swanson RA (2011) Nuclear factor erythroid 2-related factor 2 facilitates neuronal glutathione synthesis by upregulating neuronal excitatory amino acid transporter 3 expression. J Neurosci 31:7392–7401

Gao Y, Howard A, Ban K, Chandra J (2009) Oxidative stress promotes transcriptional up-regulation of Fyn in BCR-ABL1-expressing cells. J Biol Chem 284:7114–7125

Geiger JR, Lubke J, Roth A, Frotscher M, Jonas P (1997) Submillisecond AMPA receptor-mediated signaling at a principal neuron-interneuron synapse. Neuron 18:1009–1023

Gilman SC, Bonner MJ, Pellmar TC (1994) Free radicals enhance basal release of D-[3H]aspartate from cerebral cortical synaptosomes. J Neurochem 62:1757–1763

Girouard H, Wang G, Gallo EF, Anrather J, Zhou P, Pickel VM, Iadecola C (2009) NMDA receptor activation increases free radical production through nitric oxide and NOX2. J Neurosci 29:2545–2552

Gluck MR, Jayatilleke E, Shaw S, Rowan AJ, Haroutunian V (2000) CNS oxidative stress associated with the kainic acid rodent model of experimental epilepsy. Epilepsy Res 39:63–71

Grohm J, Plesnila N, Culmsee C (2010) Bid mediates fission, membrane permeabilization and peri-nuclear accumulation of mitochondria as a prerequisite for oxidative neuronal cell death. Brain Behav Immun 24:831–838

Guemez-Gamboa A, Estrada-Sanchez AM, Montiel T, Paramo B, Massieu L, Moran J (2011) Activation of NOX2 by the stimulation of ionotropic and metabotropic glutamate receptors contributes to glutamate neurotoxicity in vivo through the production of reactive oxygen species and calpain activation. J Neuropathol Exp Neurol 70:1020–1035

Hardingham GE (2009) Coupling of the NMDA receptor to neuroprotective and neurodestructive events. Biochem Soc Trans 37:1147–1160

Hardingham GE, Bading H (2010) Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nat Rev Neurosci 11:682–696

Hazell AS, Sheedy D, Oanea R, Aghourian M, Sun S, Jung JY, Wang D, Wang C (2010) Loss of astrocytic glutamate transporters in Wernicke encephalopathy. Glia 58:148–156

Holmseth S, Dehnes Y, Huang YH, Follin-Arbelet VV, Grutle NJ, Mylonakou MN, Plachez C, Zhou Y, Furness DN, Bergles DE, Lehre KP, Danbolt NC (2012) The density of EAAC1 (EAAT3) glutamate transporters expressed by neurons in the mammalian CNS. J Neurosci 32:6000–6013

Hota SK, Barhwal K, Singh SB, Sairam M, Ilavazhagan G (2008) NR1 and GluR2 expression mediates excitotoxicity in chronic hypobaric hypoxia. J Neurosci Res 86:1142–1152

Hota SK, Hota KB, Prasad D, Ilavazhagan G, Singh SB (2010) Oxidative-stress-induced alterations in Sp factors mediate transcriptional regulation of the NR1 subunit in hippocampus during hypoxia. Free Radic Biol Med 49:178–191

Hsiao YH, Kuo JR, Chen SH, Gean PW (2012) Amelioration of social isolation-triggered onset of early Alzheimer's disease-related cognitive deficit by N-acetylcysteine in a transgenic mouse model. Neurobiol Dis 45:1111–1120

Isaac MG, Quinn R, Tabet N (2008) Vitamin E for Alzheimer's disease and mild cognitive impairment. Cochrane Database Syst Rev 3, CD002854

Ishikawa Y, Satoh T, Enokido Y, Nishio C, Ikeuchi T, Hatanaka H (1999) Generation of reactive oxygen species, release of L-glutamate and activation of caspases are required for oxygen-induced apoptosis of embryonic hippocampal neurons in culture. Brain Res 824:71–80

Jane DE, Lodge D, Collingridge GL (2009) Kainate receptors: pharmacology, function and therapeutic potential. Neuropharmacology 56:90–113

Janssen-Heininger YM, Poynter ME, Baeuerle PA (2000) Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappaB. Free Radic Biol Med 28:1317–1327

Kanai Y, Hediger MA (2004) The glutamate/neutral amino acid transporter family SLC1: molecular, physiological and pharmacological aspects. Pflugers Arch 447:469–479

Kohr G (2006) NMDA receptor function: subunit composition versus spatial distribution. Cell Tissue Res 326:439–446

Kudin AP, Malinska D, Kunz WS (2008) Sites of generation of reactive oxygen species in homogenates of brain tissue determined with the use of respiratory substrates and inhibitors. Biochim Biophys Acta 1777:689–695

Kumari S, Mehta SL, Li PA (2012) Glutamate induces mitochondrial dynamic imbalance and autophagy activation: preventive effects of selenium. PLoS One 7:e39382

Labrie V, Fukumura R, Rastogi A, Fick LJ, Wang W, Boutros PC, Kennedy JL, Semeralul MO, Lee FH, Baker GB, Belsham DD, Barger SW, Gondo Y, Wong AH, Roder JC (2009) Serine racemase is associated with schizophrenia susceptibility in humans and in a mouse model. Hum Mol Genet 18:3227–3243

Lafon-Cazal M, Pietri S, Culcasi M, Bockaert J (1993) NMDA-dependent superoxide production and neurotoxicity. Nature 364:535–537

Leavesley HB, Li L, Prabhakaran K, Borowitz JL, Isom GE (2008) Interaction of cyanide and nitric oxide with cytochrome c oxidase: implications for acute cyanide toxicity. Toxicol Sci 101:101–111

Lee SG, Su ZZ, Emdad L, Gupta P, Sarkar D, Borjabad A, Volsky DJ, Fisher PB (2008) Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes. J Biol Chem 283:13116–13123

Lu C, Chan SL, Haughey N, Lee WT, Mattson MP (2001) Selective and biphasic effect of the membrane lipid peroxidation product 4-hydroxy-2,3-nonenal on N-methyl-D-aspartate channels. J Neurochem 78:577–589

Ma TM, Abazyan S, Abazyan B, Nomura J, Yang C, Seshadri S, Sawa A, Snyder SH, Pletnikov MV (2013) Pathogenic disruption of DISC1-serine racemase binding elicits schizophrenia-like behavior via D-serine depletion. Mol Psychiatry 18(5):557–567

McKenna MC (2007) The glutamate-glutamine cycle is not stoichiometric: fates of glutamate in brain. J Neurosci Res 85:3347–3358

Miralles VJ, Martinez-Lopez I, Zaragoza R, Borras E, Garcia C, Pallardo FV, Vina JR (2001) Na+dependent glutamate transporters (EAAT1, EAAT2, and EAAT3) in primary astrocyte cultures: effect of oxidative stress. Brain Res 922:21–29

Morita Y, Ujike H, Tanaka Y, Otani K, Kishimoto M, Morio A, Kotaka T, Okahisa Y, Matsushita M, Morikawa A, Hamase K, Zaitsu K, Kuroda S (2007) A genetic variant of the serine racemase gene is associated with schizophrenia. Biol Psychiatry 61:1200–1203

Mueller-Burke D, Koehler RC, Martin LJ (2008) Rapid NMDA receptor phosphorylation and oxidative stress precede striatal neurodegeneration after hypoxic ischemia in newborn piglets and are attenuated with hypothermia. Int J Dev Neurosci 26:67–76

Muir KW (2006) Glutamate-based therapeutic approaches: clinical trials with NMDA antagonists. Curr Opin Pharmacol 6:53–60

Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT (1989) Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. Neuron 2:1547–1558

Nafia I, Re DB, Masmejean F, Melon C, Kachidian P, Kerkerian-Le Goff L, Nieoullon A, Had-Aissouni L (2008) Preferential vulnerability of mesencephalic dopamine neurons to glutamate transporter dysfunction. J Neurochem 105:484–496

Nguyen D, Alavi MV, Kim KY, Kang T, Scott RT, Noh YH, Lindsey JD, Wissinger B, Ellisman MH, Weinreb RN, Perkins GA, Ju WK (2011) A new vicious cycle involving glutamate excitotoxicity, oxidative stress and mitochondrial dynamics. Cell Death Dis 2:e240

Panov A, Schonfeld P, Dikalov S, Hemendinger R, Bonkovsky HL, Brooks BR (2009) The neuromediator glutamate, through specific substrate interactions, enhances mitochondrial ATP production and reactive oxygen species generation in nonsynaptic brain mitochondria. J Biol Chem 284:14448–14456

Papadia S, Soriano FX, Leveille F, Martel MA, Dakin KA, Hansen HH, Kaindl A, Sifringer M, Fowler J, Stefovska V, McKenzie G, Craigon M, Corriveau R, Ghazal P, Horsburgh K, Yankner BA, Wyllie DJ, Ikonomidou C, Hardingham GE (2008) Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. Nat Neurosci 11:476–487

Perluigi M, Sultana R, Cenini G, Di Domenico F, Memo M, Pierce WM, Coccia R, Butterfield DA (2009) Redox proteomics identification of 4-hydroxynonenal-modified brain proteins in Alzheimer's disease: role of lipid peroxidation in Alzheimer's disease pathogenesis. Proteomics Clin Appl 3:682–693

Piani D, Frei K, Pfister HW, Fontana A (1993) Glutamate uptake by astrocytes is inhibited by reactive oxygen intermediates but not by other macrophage-derived molecules including cytokines, leukotrienes or platelet-activating factor. J Neuroimmunol 48:99–104

Pinheiro P, Mulle C (2006) Kainate receptors. Cell Tissue Res 326:457–482

Piotrowski P (2003) Are experimental models useful for analysis of pathogenesis of changes in central nervous system in human diabetes? Folia Neuropathol 41:167–174

Reynolds IJ, Hastings TG (1995) Glutamate induces the production of reactive oxygen species in cultured forebrain neurons following NMDA receptor activation. J Neurosci 15:3318–3327

Ross JR, Porter BE, Buckley PT, Eberwine JH, Robinson MB (2011) mRNA for the EAAC1 subtype of glutamate transporter is present in neuronal dendrites in vitro and dramatically increases in vivo after a seizure. Neurochem Int 58:366–375

Rothman DL, Behar KL, Hyder F, Shulman RG (2003) In vivo NMR studies of the glutamate neurotransmitter flux and neuroenergetics: implications for brain function. Annu Rev Physiol 65:401–427

Sanacora G, Treccani G, Popoli M (2012) Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacology 62:63–77

Sanchez RM, Wang C, Gardner G, Orlando L, Tauck DL, Rosenberg PA, Aizenman E, Jensen FE (2000) Novel role for the NMDA receptor redox modulatory site in the pathophysiology of seizures. J Neurosci 20:2409–2417

Scanlon JM, Reynolds IJ (1998) Effects of oxidants and glutamate receptor activation on mitochondrial membrane potential in rat forebrain neurons. J Neurochem 71:2392–2400

Serres S, Raffard G, Franconi JM, Merle M (2008) Close coupling between astrocytic and neuronal metabolisms to fulfill anaplerotic and energy needs in the rat brain. J Cereb Blood Flow Metab 28:712–724

Shepherd JD, Huganir RL (2007) The cell biology of synaptic plasticity: AMPA receptor trafficking. Annu Rev Cell Dev Biol 23:613–643

Sitcheran R, Gupta P, Fisher PB, Baldwin AS (2005) Positive and negative regulation of EAAT2 by NF-kappaB: a role for N-myc in TNFalpha-controlled repression. EMBO J 24:510–520

Sorce S, Schiavone S, Tucci P, Colaianna M, Jaquet V, Cuomo V, Dubois-Dauphin M, Trabace L, Krause KH (2010) The NADPH oxidase NOX2 controls glutamate release: a novel mechanism involved in psychosis-like ketamine responses. J Neurosci 30:11317–11325

Sullivan JM, Traynelis SF, Chen HS, Escobar W, Heinemann SF, Lipton SA (1994) Identification of two cysteine residues that are required for redox modulation of the NMDA subtype of glutamate receptor. Neuron 13:929–936

Tateno M, Sadakata H, Tanaka M, Itohara S, Shin RM, Miura M, Masuda M, Aosaki T, Urushitani M, Misawa H, Takahashi R (2004) Calcium-permeable AMPA receptors promote misfolding of mutant SOD1 protein and development of amyotrophic lateral sclerosis in a transgenic mouse model. Hum Mol Genet 13:2183–2196

Tretter L, Adam-Vizi V (2002) Glutamate release by an Na+load and oxidative stress in nerve terminals: relevance to ischemia/reperfusion. J Neurochem 83:855–862

Vincent P, Mulle C (2009) Kainate receptors in epilepsy and excitotoxicity. Neuroscience 158:309–323

Volterra A, Trotti D, Floridi S, Racagni G (1994a) Reactive oxygen species inhibit high-affinity glutamate uptake: molecular mechanism and neuropathological implications. Ann N Y Acad Sci 738:153–162

Volterra A, Trotti D, Tromba C, Floridi S, Racagni G (1994b) Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. J Neurosci 14:2924–2932

Wallis N, Zagami CJ, Beart PM, O'Shea RD (2012) Combined excitotoxic-oxidative stress and the concept of non-cell autonomous pathology of ALS: insights into motoneuron axonopathy and astrogliosis. Neurochem Int 61:523–530

Wang W, Barger SW (2012) Cross-linking of serine racemase dimer by reactive oxygen species and reactive nitrogen species. J Neurosci Res 90:1218–1229

Won MH, Kang T, Park S, Jeon G, Kim Y, Seo JH, Choi E, Chung M, Cho SS (2001) The alterations of N-Methyl-D-aspartate receptor expressions and oxidative DNA damage in the CA1 area at the early time after ischemia-reperfusion insult. Neurosci Lett 301:139–142

Wu YC, Wang YJ, Tseng GF (2011) Ascorbic acid and alpha-tocopherol supplement starting prenatally enhances the resistance of nucleus tractus solitarius neurons to hypobaric hypoxic challenge. Brain Struct Funct 216:105–122

Yang CS, Tsai PJ, Chen WY, Kuo JS (2003) Ionotropic glutamate receptors are involved in malondialdehyde production in anesthetized rat brain cortex: a microdialysis study. Redox Rep 8:35–39

Yang JL, Tadokoro T, Keijzers G, Mattson MP, Bohr VA (2010) Neurons efficiently repair glutamate-induced oxidative DNA damage by a process involving CREB-mediated up-regulation of apurinic endonuclease 1. J Biol Chem 285:28191–28199

Zarate CA Jr, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, Charney DS, Manji HK (2006) A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 63:856–864