Therapeutic targeting of the pathological triad of extrasynaptic NMDA receptor signaling in neurodegenerations

Hilmar Bading

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

Activation of extrasynaptic *N*-methyl-D-aspartate (NMDA) receptors causes neurodegeneration and cell death. The disease mechanism involves a pathological triad consisting of mitochondrial dysfunction, loss of integrity of neuronal structures and connectivity, and disruption of excitation–transcription coupling caused by CREB (cyclic adenosine monophosphate–responsive element-binding protein) shut-off and nuclear accumulation of class IIa histone deacetylases. Interdependency within the triad fuels an accelerating disease progression that culminates in failure of mitochondrial energy production and cell loss. Both acute and slowly progressive neurodegenerative conditions, including stroke, Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease, share increased death signaling by extrasynaptic NMDA receptors caused by elevated extracellular glutamate concentrations or relocalization of NMDA receptors to extrasynaptic sites. Six areas of therapeutic objectives are defined, based on which a broadly applicable combination therapy is proposed to combat the pathological triad of extrasynaptic NMDA receptor signaling that is common to many neurodegenerative diseases.

Neurodegenerations: Convergence of pathomechanisms

Cell death is ultimately the result of energy failure. Therefore, damage to mitochondria that house the machinery for efficient ATP synthesis puts cells at risk for death. Indeed, mutations of genes encoding proteins required for functional integrity of mitochondria have been linked to neurodegenerative conditions (Schon and Przedborski, 2011). However, slowly progressive, adult-onset neurodegenerations, such as Alzheimer's disease (AD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD), are rarely caused primarily by a defect of mitochondria but are caused by deregulation of one or several generic cellular processes, in particular processing, folding, quality control, and subcellular trafficking of proteins (Bossy-Wetzel et al., 2004; Schon and Przedborski, 2011). Diseases associated with an acute loss of neurons are most commonly the result of reduced blood flow to the brain and trauma or are a consequence of inflammation. Thus, neurodegenerations comprise a large and heterogeneous collection of diseases that differ in their biochemical origin, progressiveness, and in the neuronal cell type affected (known as selective vulnerability; Saxena and Caroni, 2011). Here, it is proposed that extrasynaptic *N*-methyl-D-aspartate (NMDA) receptors and the induction of a pathological triad represent a common converging point in the pathomechanisms of diverse neurodegenerative disorders that ultimately is responsible for the disruption of mitochondrial bioenergetics and cell death.

Extrasynaptic NMDA receptors: initiators of a pathological triad

NMDA receptors are glutamate- and voltage-gated ion channels that are permeable for calcium (Paoletti et al., 2013). They can be categorized according to their subcellular location as synaptic and extrasynaptic NMDA receptors (Tovar and Westbrook, 2002; Petralia et al., 2010; Gladding and Raymond, 2011). The subunit composition of the receptors within and outside synaptic contacts is similar, although, in addition to carrying the common GluN1 subunit, extrasynaptic NMDA receptors contain preferentially the GluN2B subunit, whereas GluN2A is the predominant subunit in synaptic NMDA receptors (Paoletti et al., 2013). The cellular consequences of synaptic versus extrasynaptic NMDA receptor stimulation are dramatically different (Hardingham et al., 2002; Hardingham and Bading, 2010). Synaptic NMDA receptors initiate physiological changes in the efficacy of synaptic transmission (Petralia et al., 2010; Morris, 2013; Paoletti et al., 2013). They also trigger calcium signaling pathways to the cell nucleus that activate gene expression responses critical for the long-term implementation of virtually all behavioral adaptations (West and Greenberg, 2011; Bading, 2013). Most importantly,

Correspondence to Hilmar Bading: Bading@nbio.uni-heidelberg.de

Abbreviations used: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; BDNF, brain-derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; CREB, cAMP-responsive element-binding protein; DAPK1, death-associated protein kinase 1; Drp1, dynamin-related protein 1; ERK, extracellular signal–regulated kinase; HD, Huntington's disease; HDAC, histone deacetylase; MCU, mitochondrial calcium uniporter; MS, multiple sclerosis; NCLX, sodium/calcium/lithium exchanger; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; PD, Parkinson's disease; PDE, phosphodiesterase; PKA, cAMP-dependent protein kinase; VEGFD, vascular endothelial growth factor D.

© 2017 Bading This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms/). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at https://creativecommons.org/licenses/by-nc-sa/4.0/).

The Rockefeller University Press $30.00
J. Exp. Med. 2017 Vol. 214 No. 3 569–578
https://doi.org/10.1084/jem.20161673

The Journal of Experimental Medicine

Downloaded from http://rupress.org/jem/article-pdf/214/3/569/1764089/jem_20161673.pdf by guest on 04 December 2025

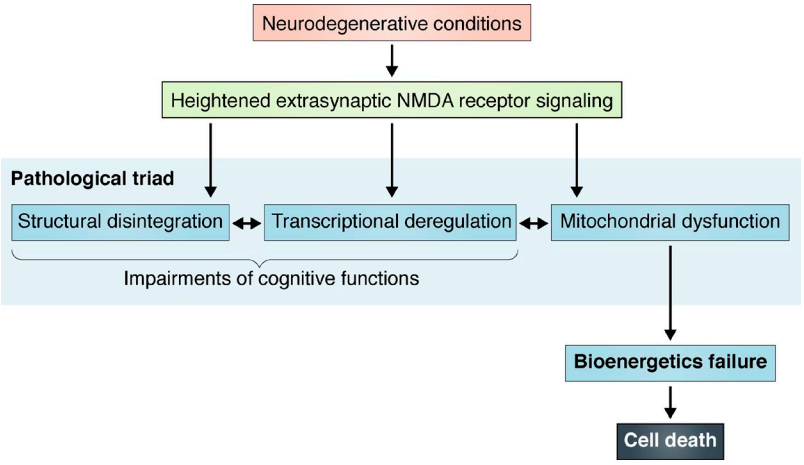

Figure 1. **The pathological triad of extrasynaptic NMDA receptor signaling as a common converging point in neurodegenerative conditions.** The pathological triad leads to impairments of cognitive functions and culminates in bioenergetics failure and neuronal cell death.

synaptic NMDA receptors, acting via nuclear calcium, are strong activators of neuronal structure-protective and survival-promoting genes (Zhang et al., 2007, 2009; Mauceri et al., 2011). In striking contrast, extrasynaptic NMDA receptors trigger cell death pathways (Hardingham et al., 2002; Hardingham and Bading, 2010). Within minutes after extrasynaptic NMDA receptor activation, the mitochondrial membrane potential breaks down, followed by mitochondrial permeability transition (Hardingham et al., 2002). Extrasynaptic NMDA receptors also strongly antagonize excitation–transcription coupling and disrupt nuclear calcium-driven adaptogenomics because they trigger a cyclic adenosine monophosphate (cAMP)–responsive element-binding protein (CREB) shut-off pathway (Hardingham et al., 2002), inactivate extracellular signal–regulated kinase (ERK)–MAPK signaling (Ivanov et al., 2006), and lead to nuclear import of class IIa histone deacetylases (HDACs) and the proapoptotic transcription factor Foxo3A (Chawla et al., 2003; Dick and Bading, 2010). This affects activity regulation of many genes, including *brain-derived neurotrophic factor* (*bdnf*) and *vascular endothelial growth factor D* (*vegfd*; Hardingham et al., 2002; Zhang et al., 2007), that are vital for the maintenance of complex dendritic architecture and synaptic connectivity as well as the buildup of a neuroprotective shield (Zhang et al., 2009; Mauceri et al., 2011; Bading, 2013). In addition, given the short reach of activated ERK1/2 (Wiegert et al., 2007), their shut-off by extrasynaptic NMDA receptors disrupts important local signaling events including dendritic mRNA translation and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor trafficking that controls the efficacy of synaptic transmission (Steward and Schuman, 2001; Kelleher et al., 2004; Kim et al., 2005; Costa-Mattioli et al., 2009). Thus, extrasynaptic NMDA receptor signaling is characterized by the initiation of a pathological triad with mitochondrial dysfunction, deregulation of transcription, and loss of integrity of neuronal structures and connectivity. Reciprocal reinforcements of the impairments underlie disease progression leading to both cognitive decline and increasing deficits in cellular energy production that end in cell death (Fig. 1).

Progression of cell pathology: acute versus chronic neurodegeneration

The strength and duration of extrasynaptic NMDA receptor stimulation determines how fast the pathological triad arrives at bioenergetics failure. Massive activation of extrasynaptic NMDA receptors, for example as a result of cerebral ischemia (see also the Heightened extrasynaptic NMDA receptor signaling in neurodegenerations section), triggers, virtually immediately, a shutdown of mitochondrial function leading to severe energy deficiencies not compensated by glycolysis. In those conditions, which are furthermore accompanied by dendritic swelling and destruction of cytoskeletal components (known as dendrotoxicity; Olney et al., 1979; Ahlgren et al., 2014), neurons die within minutes to hours. Chronically elevated, not acutely toxic levels of extrasynaptic NMDA receptor activation, for example in AD and HD (see also the Heightened extrasynaptic NMDA receptor signaling in neurodegenerations section), impair but do not abrogate mitochondrial ATP synthesis. However, given the concomitant transcriptional deregulation of genes critical for survival and maintenance of structural integrity, neurons enter a feed-forward destruction process and inevitably undergo cell death, although they may sustain a progressively deteriorating state for a long time.

Heightened extrasynaptic NMDA receptor signaling in neurodegenerations

The cell-destructive consequences of extrasynaptic NMDA receptor activation are well known (Hardingham et al., 2002; Hardingham and Bading, 2010). Yet, their relevance for human neurodegenerative diseases only emerged in recent years (Parsons and Raymond, 2014). What has been investigated in detail for six decades are the toxic effects of glutamate, which were first described in the retina (Lucas and Newhouse, 1957) but can occur throughout the brain (Olney, 1969). Glutamate excitotoxicity is responsible for the death of neurons after stroke and traumatic brain or spinal cord injuries and has been implicated in all most common neurodegenerative and neuroinflammatory conditions including AD, HD, ALS, PD, and

Downloaded from http://rupress.org/jem/article-pdf/214/3/569/1764089/jem_20161673.pdf by guest on 04 December 2025

multiple sclerosis (MS; Lipton and Rosenberg, 1994; Lau and Tymianski, 2010). Pharmacology studies have identified the NMDA receptor as the mediator of glutamate excitotoxicity (Choi, 1987; Rothman and Olney, 1995). However, NMDA receptors were found equally important for physiological processes, in particular learning and memory (Morris, 2013; Paoletti et al., 2013), which seemed difficult to reconcile with mediating toxic effects. The explanation offered initially was that excessive activation of NMDA receptors would kill neurons, whereas weak or moderate stimulation would induce physiological plasticity (Lau and Tymianski, 2010). The discovery of differential signaling by synaptic and extrasynaptic NMDA receptors provided a novel and cell biologically more plausible conceptual framework that resolved the apparent NMDA receptor paradox and identified the extrasynaptic NMDA receptor as a death receptor (Hardingham et al., 2002). In light of this new perspective on excitotoxicity, extrasynaptic NMDA receptors appeared as the converging point on the path to death that is shared by etiologically distinct neurodegenerative diseases. There are still gaps in understanding precisely how the primary causes of the different diseases lead to increased extrasynaptic NMDA receptor signaling. However, a common theme is emerging that is characterized by two pathological biochemical states: elevated levels of glutamate in the extracellular space and increases in the number of NMDA receptors located extrasynaptically, possibly accompanied by a decrease in survival-promoting synaptic NMDA receptors. Extracellular glutamate concentrations reach excessively high levels in stroke, which is caused by the reverse operation of neuronal and glial glutamate transporters in hypoxic/ischemic conditions (Rossi et al., 2000). In spinal cord or brain injuries, cell rupture and deregulated glutamate uptake lead to acute increases in extracellular glutamate (Katayama et al., 1990; Yokobori and Bullock, 2012). In AD, a slowly progressing buildup of extracellular glutamate is the result of amyloid β promoting glutamate release from both neurons and glial cells (Li et al., 2009, 2011; Talantova et al., 2013). Elevated glutamate levels have also been detected in the cerebral spinal fluid of ALS patients, which may result from decreased glutamate uptake into neurons and/or glial cells (Plaitakis and Caroscio, 1987; Rothstein et al., 1990, 1992). Astrocytes and activated immune cells are potential sources for the observed increased glutamate levels in acute MS lesions (Frigo et al., 2012). Even in neurodegenerative conditions caused by infections with the protozoan parasites *Plasmodium falciparum* or *Toxoplasma gondii*, increased cerebral spinal fluid glutamate concentrations have been measured, which possibly represent a major cause of cerebral malaria and toxoplasmosis-associated brain damage (Miranda et al., 2010; David et al., 2016). The evidence for elevated extracellular levels of glutamate in PD is sparse, although in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD, glutamate concentrations in the substantia nigra were significantly increased (Meredith et al., 2009). Possibly more disease-relevant, at least in animal models of parkinsonian dyskinesia, is a shift in the subcellular distribution of NMDA receptors toward extrasynaptic sites (Gardoni et al., 2006). Such redistribution of NMDA receptors from synaptic to extrasynaptic locations is best documented in HD (Okamoto et al., 2009; Milnerwood et al., 2010).

The extrasynaptic NMDA receptor–mitotoxicity link

Why do extrasynaptic NMDA receptors and not those located within synapses initiate cell-destructive pathways and, in particular, disrupt the mitochondrial membrane potential that represents an early step toward bioenergetics failure? The answer does not appear to lie within the nature of the evoked increases in the cytosolic calcium concentrations. A comparative analysis of cytosolic calcium transients with virtually identical amplitude and duration revealed that calcium signals initiated by extrasynaptic NMDA receptors cause a rapid loss of the mitochondrial membrane potential, whereas those triggered by synaptic NMDA receptors did not at all harm mitochondria (Hardingham et al., 2002). This difference correlates with the dynamics of mitochondrial calcium: extrasynaptic NMDA receptors cause a sustained rise in mitochondrial calcium, whereas calcium rises in response to synaptic NMDA receptor activation are transient because of a rapid calcium clearance process mediated primarily by sodium/calcium/lithium exchanger (NCLX; Palty et al., 2010; Palty and Sekler, 2012; Kostic et al., 2015; unpublished data). Mitochondrial calcium rises can tune ATP production by modulating enzymes involved in oxidative phosphorylation (Glancy and Balaban, 2012). At the same time, they have the potential to initiate death pathways and thus are dangerous (Bhosale et al., 2015). Indeed, lowering calcium entry into mitochondria in hippocampal neurons by down-regulating the mitochondrial calcium uniporter (MCU) significantly reduces excitotoxic cell death (Qiu et al., 2013). Conversely, changing the calcium clearance through lowering expression of NCLX increases vulnerability (Palty et al., 2010; Palty and Sekler, 2012; Kostic et al., 2015; unpublished data). Thus, the mitochondrial calcium load, specified by the amplitude and duration of mitochondrial calcium increases, acts as a cell-fate checkpoint at which it is being decided whether energy metabolism is physiologically adjusted according to the status of neuronal activity or—induced by extrasynaptic NMDA receptors—mitochondria are rendered dysfunctional, and energy production fails.

Proximity of extrasynaptic NMDA receptors to mitochondria

One possible reason why extrasynaptic NMDA receptors but not synaptic NMDA receptors link to mitotoxicity could be their proximity to mitochondria. Extrasynaptic NMDA receptors may be in close contact with mitochondria, whereas the postsynaptic scaffold, and also the spine structure as such, keeps mitochondria at a distance to synaptic NMDA receptors (spines usually lack mitochondria; Peters et al., 1991). Thus, upon stimulation of extrasynaptic but not synaptic NMDA receptors, mitochondria are exposed to calcium rises

Downloaded from http://rupress.org/jem/article-pdf/214/3/569/1764089/jem_20161673.pdf by guest on 04 December 2025

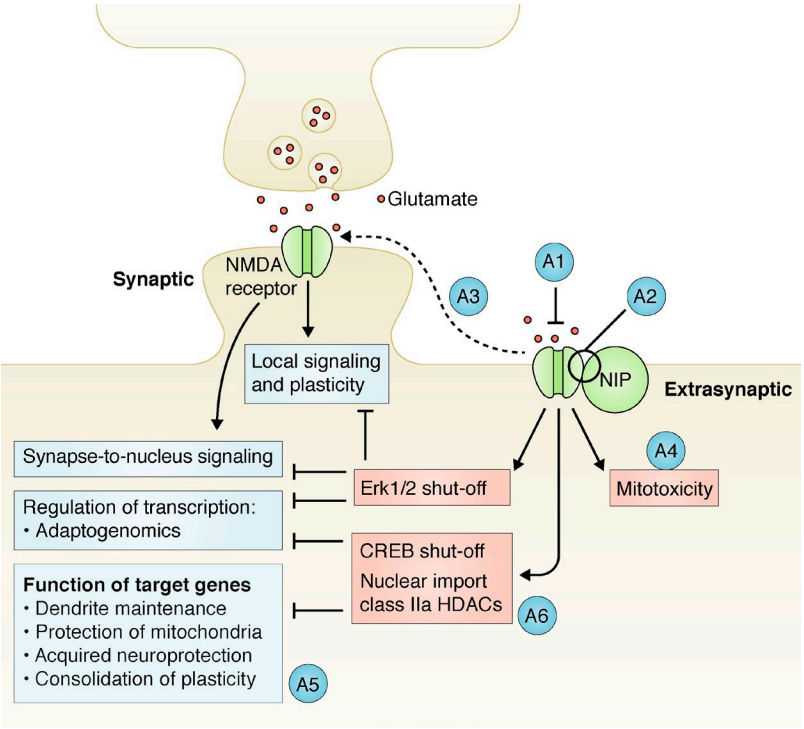

Figure 2. **Schematic illustration of the antagonism by extrasynaptic NMDA receptors of both local signaling and synapse-to-nucleus signaling activated by synaptic NMDA receptors.** Not depicted, for simplicity, is the contribution of back-propagating action potential firing and opening of L-type voltage-gated calcium channels to activity-driven, synaptic NMDA receptor–dependent gene expression (Bading, 2013). Synapse-to-nucleus signaling is mediated by a propagating calcium signal (Bading, 2013) but in addition can involve protein-based communication pathways including the ERK-MAPK cascade, Jacob, and TORC1/2 (Hagenston and Bading, 2011; Panayotis et al., 2015). NMDA receptor–interacting protein (NIP) indicates a putative extrasynaptic NMDA receptor–interacting protein that may be part of the death-signaling complex. Local signaling and plasticity refers in particular to dendritic mRNA translation, AMPA receptor trafficking, and control of synaptic efficacy (Steward and Schuman, 2001; Kelleher et al., 2004; Kim et al., 2005; Costa-Mattioli et al., 2009). The six areas of therapeutic objectives described in this paper are indicated as A1–A6.

in the vicinity of the mouth of the receptors, which may be very high and possibly damaging for mitochondria. Physical separation may also shield mitochondria from other potentially harmful signaling molecules that are generated in the vicinity of activated NMDA receptors. This may in particular apply to nitric oxide (NO), a highly reactive compound with a spatially very limited range of action that is produced upon NMDA receptor activation. NO is a physiological signaling molecule but can also cause neuronal injury and death (Dawson et al., 1994; Garthwaite, 2008). The latter may be mediated by NO-induced S-nitrosylation of dynamin-related protein 1 (Drp1), which damages mitochondria by causing their excessive fragmentation (Cho et al., 2009). According to the proximity to mitochondria hypothesis, only NO produced by extrasynaptic NMDA receptor activation may reach mitochondria at sufficiently high concentrations to initiate damage through nitrosylating Drp1 or possibly other molecules that control mitochondrial calcium dynamics. This model offers an explanation for the increased levels of nitrosylated Drp1 and mitochondrial damage observed in the brains of AD patients (Cho et al., 2009), which may be caused by β amyloid protein acting by increasing extracellular glutamate levels resulting in higher extrasynaptic NMDA receptor activity (Li et al., 2009, 2011; Talantova et al., 2013). It could also explain why NO generated upon synaptic NMDA receptor stimulation is not causing damage to mitochondria but, instead, can serve physiological functions in neuronal plasticity (Dawson et al., 1994; Garthwaite, 2008). Alternatively, differences in the mitotoxic potential of synaptic and extrasynaptic NMDA receptors could also be a consequence of specific interactions with other proteins. It is conceivable that extrasynaptic NMDA receptors act in concert with a co-receptor or other proteins in a mitotoxic death-signaling complex.

Defining six areas of therapeutic objectives to combat the pathological triad

Increased extrasynaptic NMDA receptor signaling causing mitotoxicity, transcriptional shut-off, and structural disintegration defines a converging point in the pathomechanisms of neurodegenerative processes (Fig. 1). It provides a conceptual framework for the development of novel, broadly applicable neuroprotective treatments. For those to be effective, it is important to target each component of the pathological triad to disrupt their reciprocal reinforcements that are responsible for disease progression. This can be achieved by combining therapeutic means developed in six areas of therapeutic objectives, referred to as A1–A6 (Fig. 2).

A1: Innovative extrasynaptic NMDA receptor pharmacology

Several attempts have been made to use blockers of NMDA receptors for treatments of neurological conditions. In general, the results of clinical studies were disappointing largely because of serious side effects caused by interference of the blockers with the physiological function of synaptically localized NMDA receptors (Ogden and Traynelis, 2011). One notable exception is the NMDA receptor antagonist memantine (Bormann, 1989). Beneficial effects of low-dose treatments with memantine have been observed in several animal models of neurodegeneration, which include AD, HD, ALS, and the experimental autoimmune encephalomyelitis model

Downloaded from http://rupress.org/jem/article-pdf/214/3/569/1764089/jem_20161673.pdf by guest on 04 December 2025

of MS (Parsons et al., 1999; Wang and Zhang, 2005; Lipton, 2006; Okamoto et al., 2009; Milnerwood et al., 2010; Talantova et al., 2013; Sühs et al., 2014). Moreover, memantine is approved since 2002 by the European Medicines Agency and the US Food and Drug Administration (FDA) for the treatment of AD patients (Reisberg et al., 2003). The discovery that memantine in a certain concentration range blocks preferentially extrasynaptic NMDA receptors (Xia et al., 2010) explains why it is effective in a wide range of neurodegenerative conditions that share toxic extrasynaptic NMDA receptor signaling as a pathomechanism. Thus, means of selectively attenuating extrasynaptic NMDA receptor activity hold great potential for the development of broadly effective, well-tolerated neuroprotective therapeutics. Generating improved, more effective variants of memantine, such as nitromemantine (Talantova et al., 2013), is one task for the future. It is equally important to pursue novel innovative paths. By exploiting the differences in the cell-surface distribution of NMDA receptors, it may be possible to specifically target extrasynaptic NMDA receptors, also sparing synaptic NMDA receptors. One option is to attach an NMDA receptor blocker to nanoparticles with a diameter larger than the width of the gap between the pre- and postsynaptic membranes, which is ∼20 nm. A bead-conjugated receptor blocker would be unable to enter the synaptic cleft. Consequently, it would have no access to synaptic NMDA receptors, and its inhibitory action would be restricted to extrasynaptic NMDA receptors. Prototypes of such compounds have been developed (Maus et al., 2010; Savchenko et al., 2016). They consist of 30–35-nm diameter gold nanoparticles conjugated either with memantine (Savchenko et al., 2016) or conantokin G (Maus et al., 2010), a peptide toxin that blocks NR2B subunit–containing NMDA receptors (Donevan and McCabe, 2000). The choice of an NR2B-specific blocker such as conantokin G for the coupling to beads further contributes to the selective targeting, as NR2B is found preferentially in extrasynaptic NMDA receptors (Paoletti et al., 2013). The coupling to beads of other NR2B-selective drugs, including small molecules, is conceivable. The spectrum of subunit-specific antagonists is constantly growing owing to huge research efforts to dissect the diversity of NMDA receptor complexes in the hope to develop clinically suitable drugs that differentially modulate the physiological and pathological consequences of NMDA receptor activation (Ogden and Traynelis, 2011). However, subunit selectivity of an antagonist is not per se sufficient to warrant selective blockade of extrasynaptic NMDA receptors because the synaptic versus extrasynaptic localization of the NMDA receptor is only partially reflected by the subunit composition (Paoletti et al., 2013). Consequently, even a drug that is highly selective for NR2B would neither eliminate extrasynaptic NMDA receptor activity nor would it leave synaptic NMDA receptors unaffected. Thus, the coupling to beads to limit access to extrasynaptic NMDA receptors or other means of guided inhibition is necessary to obtain the desired pharmacological effects. One possibility involves the design of two-arm blockers that consist of a small molecule or peptide-based NMDA receptor blocker that is connected by a chemical linker or a short peptide sequence to a targeting module. Guided inhibition may be accomplished by the interaction of the targeting module with an extracellular molecule or a protein domain that is in close proximity to extrasynaptic but not synaptic NMDA receptors. Components of the extracellular matrix or nonsynaptic membrane proteins may serve as guides.

A2: Interactors of extrasynaptic NMDA receptors in a death-signaling complex

Many NMDA receptor–interacting proteins have been identified (Husi et al., 2000). However, it remains a challenge for the future to determine which interacting proteins associate specifically with extrasynaptic NMDA receptors. Interactors of extrasynaptic NMDA receptors may not only serve as guides for two-arm blockers (see the A1: Innovative extrasynaptic NMDA receptor pharmacology section), but may also act in concert with extrasynaptic NMDA receptors in the death-signaling complex. Disrupting such interactions using competing peptides or small molecules may confer robust neuroprotection. One promising candidate is the death-associated protein kinase 1 (DAPK1) that associates with NR2B in ischemic conditions, thereby potentiating NMDA receptor activity at extrasynaptic sites (Tu et al., 2010). Blockade of the NR2B–DAPK1 interaction or deletion of the *dapk1* gene provides protection against brain damage in a mouse stroke model (Tu et al., 2010).

A3: Relocalization strategies of extrasynaptic NMDA receptors

Another strategy to minimize extrasynaptic NMDA receptor signaling is to promote their internalization or relocalization to synaptic plasma membrane sites. Trafficking of NMDA receptors and their lateral mobility is well studied (Tovar and Westbrook, 2002; Triller and Choquet, 2005), but no druggable target has been identified. However, the search for survival-promoting genes activated by synaptic NMDA receptors (Zhang et al., 2007, 2009) or upon BDNF treatment revealed that expression of *inhba*, the gene encoding inhibin βA, also known as activin A, can reduce toxic extrasynaptic NMDA receptor signaling (Lau et al., 2015). Activin A can be delivered to the brain via various routes, including the application of nasal drops; it significantly reduces stroke-induced brain damage in mice, even when given after the trauma (Tretter et al., 2000; Mukerji et al., 2009; Lau et al., 2015; unpublished data). As a body's own natural reducer of extrasynaptic NMDA receptor signaling that, moreover, can be delivered via a simple, noninvasive nose-to-brain delivery route, activin A appears to be an ideal component of a broadly applicable neuroprotective therapy scheme for both acute and chronic degenerative conditions.

A4: Mitoprotection

An important target of pharmacological intervention downstream of extrasynaptic NMDA receptors is the

Downloaded from http://rupress.org/jem/article-pdf/214/3/569/1764089/jem_20161673.pdf by guest on 04 December 2025

mitochondrial calcium dynamics. The goal is to prevent the extrasynaptic NMDA receptor–induced pathological calcium load either by blocking entry of calcium into mitochondria or by boosting calcium clearance. With the identification of the MCU complex (Kamer and Mootha, 2015) and NCLX (Palty et al., 2010; Palty and Sekler, 2012), key molecules of the mitochondrial calcium entry and exit routes are available for screens for mitoprotective compounds. Attenuation of extrasynaptic NMDA receptor–induced mitochondrial calcium load using RNA interference–mediated knockdown of MCU does indeed provide robust neuroprotection (Qiu et al., 2013). However, this may happen at the expense of losing, at least in part, the possibility of physiologically adapting the rates of oxidative phosphorylation through signal-induced mitochondrial calcium increases in conditions of high-energy demands. Mitoprotection can also be built up via synaptic activity. This body's own process is mediated by Npas4, a transcription factor induced by synaptic NMDA receptors and nuclear calcium signaling (Zhang et al., 2009) that reduces MCU expression (Qiu et al., 2013). Lowering mitochondrial calcium load by enhancing NCLX-mediated calcium clearance may also be accomplished via a physiological pathway. NCLX is phosphorylated on serine 258 by the cAMP-dependent protein kinase (PKA), which increases NCLX activity and rescues the impairments of NCLX function observed after partial loss of mitochondrial membrane potential (Kostic et al., 2015). Therefore, pharmacologically evoked elevation of cAMP levels activating PKA signaling may restore or even boost NCLX function under conditions of increased extrasynaptic NMDA receptor signaling that drives the breakdown of the mitochondrial membrane potential. Clinically applicable compounds that increase cAMP levels include rolipram, an FDA approved inhibitor of phosphodiesterase 4 (PDE4); BPN14770, a different PDE4 inhibitor at present in phase I safety trial (https://clinicaltrials.gov/ct2/show/NCT02648672); and PF-02545920, an inhibitor of PDE10A, currently in phase II clinical trial for HD (https://clinicaltrials.gov/ct2/show/NCT02197130). Rolipram, BPN14770, and PF-02545920 may have an add-on survival-promoting effect owing to the transcriptional responses evoked by cAMP-PKA signaling. cAMP and nuclear calcium represent the two principal activators of CREB/CREB-binding protein–mediated gene expression (Mayr and Montminy, 2001; Bading, 2013). Therefore, components of the nuclear calcium-regulated gene program responsible for acquired neuroprotection (Zhang et al., 2009) may also be induced by treatment with rolipram, BPN14770, or PF-02545920.

A5: Supplementation of structure-protective and prosurvival gene products

It is important to counteract the consequences of the disruption of excitation–transcription coupling by extrasynaptic NMDA receptors. The expression of hundreds of genes is under tight control of neuronal activity and synaptic NMDA receptors (Zhang et al., 2007). This includes many survival-promoting genes and the neurotrophin BDNF, as well as the dendrite maintenance factor, VEGFD, and the complement factor C1q, a synapse-pruning factor whose expression is suppressed by synaptic activity (Zhang et al., 2007, 2009; Mauceri et al., 2011; West and Greenberg, 2011; Bading, 2013; Simonetti et al., 2013). Collectively, the transcriptional responses induced in synaptically activated neurons build up a neuroprotective shield and help maintain proper neuronal structures (Bading, 2013). Extrasynaptic NMDA receptors antagonize the synapse-to-nucleus communication axis and thus compromise the coupling of synaptic activity to the activation of vital genomic events (Hardingham et al., 2002; Zhang et al., 2007; Hardingham and Bading, 2010). The consequences are inappropriate basal or induced expression levels of survival-promoting and structure-preserving genes, which result in increased vulnerability, a reduction in length and complexity of dendrites, and synapse loss. In addition, because of disruption of activity regulation of the activin A encoding *inhba*, neurons may lose an intrinsic mechanism to reduce the number of NMDA receptors at extrasynaptic plasma membrane sites (see also the A3: Relocalization strategies of extrasynaptic NMDA receptors section). The loss of structural integrity and connectivity and increased extrasynaptic NMDA receptor signaling fuels malfunctioning of activity-dependent transcription and drives neurons into a detrimental feed-forward process that further promotes structural and functional disintegration and accelerates disease progression. It is important to disrupt this chain of events. One therapeutic direction is the supplementation of important gene products. In particular, the delivery of activin A to reduce extrasynaptic NMDA receptor signaling attenuates excitotoxicity and generates significant therapeutic benefits in a mouse stroke model (Lau et al., 2015). Supplementing VEGFD through delivery of the recombinant protein or VEGFD-derived peptide mimetics is expected to reduce the neurodegeneration-associated loss of dendritic structures, thereby preserving synaptic connectivity and promoting the survival of neurons. By neutralizing C1q using monoclonal antibodies together with Brain Shuttle technology (Phuan et al., 2013; Niewoehner et al., 2014), it may be possible to attenuate synapse loss.

A6: Rescue of excitation–transcription coupling and boosting nuclear calcium signaling

Inhibitors of HDACs may be suitable to render the neurons' chromatin more permissive for initiation of transcription, which may help restore activity-dependent gene expression under conditions of heightened extrasynaptic NMDA receptor signaling. Through blocking the transcription-repressing function of HDACs, compounds such as the FDA-approved Vorinostat (suberanilohydroxamic acid) would cause a broadly occurring facilitation of gene expression. In addition, such a treatment would counteract the consequences

Downloaded from http://rupress.org/jem/article-pdf/214/3/569/1764089/jem_20161673.pdf by guest on 04 December 2025

of the nuclear import of class IIa HDACs, which occurs under conditions of extrasynaptic NMDA receptor stimulation (Chawla et al., 2003) and could represent one reason for decreased expression levels of VEGFD and increased levels of C1q (Zhang et al., 2007; Schlumm et al., 2013; Mauceri et al., 2015). Another key therapeutic target is CREB that is being shut off upon extrasynaptic NMDA receptor stimulation through a process that leads to the dephosphorylation of phospho–serine 133 of the activated, i.e., serine 133–phosphorylated, form of CREB (Hardingham et al., 2002). The loss of this phosphorylation event may be compensated by means of increasing cognitive activities to stimulate synaptic transmission and to promote CREB serine 133 phosphorylation via synaptic NMDA receptor–nuclear calcium-CaMKIV signaling. One possible pharmacological strategy is the use of so-called AMPA-kines that facilitate excitatory glutamatergic synaptic transmission (Lynch et al., 2014). An elegant alternative mechanism to increase CREB-dependent gene expression that, at the same time, promotes nuclear export of class IIa HDACs would involve enhancement of the amplitude and/or duration of synaptic activity–evoked nuclear calcium signals, for example by slowing down the clearance of calcium from the cell nucleus. This could be accomplished by the design of inhibitors of those sodium/calcium exchangers that are localized to the inner leaflet of the nuclear envelope and transfer calcium from the nucleoplasm into the lumen of the endoplasmic reticulum (Wu et al., 2009). Currently no suitable compounds are available to boost nuclear calcium signaling to promote the expression of the endogenous neuroprotective gene program (Zhang et al., 2007, 2009). However, because phosphorylation of CREB on its activator site serine 133 can be catalyzed by numerous protein kinases including PKA (Mayr and Montminy, 2001), therapeutic benefits may also be obtained by pharmacologically activating alternative routes to CREB phosphorylation, for example using rolipram, BPN14770, or PF-02545920 that stimulate cAMP-PKA signaling (see also the A4: Mitoprotection section).

Neuroprotective combination therapy

An effective, broadly applicable neuroprotective treatment may ideally use a combination of interventions to simultaneously address multiple areas of therapeutic objectives. A prototypical therapy scheme for both acute and slowly progressing neurodegenerations may include the following compounds (the corresponding areas of therapeutic objectives are given in brackets): oral application of memantine (A1), rolipram (A4 and A6), and vorinostat (A6), plus administration of activin A (A3 and A5) and VEGFD or VEGFD-derived peptide mimetics (A5) via nasal spray or an intrathecal catheter. An additional ingredient of a neuroprotective therapy is an active lifestyle to keep up brain activity that fuels the synapse-to-nucleus communication axis and drives gene programs that help maintain an intact neuronal architecture and strengthen the body's own neuroprotective shield (Fig. 2; Bading, 2013).

ACKNOWLEDGMENTS

I would like to thank the members of my laboratory for comments on the manuscript.

The work in the author's laboratory is supported by a European Research Council (ERC) Advanced Grant (233024), an ERC Proof-of-Concept Grant (640892), the German-Israeli Project Cooperation (grant no. BA 1007/7-1), the Sonderforschungsbereich 1134 and 1158 of the Deutsche Forschungsgemeinschaft (DFG), and the DFG Forschergruppe 2289. The author is a member of the Excellence Cluster CellNetworks at Heidelberg University.

The author declares no competing financial interests.

Submitted: 3 October 2016
Revised: 2 January 2017
Accepted: 5 January 2017

Downloaded from http://rupress.org/jem/article-pdf/214/3/569/1764089/jem_20161673.pdf by guest on 04 December 2025

REFERENCES

Ahlgren, H., C. Bas-Orth, H.E. Freitag, A. Hellwig, O.P. Ottersen, and H. Bading. 2014. The nuclear calcium signaling target, activating transcription factor 3 (ATF3), protects against dendrotoxicity and facilitates the recovery of synaptic transmission after an excitotoxic insult. *J. Biol. Chem.* 289:9970–9982. http://dx.doi.org/10.1074/jbc.M113.502914

Bading, H. 2013. Nuclear calcium signalling in the regulation of brain function. *Nat. Rev. Neurosci.* 14:593–608. http://dx.doi.org/10.1038/nrn3531

Bhosale, G., J.A. Sharpe, S.Y. Sundier, and M.R. Duchen. 2015. Calcium signaling as a mediator of cell energy demand and a trigger to cell death. *Ann. N. Y. Acad. Sci.* 1350:107–116. http://dx.doi.org/10.1111/nyas.12885

Bormann, J. 1989. Memantine is a potent blocker of N-methyl-D-aspartate (NMDA) receptor channels. *Eur. J. Pharmacol.* 166:591–592. http://dx.doi.org/10.1016/0014-2999(89)90385-3

Bossy-Wetzel, E., R. Schwarzenbacher, and S.A. Lipton. 2004. Molecular pathways to neurodegeneration. *Nat. Med.* 10:S2–S9. http://dx.doi.org/10.1038/nm1067

Chawla, S., P. Vanhoutte, F.J.L. Arnold, C.L.-H. Huang, and H. Bading. 2003. Neuronal activity-dependent nucleocytoplasmic shuttling of HDAC4 and HDAC5. *J. Neurochem.* 85:151–159. http://dx.doi.org/10.1046/j.1471-4159.2003.01648.x

Cho, D.H., T. Nakamura, J. Fang, P. Cieplak, A. Godzik, Z. Gu, and S.A. Lipton. 2009. S-nitrosylation of Drp1 mediates β-amyloid-related mitochondrial fission and neuronal injury. *Science.* 324:102–105. http://dx.doi.org/10.1126/science.1171091

Choi, D.W. 1987. Ionic dependence of glutamate neurotoxicity. *J. Neurosci.* 7:369–379.

Costa-Mattioli, M., W.S. Sossin, E. Klann, and N. Sonenberg. 2009. Translational control of long-lasting synaptic plasticity and memory. *Neuron.* 61:10–26. http://dx.doi.org/10.1016/j.neuron.2008.10.055

David, C.N., E.S. Frias, J.I. Szu, P.A. Vieira, J.A. Hubbard, J. Lovelace, M. Michael, D. Worth, K.E. McGovern, I.M. Ethell, et al. 2016. GLT-1-dependent disruption of CNS glutamate homeostasis and neuronal function by the protozoan parasite *Toxoplasma gondii*. *PLoS Pathog.* 12:e1005643. http://dx.doi.org/10.1371/journal.ppat.1005643

Dawson, T.M., J. Zhang, V.L. Dawson, and S.H. Snyder. 1994. Nitric oxide: cellular regulation and neuronal injury. *Prog. Brain Res.* 103:365–369. http://dx.doi.org/10.1016/S0079-6123(08)61150-4

Dick, O., and H. Bading. 2010. Synaptic activity and nuclear calcium signaling protect hippocampal neurons from death signal-associated nuclear translocation of FoxO3a induced by extrasynaptic *N*-methyl-D-aspartate receptors. *J. Biol. Chem.* 285:19354–19361. http://dx.doi.org/10.1074/jbc.M110.127654

Donevan, S.D., and R.T. McCabe. 2000. Conantokin G is an NR2B-selective competitive antagonist of N-methyl-D-aspartate receptors. *Mol. Pharmacol.* 58:614–623.

Frigo, M., M.G. Cogo, M.L. Fusco, M. Gardinetti, and B. Frigeni. 2012. Glutamate and multiple sclerosis. *Curr. Med. Chem.* 19:1295–1299. http://dx.doi.org/10.2174/092986712799462559

Gardoni, F., B. Picconi, V. Ghiglieri, F. Polli, V. Bagetta, G. Bernardi, F. Cattabeni, M. Di Luca, and P. Calabresi. 2006. A critical interaction between NR2B and MAGUK in L-DOPA induced dyskinesia. *J. Neurosci.* 26:2914–2922. http://dx.doi.org/10.1523/JNEUROSCI.5326-05.2006

Garthwaite, J. 2008. Concepts of neural nitric oxide-mediated transmission. *Eur. J. Neurosci.* 27:2783–2802. http://dx.doi.org/10.1111/j.1460-9568.2008.06285.x

Gladding, C.M., and L.A. Raymond. 2011. Mechanisms underlying NMDA receptor synaptic/extrasynaptic distribution and function. *Mol. Cell. Neurosci.* 48:308–320. http://dx.doi.org/10.1016/j.mcn.2011.05.001

Glancy, B., and R.S. Balaban. 2012. Role of mitochondrial Ca^{2+} in the regulation of cellular energetics. *Biochemistry.* 51:2959–2973. http://dx.doi.org/10.1021/bi2018909

Hagenston, A.M., and H. Bading. 2011. Calcium signaling in synapse-to-nucleus communication. *Cold Spring Harb. Perspect. Biol.* 3:a004564. http://dx.doi.org/10.1101/cshperspect.a004564

Hardingham, G.E., and H. Bading. 2010. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat. Rev. Neurosci.* 11:682–696. http://dx.doi.org/10.1038/nrn2911

Hardingham, G.E., Y. Fukunaga, and H. Bading. 2002. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat. Neurosci.* 5:405–414.

Husi, H., M.A. Ward, J.S. Choudhary, W.P. Blackstock, and S.G. Grant. 2000. Proteomic analysis of NMDA receptor–adhesion protein signaling complexes. *Nat. Neurosci.* 3:661–669. http://dx.doi.org/10.1038/76615

Ivanov, A., C. Pellegrino, S. Rama, I. Dumalska, Y. Salyha, Y. Ben-Ari, and I. Medina. 2006. Opposing role of synaptic and extrasynaptic NMDA receptors in regulation of the extracellular signal-regulated kinases (ERK) activity in cultured rat hippocampal neurons. *J. Physiol.* 572:789–798. http://dx.doi.org/10.1113/jphysiol.2006.105510

Kamer, K.J., and V.K. Mootha. 2015. The molecular era of the mitochondrial calcium uniporter. *Nat. Rev. Mol. Cell Biol.* 16:545–553. http://dx.doi.org/10.1038/nrm4039

Katayama, Y., D.P. Becker, T. Tamura, and D.A. Hovda. 1990. Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. *J. Neurosurg.* 73:889–900. http://dx.doi.org/10.3171/jns.1990.73.6.0889

Kelleher, R.J. III, A. Govindarajan, H.Y. Jung, H. Kang, and S. Tonegawa. 2004. Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell.* 116:467–479. http://dx.doi.org/10.1016/S0092-8674(04)00115-1

Kim, M.J., A.W. Dunah, Y.T. Wang, and M. Sheng. 2005. Differential roles of NR2A- and NR2B-containing NMDA receptors in Ras-ERK signaling and AMPA receptor trafficking. *Neuron.* 46:745–760. http://dx.doi.org/10.1016/j.neuron.2005.04.031

Kostic, M., M.H. Ludtmann, H. Bading, M. Hershfinkel, E. Steer, C.T. Chu, A.Y. Abramov, and I. Sekler. 2015. PKA Phosphorylation of NCLX reverses mitochondrial calcium overload and depolarization, promoting survival of PINK1-deficient dopaminergic neurons. *Cell Reports.* 13:376–386. http://dx.doi.org/10.1016/j.celrep.2015.08.079

Lau, A., and M. Tymianski. 2010. Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Arch.* 460:525–542. http://dx.doi.org/10.1007/s00424-010-0809-1

Lau, D., C.P. Bengtson, B. Buchthal, and H. Bading. 2015. BDNF reduces toxic extrasynaptic NMDA receptor signaling via synaptic NMDA receptors and nuclear calcium-induced transcription of *inhba*/activin A. *Cell Reports.* 12:1353–1366. http://dx.doi.org/10.1016/j.celrep.2015.07.038

Li, S., S. Hong, N.E. Shepardson, D.M. Walsh, G.M. Shankar, and D. Selkoe. 2009. Soluble oligomers of amyloid β protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron.* 62:788–801. http://dx.doi.org/10.1016/j.neuron.2009.05.012

Li, S., M. Jin, T. Koeglsperger, N.E. Shepardson, G.M. Shankar, and D.J. Selkoe. 2011. Soluble Aβ oligomers inhibit long-term potentiation through a mechanism involving excessive activation of extrasynaptic NR2B-containing NMDA receptors. *J. Neurosci.* 31:6627–6638. http://dx.doi.org/10.1523/JNEUROSCI.0203-11.2011

Lipton, S.A. 2006. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. *Nat. Rev. Drug Discov.* 5:160–170. http://dx.doi.org/10.1038/nrd1958

Lipton, S.A., and P.A. Rosenberg. 1994. Excitatory amino acids as a final common pathway for neurologic disorders. *N. Engl. J. Med.* 330:613–622. http://dx.doi.org/10.1056/NEJM199403033300907

Lucas, D.R., and J.P. Newhouse. 1957. The toxic effect of sodium L-glutamate on the inner layers of the retina. *AMA Arch. Opthalmol.* 58:193–201. http://dx.doi.org/10.1001/archopht.1957.00940010205006

Lynch, G., C.D. Cox, and C.M. Gall. 2014. Pharmacological enhancement of memory or cognition in normal subjects. *Front. Syst. Neurosci.* 8:90. http://dx.doi.org/10.3389/fnsys.2014.00090

Mauceri, D., H.E. Freitag, A.M.M. Oliveira, C.P. Bengtson, and H. Bading. 2011. Nuclear calcium-VEGFD signaling controls maintenance of dendrite arborization necessary for memory formation. *Neuron.* 71:117–130. http://dx.doi.org/10.1016/j.neuron.2011.04.022

Mauceri, D., A.M. Hagenston, K. Schramm, U. Weiss, and H. Bading. 2015. Nuclear calcium buffering capacity shapes neuronal architecture. *J. Biol. Chem.* 290:23039–23049. http://dx.doi.org/10.1074/jbc.M115.654962

Maus, L., O. Dick, H. Bading, J.P. Spatz, and R. Fiammengo. 2010. Conjugation of peptides to the passivation shell of gold nanoparticles for targeting of cell-surface receptors. *ACS Nano.* 4:6617–6628. http://dx.doi.org/10.1021/nn101867w

Mayr, B., and M. Montminy. 2001. Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat. Rev. Mol. Cell Biol.* 2:599–609. http://dx.doi.org/10.1038/35085068

Meredith, G.E., S. Totterdell, M. Beales, and C.K. Meshul. 2009. Impaired glutamate homeostasis and programmed cell death in a chronic MPTP mouse model of Parkinson's disease. *Exp. Neurol.* 219:334–340. http://dx.doi.org/10.1016/j.expneurol.2009.06.005

Milnerwood, A.J., C.M. Gladding, M.A. Pouladi, A.M. Kaufman, R.M. Hines, J.D. Boyd, R.W.Y. Ko, O.C. Vasuta, R.K. Graham, M.R. Hayden, et al. 2010. Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron.* 65:178–190. http://dx.doi.org/10.1016/j.neuron.2010.01.008

Miranda, A.S., L.B. Vieira, N. Lacerda-Queiroz, A.H. Souza, D.H. Rodrigues, M.C. Vilela, M.V. Gomez, F.S. Machado, M.A. Rachid, and A.L. Teixeira. 2010. Increased levels of glutamate in the central nervous system are associated with behavioral symptoms in experimental malaria. *Braz. J. Med. Biol. Res.* 43:1173–1177. http://dx.doi.org/10.1590/S0100-879X2010007500130

Morris, R.G. 2013. NMDA receptors and memory encoding. *Neuropharmacology.* 74:32–40. http://dx.doi.org/10.1016/j.neuropharm.2013.04.014

Mukerji, S.S., R.N. Rainey, J.L. Rhodes, and A.K. Hall. 2009. Delayed activin A administration attenuates tissue death after transient focal cerebral ischemia and is associated with decreased stress-responsive kinase activation. *J. Neurochem.* 111:1138–1148. http://dx.doi.org/10.1111/j.1471-4159.2009.06406.x

Downloaded from http://rupress.org/jem/article-pdf/214/3/569/1764089/jem_20161673.pdf by guest on 04 December 2025

Niewoehner, J., B. Bohrmann, L. Collin, E. Urich, H. Sade, P. Maier, P. Rueger, J.O. Stracke, W. Lau, A.C. Tissot, et al. 2014. Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. *Neuron*. 81:49–60. http://dx.doi.org/10.1016/j.neuron.2013.10.061

Ogden, K.K., and S.F. Traynelis. 2011. New advances in NMDA receptor pharmacology. *Trends Pharmacol. Sci.* 32:726–733. http://dx.doi.org/10.1016/j.tips.2011.08.003

Okamoto, S., M.A. Pouladi, M. Talantova, D. Yao, P. Xia, D.E. Ehrnhoefer, R. Zaidi, A. Clemente, M. Kaul, R.K. Graham, et al. 2009. Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. *Nat. Med.* 15:1407–1413. http://dx.doi.org/10.1038/nm.2056

Olney, J.W. 1969. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science.* 164:719–721. http://dx.doi.org/10.1126/science.164.3880.719

Olney, J.W., T. Fuller, and T. de Gubareff. 1979. Acute dendrotoxic changes in the hippocampus of kainate treated rats. *Brain Res.* 176:91–100. http://dx.doi.org/10.1016/0006-8993(79)90872-2

Palty, R., and I. Sekler. 2012. The mitochondrial Na^+/Ca^{2+} exchanger. *Cell Calcium*. 52:9–15. http://dx.doi.org/10.1016/j.ceca.2012.02.010

Palty, R., W.F. Silverman, M. Hershfinkel, T. Caporale, S.L. Sensi, J. Parnis, C. Nolte, D. Fishman, V. Shoshan-Barmatz, S. Herrmann, et al. 2010. NCLX is an essential component of mitochondrial Na^+/Ca^{2+} exchange. *Proc. Natl. Acad. Sci. USA*. 107:436–441. http://dx.doi.org/10.1073/pnas.0908099107

Panayotis, N., A. Karpova, M.R. Kreutz, and M. Fainzilber. 2015. Macromolecular transport in synapse to nucleus communication. *Trends Neurosci.* 38:108–116. http://dx.doi.org/10.1016/j.tins.2014.12.001

Paoletti, P., C. Bellone, and Q. Zhou. 2013. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nat. Rev. Neurosci.* 14:383–400. http://dx.doi.org/10.1038/nrn3504

Parsons, M.P., and L.A. Raymond. 2014. Extrasynaptic NMDA receptor involvement in central nervous system disorders. *Neuron*. 82:279–293. http://dx.doi.org/10.1016/j.neuron.2014.03.030

Parsons, C.G., W. Danysz, and G. Quack. 1999. Memantine is a clinically well tolerated *N*-methyl-D-aspartate (NMDA) receptor antagonist—a review of preclinical data. *Neuropharmacology.* 38:735–767. http://dx.doi.org/10.1016/S0028-3908(99)00019-2

Peters, A., S.L. Palay, and H.F. Webster. 1991. Fine Structure of the Nervous System: Neurons and Their Supporting Cells. Oxford University Press, New York. 528 pp.

Petralia, R.S., Y.X. Wang, F. Hua, Z. Yi, A. Zhou, L. Ge, F.A. Stephenson, and R.J. Wenthold. 2010. Organization of NMDA receptors at extrasynaptic locations. *Neuroscience.* 167:68–87. http://dx.doi.org/10.1016/j.neuroscience.2010.01.022

Phuan, P.W., H. Zhang, N. Asavapanumas, M. Leviten, A. Rosenthal, L. Tradtrantip, and A.S. Verkman. 2013. C1q-targeted monoclonal antibody prevents complement-dependent cytotoxicity and neuropathology in in vitro and mouse models of neuromyelitis optica. *Acta Neuropathol.* 125:829–840. http://dx.doi.org/10.1007/s00401-013-1128-3

Plaitakis, A., and J.T. Caroscio. 1987. Abnormal glutamate metabolism in amyotrophic lateral sclerosis. *Ann. Neurol.* 22:575–579. http://dx.doi.org/10.1002/ana.410220503

Qiu, J., Y.-W. Tan, A.M. Hagenston, M.-A. Martel, N. Kneisel, P.A. Skehel, D.J.A. Wyllie, H. Bading, and G.E. Hardingham. 2013. Mitochondrial calcium uniporter Mcu controls excitotoxicity and is transcriptionally repressed by neuroprotective nuclear calcium signals. *Nat. Commun.* 4:2034. http://dx.doi.org/10.1038/ncomms3034

Reisberg, B., R. Doody, A. Stöffler, F. Schmitt, S. Ferris, and H.J. Möbius. Memantine Study Group. 2003. Memantine in moderate-to-severe Alzheimer's disease. *N. Engl. J. Med.* 348:1333–1341. http://dx.doi.org/10.1056/NEJMoa013128

Rossi, D.J., T. Oshima, and D. Attwell. 2000. Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature.* 403:316–321. http://dx.doi.org/10.1038/35002090

Rothman, S.M., and J.W. Olney. 1995. Excitotoxicity and the NMDA receptor—still lethal after eight years. *Trends Neurosci.* 18:57–58.

Rothstein, J.D., G. Tsai, R.W. Kuncl, L. Clawson, D.R. Cornblath, D.B. Drachman, A. Pestronk, B.L. Stauch, and J.T. Coyle. 1990. Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. *Ann. Neurol.* 28:18–25. http://dx.doi.org/10.1002/ana.410280106

Rothstein, J.D., L.J. Martin, and R.W. Kuncl. 1992. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *N. Engl. J. Med.* 326:1464–1468. http://dx.doi.org/10.1056/NEJM199205283262204

Savchenko, A., G.B. Braun, and E. Molokanova. 2016. Nanostructured antagonist of extrasynaptic NMDA receptors. *Nano Lett.* 16:5495–5502. http://dx.doi.org/10.1021/acs.nanolett.6b01988

Saxena, S., and P. Caroni. 2011. Selective neuronal vulnerability in neurodegenerative diseases: from stressor thresholds to degeneration. *Neuron*. 71:35–48. http://dx.doi.org/10.1016/j.neuron.2011.06.031

Schlumm, F., D. Mauceri, H.E. Freitag, and H. Bading. 2013. Nuclear calcium signaling regulates nuclear export of a subset of class IIa histone deacetylases following synaptic activity. *J. Biol. Chem.* 288:8074–8084. http://dx.doi.org/10.1074/jbc.M112.432773

Schon, E.A., and S. Przedborski. 2011. Mitochondria: the next (neurode)generation. *Neuron*. 70:1033–1053. http://dx.doi.org/10.1016/j.neuron.2011.06.003

Simonetti, M., A.M. Hagenston, D. Vardeh, H.E. Freitag, D. Mauceri, J. Lu, V.P. Satagopam, R. Schneider, M. Costigan, H. Bading, and R. Kuner. 2013. Nuclear calcium signaling in spinal neurons drives a genomic program required for persistent inflammatory pain. *Neuron*. 77:43–57. http://dx.doi.org/10.1016/j.neuron.2012.10.037

Steward, O., and E.M. Schuman. 2001. Protein synthesis at synaptic sites on dendrites. *Annu. Rev. Neurosci.* 24:299–325. http://dx.doi.org/10.1146/annurev.neuro.24.1.299

Sühs, K.W., R. Fairless, S.K. Williams, K. Heine, A. Cavalié, and R. Diem. 2014. *N*-methyl-d-aspartate receptor blockade is neuroprotective in experimental autoimmune optic neuritis. *J. Neuropathol. Exp. Neurol.* 73:507–518. http://dx.doi.org/10.1097/NEN.0000000000000073

Talantova, M., S. Sanz-Blasco, X. Zhang, P. Xia, M.W. Akhtar, S. Okamoto, G. Dziewczapolski, T. Nakamura, G. Cao, A.E. Pratt, et al. 2013. Aβ induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. *Proc. Natl. Acad. Sci. USA*. 110:E2518–E2527. http://dx.doi.org/10.1073/pnas.1306832110

Tovar, K.R., and G.L. Westbrook. 2002. Mobile NMDA receptors at hippocampal synapses. *Neuron*. 34:255–264. http://dx.doi.org/10.1016/S0896-6273(02)00658-X

Tretter, Y.P., M. Hertel, B. Munz, G. ten Bruggencate, S. Werner, and C. Alzheimer. 2000. Induction of activin A is essential for the neuroprotective action of basic fibroblast growth factor in vivo. *Nat. Med.* 6:812–815. http://dx.doi.org/10.1038/77548

Triller, A., and D. Choquet. 2005. Surface trafficking of receptors between synaptic and extrasynaptic membranes: and yet they do move! *Trends Neurosci.* 28:133–139. http://dx.doi.org/10.1016/j.tins.2005.01.001

Tu, W., X. Xu, L. Peng, X. Zhong, W. Zhang, M.M. Soundarapandian, C. Balel, M. Wang, N. Jia, W. Zhang, et al. 2010. DAPK1 interaction with NMDA receptor NR2B subunits mediates brain damage in stroke. *Cell*. 140:222–234. http://dx.doi.org/10.1016/j.cell.2009.12.055

Downloaded from http://rupress.org/jem/article-pdf/214/3/569/1764089/jem_20161673.pdf by guest on 04 December 2025

Wang, R., and D. Zhang. 2005. Memantine prolongs survival in an amyotrophic lateral sclerosis mouse model. *Eur. J. Neurosci.* 22:2376–2380. http://dx.doi.org/10.1111/j.1460-9568.2005.04431.x

West, A.E., and M.E. Greenberg. 2011. Neuronal activity-regulated gene transcription in synapse development and cognitive function. *Cold Spring Harb. Perspect. Biol.* 3:a005744. http://dx.doi.org/10.1101/cshperspect.a005744

Wiegert, J.S., C.P. Bengtson, and H. Bading. 2007. Diffusion and not active transport underlies and limits ERK1/2 synapse-to-nucleus signaling in hippocampal neurons. *J. Biol. Chem.* 282:29621–29633. http://dx.doi.org/10.1074/jbc.M701448200

Wu, G., X. Xie, Z.H. Lu, and R.W. Ledeen. 2009. Sodium-calcium exchanger complexed with GM1 ganglioside in nuclear membrane transfers calcium from nucleoplasm to endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA.* 106:10829–10834. http://dx.doi.org/10.1073/pnas.0903408106

Xia, P., H.S. Chen, D. Zhang, and S.A. Lipton. 2010. Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. *J. Neurosci.* 30:11246–11250. http://dx.doi.org/10.1523/JNEUROSCI.2488-10.2010

Yokobori, S., and M.R. Bullock. 2012. Pathobiology of traumatic brain injury. *In* Brain Injury Medicine. Second edition. D.I. Katz, and R.D. Zafonte, editors. Demos Medical Publishing, Zasler, N.D. pp. 137–147.

Zhang, S.-J., M.N. Steijaert, D. Lau, G. Schütz, C. Delucinge-Vivier, P. Descombes, and H. Bading. 2007. Decoding NMDA receptor signaling: identification of genomic programs specifying neuronal survival and death. *Neuron.* 53:549–562. http://dx.doi.org/10.1016/j.neuron.2007.01.025

Zhang, S.-J., M. Zou, L. Lu, D. Lau, D.A.W. Ditzel, C. Delucinge-Vivier, Y. Aso, P. Descombes, and H. Bading. 2009. Nuclear calcium signaling controls expression of a large gene pool: identification of a gene program for acquired neuroprotection induced by synaptic activity. *PLoS Genet.* 5:e1000604. http://dx.doi.org/10.1371/journal.pgen.1000604

Downloaded from http://rupress.org/jem/article-pdf/214/3/569/1764089/jem_20161673.pdf by guest on 04 December 2025