White matter NMDA receptors: an unexpected new therapeutic target?

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Axons, their ensheathing myelin and supporting glia that make up the white matter in the mammalian brain and spinal cord are fundamentally important for the normal operation of the central nervous system. Prevalent human disorders such as stroke, vascular dementia, multiple sclerosis, brain and spinal cord trauma, HIV-associated dementia, periventricular leukomalacia of premature infants, and seemingly traditional ‘gray matter disorders’ such as Alzheimer’s disease and schizophrenia, exhibit white matter pathology that contributes to morbidity and mortality. N-Methyl-D-aspartate (NMDA) receptors have been shown to have an important role in mediating Ca²⁺-dependent injury of oligodendrocytes and the myelin sheath; newly recognized family members of the NMDA receptor, known as NR3 subunits, seem to be involved. Recently developed competitive NMDA channel blockers such as memantine hold therapeutic promise because these agents are well tolerated clinically and might prove to be effective at protecting certain white matter elements from a variety of insults.

Introduction

The mammalian central nervous system (CNS) consists of two fundamentally different types of tissue. Gray and white matter make up roughly equal proportions of the human brain [1], and each subserves unique functions: most of the neurons and synapses of the brain are contained in the gray matter, whereas axons and their supporting glial cells are concentrated in the white matter regions. The latter support vital communication between neurons in the CNS, and convey information to and from the body through millions of afferent and efferent axons in the white matter tracts of the spinal cord. Because of the important role of white matter axons and their high packing densities in the CNS, even small lesion volumes, located in strategic areas, can produce devastating neurological deficits. Many different human neurological disorders, reflecting diverse underlying etiologies, adversely affect white matter; prominent examples include stroke, vascular dementia, multiple sclerosis (MS) and other demyelinating diseases, traumatic injury to the brain (‘diffuse axonal injury’) and spinal cord (more localized and severe damage), periventricular leukomalacia affecting premature newborns, HIV-induced encephalomyelitis, Alzheimer’s disease and numerous inherited leukodystrophies. Although white matter is rarely damaged in isolation, the common thread in these diseases is the permanent physical and/or functional interruption of axonal pathways, significantly contributing to morbidity and mortality. Therefore, developing pharmacotherapeutic agents aimed at mitigating acute or ongoing damage to white matter axons and supporting glia (astrocytes and oligodendrocytes) might represent an important advance in our ability to treat several prevalent neurological disorders. This aspect of ‘neuroprotection’ has received surprisingly little attention over the years, and might be one reason why many cytoprotective treatments have failed in clinical trials for neurological diseases.

Glutamate receptors in health and disease

The CNS depends on dozens of different neurotransmitters and receptors for normal operation. Arguably, the most prominent class of excitatory transmitter is the glutamatergic system, which uses the amino acid glutamate as the native neurotransmitter. Glutamate is the natural ligand for two main classes of receptors: those coupled to ion-permeable (ionotropic) channels consisting of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate and N-methyl-D-aspartate (NMDA) receptors [2,3], and G-protein-coupled metabotropic receptors that do not conduct current directly but instead signal through phospholipase C to release Ca²⁺ from intracellular stores, or through adenylate cyclase, to modulate other ion channels and intracellular biochemical pathways [4]. The ionotropic glutamate receptors have in common substantial permeability to both Na⁺ and K⁺, and varying permeability to Ca²⁺ ions. Most rapidly activating and desensitizing AMPA receptors, in addition to kainate receptors, traditionally manifest modest Ca²⁺ permeability, whereas NMDA receptors (NMDARs) have slower kinetics but PCa/PNa (permeability ratios of Ca²⁺ to Na⁺) as high as 10:1 [5]. Together, the ionotropic receptors have fundamentally important roles in excitatory neurotransmission in the CNS, and affect plasticity, memory and learning, in addition to neuronal development.

Given the ubiquitous distribution of glutamate receptors in the CNS and their ability to flux large ionic loads – particularly deleterious Ca²⁺ loads – it is no surprise that modulators of glutamate receptors have been the subject of intense research for a wide variety of conditions, ranging from acute ischemia and trauma, to more chronic...
neurodegenerative disorders such as Huntington’s, Parkinson’s and Alzheimer’s diseases; epilepsy; infectious diseases such as HIV-associated dementia (HAD), and, more recently, even psychiatric disorders have been proposed to be amenable to glutamatergic modulation [6]. Unfortunately, with few exceptions, glutamate antagonists have to date been disappointing for the treatment of acute and chronic degenerative CNS disorders, owing to a combination of lack of clinical efficacy and/or excessive side effects [7].

Of note, targeting glutamate receptors in the above disorders was designed to protect neuronal populations, with little regard to white matter, because the latter was thought not to support glutamatergic transmission, or to be susceptible to glutamate-mediated excitotoxicity. Recent data have convincingly dispelled this notion: we now know that astrocytes express AMPA receptors, and that oligodendrocytes additionally express kainate receptors [8,9]. Moreover, glutamate release has been shown to occur from white matter axons under physiological [10,11] and pathological [12,13] conditions. Perhaps most surprisingly, recent studies have conclusively demonstrated the presence of NMDARs on both astrocytes and oligodendrocytes, and also in the compact myelin sheath surrounding central myelinated fibers [9,14–16]. Therefore, given the widespread distribution of NMDARs on neurons and glia, together with their propensity to flux potentially damaging Ca²⁺ loads, this class of receptor might represent an attractive target for protecting not only neurons, but also white matter regions where glia and myelin predominate.

**NMDAR structure and function**

NMDARs are made up of at least two different subunits composed of NR1 (whose presence is mandatory), NR2A-D and, in some cases, NR3A or -B subunits, which were recently cloned and characterized [17,18]. The receptor is probably composed of a tetramer of these subunits, whose composition determines the pharmacology and other physiological parameters of the receptor–ion channel complex. Conventional NMDARs are composed of NR1 and NR2 subunits, and activation requires dual agonists, glutamate and glycine. Several modulatory sites of channel activity also exist on the NMDAR (Figure 1). Alternative splicing of some subunits, such as NR1, further contributes to the diversity of pharmacological properties of the receptor [19]. The subunits are differentially expressed, both regionally in the brain and temporally during development. The activity of the NMDAR-associated channel is modulated via voltage-dependent block by Mg²⁺ [20,21], and the channel manifests high permeability to Ca²⁺ [22]. Physiological NMDAR activity is essential for normal neuronal function [23], and therefore must be preserved, even in the face of excessive pathological activity in other areas of the brain.

NR3A and -3B represent the third and final group of subunits in the NMDAR family [17]. NR3A subunits have been found on glia in addition to neurons, and even in mature central myelin [14]. NR1 and NR3A and/or -B subunits, in the absence of NR2 subunits, functionally assemble to form excitatory glycine receptors because they require glycine alone for activation in the absence of glutamate or NMDA. Receptors comprised of NR1 and NR3 subunits are more sensitive to glycine than receptors containing NR1 and NR2 subunits, desensitize at high glycine concentrations, are less permeable to Ca²⁺ and are relatively resistant to Mg²⁺ blockade. The physiological importance of NR1/NR3 glycine ‘only’ receptors is still a matter of debate and is under intensive investigation [17,24]. Interestingly, CNS myelin contains NR1/NR3 complexes (McRory, J., Zamponi, G. and Stys, P.K., unpublished), which might suggest the presence of receptors responsive to glycine alone. The function of these putative myelinic ‘glycine’ receptors is unknown but, given their unique pharmacology [17], they might represent a potential therapeutic target for demyelinating disease once their pathophysiological role, if any, is elucidated.

Additionally, when coexpressed with NR1 and NR2 subunits, NR3 subunits modulate NMDAR activity by decreasing subunit conductance, Ca²⁺ permeability and Mg²⁺ sensitivity, thus acting in a ‘dominant-negative’ fashion to decrease NMDAR currents [17,24]. However, because Mg²⁺ blockade is decreased by the presence of NR3 subunits, it is possible that, under some circumstances, NR3-containing receptors might be more susceptible to activation – for example, if Mg²⁺ block would otherwise have had a prominent role in damping down excessive NMDAR activity. In general, however, the fast desensitization, decreased conductance and low Ca²⁺ permeability of NR3-containing receptors effectively antagonizes NMDARs and decreases overall Ca²⁺ entry into the cells following receptor activation. Consistent with these properties of NR3-containing receptors, mice genetically lacking NR3A manifest increased NMDA-induced currents and dendritic spine density on neurons [18,25]. Lack of highly
specific and selective agonists for the NR3 family of subunits has held this field back, but the emergence of NR3 knockout and transgenic mice should cast additional light on the importance of this subunit in the near future.

NMDARs in central glia
In the past two decades, several studies from various laboratories have provided irrefutable proof of the existence of AMPA and kainate receptors in astrocytes and oligodendroglia [8]. By contrast, expression of NMDARs on central neuroglia has been less clear [26]. Evidence of astrocytic NMDARs was obtained by experiments showing inward currents and Ca\(^{2+}\) rises elicited in response to NMDA application to astrocytes in culture or brain slices [27] (and references therein) but controversy existed as to whether these were direct or indirect responses. Nevertheless, recent reports provide compelling evidence for the presence of NMDARs, AMPA receptors and glutamate transporter-mediated inward currents in cortical astrocytes in response to glutamate application [27]. In oligodendrocytes, perhaps one of the first hints of functional NMDARs was obtained by electrophysiological recording of currents in cells from the spinal cord in young rats (prior to postnatal day 10). Gray matter, and to a lesser extent white matter, oligodendrocytes exhibited inward currents in response to NMDA application, which largely disappeared as the animals matured [28]. This observation lay dormant until recently, when three complementary papers reported the unequivocal presence of functional NMDARs in oligodendrocyte cell bodies, their processes and, perhaps most surprisingly, in the inner and outer loops of the compact myelin sheath [14–16]. NMDA-evoked inward currents were observed in precursor, immature and mature oligodendrocytes, exhibiting approximately tenfold lower sensitivity to Mg\(^{2+}\) block compared with their neuronal counterparts [15]. This unique property might enable these receptors to be activated by glutamate without the prerequisite relief of Mg\(^{2+}\) block by depolarization, typically provided by AMPA receptors at glutamatergic neuronal synapses [22]. Chemical ischemia induced an NMDA-dependent inward current in oligodendrocyte somata [15] and resulted in damage to the cell bodies and processes. Interestingly, although somatic injury was reduced by AMPA/kainate receptor antagonists, processes were not spared unless NMDAR blockade was applied [16]. Ischemia-induced Ca\(^{2+}\) increases were measured in the nanoscopic cytoplasmic compartment of compact CNS myelin [29], and were found to be mediated mainly by activation of myelinic NMDARs [14], in line with the preferential role of these receptors in mediating damage to oligodendroglial processes. During ischemia, activation of myelinic NMDARs occurs by glutamate and glycine coreleased by reverse operation of Na\(^{+}\)-dependent transporters under conditions of axonal Na\(^{+}\) loading and depolarization [29] (Figure 2). Notably, ischemic pathology of oligodendrocytic processes [16] and the myelin sheath [14] was significantly ameliorated by NMDAR antagonists. Equally important was the observation that ischemia-induced axonal Ca\(^{2+}\) overload was not reduced by modulating these receptors [14], reflecting important variability in the mechanisms of Ca\(^{2+}\)-mediated injury among white matter elements that are intimately associated, such as axons and their ensheathing myelin.

Present and future pharmacology of protection from NMDAR overactivation
Importantly, elevations in extracellular glutamate are not necessary to invoke an excitotoxic mechanism. Excitotoxicity can come into play even with normal levels of glutamate if NMDAR activity is increased – for example, when

![Figure 2](https://www.sciencedirect.com)
neurons or glia are injured and thus become depolarized; this condition relieves the normal Mg\(^{2+}\)-induced block of the ion channel and thus abnormally increases NMDAR activity [30]. Moreover, if NR3 subunits are present, as seems to be the case on many glia, Mg\(^{2+}\) block is much less evident.

Protection from excessive NMDAR activity for both white matter (glia and myelin) and gray matter (neurons) is of paramount importance to maintain the integrity of the nervous system in the face of a variety of insults. However, potential neuro- and glial-protective agents that manifest a high affinity for NMDARs block virtually all activity, including normal (physiological) signaling, and will therefore probably have unacceptable clinical side effects. For this reason, many previous NMDAR antagonists have disappointingly failed advanced clinical trials for several neurodegenerative disorders. By contrast, studies have shown that the adamantane derivative, memantine, preferentially blocks excessive NMDAR activity without disrupting normal function [7]. Memantine does this through its action as a low-affinity (but still highly selective), uncompetitive, open-channel blocker with a relatively rapid off-rate from the channel [7]. Recently, this uncompetitive, fast off-rate mechanism of action has been termed a ‘UFO’ drug, consistent with the observation that this type of antagonist is present at its site of action for only a short time and then ‘disappears’ [31]. The open-channel, uncompetitive nature of memantine block means that increasing NMDAR activity, with resultant excessive channel openings, is inhibited to a greater degree than is the case with less receptor activity; hence, excessive (pathological) NMDAR activity is inhibited to a greater extent than with normal (physiological) activity. Moreover, the relatively fast off-rate of memantine prevents the drug from accumulating in NMDAR-operated channels, so subsequent physiological neurotransmission can proceed in a normal fashion [32,33]. It is believed that this fast off-rate property contributes to the favorable profile of memantine in terms of its clinical tolerability [34,35].

Intriguingly, in some sense the effect of NR3 on the outer vestibule of the channel resembles the antagonist effect of memantine in the channel to limit excessive current flux. For example, recent work has shown that NR3A alters the outer vestibule of the ion channel to limit the influx of Ca\(^{2+}\) and other ions[36]. Thus, NR3 subunits represent in some sense natural ‘antagonists’ to decrease the activity of NMDARs in vivo [36]. Because NR3A subunits are predominantly present during early development, this mechanism might also possibly serve as a normal protection device from excitotoxic insults because NMDARs rapidly increase in numbers during postnatal development [37].

**Disorders of white matter: modulating NMDARs**

The human CNS contains a much higher proportion of white matter compared with lower mammals such as rodents [1]; therefore, in humans, one could argue that white matter damage might be more responsible for overt neurological dysfunction. Many common disorders exhibit a component of white matter injury that might be amenable to treatment. White matter damage is a pathologically recognized component, yet we know far less about the pathogenesis of injury to this tissue, and therefore, we are hard-pressed to propose, let alone design, rational pharmacotherapy. Indeed, an argument could be made that the disappointing failures of neuroprotective interventions for some of the more acute disorders could be due, at least in part, to our failure to understand and design interventions tailored to mitigate the degeneration of white matter elements. Emergence of new data on the pathogenesis of white matter injury is beginning to set the stage for rational drug design aimed at protecting this vital component of the CNS. For instance, MS and its animal model [experimental autoimmune encephalomyelitis (EAE)] might involve abnormal glutamatergic signaling. MS patients have higher levels of glutamate in the cerebrospinal fluid [38], and MR spectroscopy has revealed elevated glutamate concentrations in the brains of MS patients [39]. Further evidence for a role of glutamate receptors in the pathogenesis of EAE (and, by extension, possibly MS) is provided by reports showing a beneficial effect of AMPA/kainate receptor antagonists [40,41]. Interestingly, Wallstrom et al. [42] found that the uncompetitive NMDAR antagonist memantine reduced the clinical deficits in a Lewis rat EAE model, ascribing the improvement to a neuron-sparing effect of the drug (on the understanding at that time that only neurons, and not glia, expressed NMDARs). Given the recent data on NMDARs in oligodendrocytes and myelin, it is highly likely that their observations were also due to a direct protective effect on these myelinating cells, which are prime targets in EAE and MS. Although EAE is an imperfect model of MS, these results suggest that glutamate receptors might indeed have a role in demyelinating disorders.

Ischemia, both acute (stroke) and chronic (e.g. vascular dementia), invariably causes white matter damage which depends, in part, on glutamate receptor activation; however, the role of NMDARs in ischemic white matter damage is questionable [43]. HIV infection produces an array of pathology, involving both the CNS and peripheral nervous system [44], with white matter pathology being common both in the brain (related to HAD) and spinal cord (vacular myelopathy). The pathogenesis is not clear, although for HAD, a presumed excitotoxic mechanism, possibly mediated by NMDARs, has been suggested [45].

Taken together, numerous reports over the years have implicated excitotoxic mechanisms in the genesis of a wide variety of white matter disorders. Recent data now provide sound molecular reasons for suspecting a role for NMDARs as mediators of at least part of the pathology, particularly concerning oligodendrocyte death and demyelination, the latter being either secondary to degeneration of the myelinating cell, or as a primary target, given the known expression of NMDARs in the sheath itself. For these reasons, NMDAR antagonism might be a reasonable option for mitigating at least a component of white matter pathology.

Until recently, all drugs that showed promise as inhibitors of excitotoxicity also blocked normal neuronal function and consequently had severe and unacceptable side effects, so clinical trials for stroke, traumatic brain injury and Huntington’s disease all failed. Memantine
represents a class of drugs that have relatively low affinity for the NMDAR and function as uncompetitive, open-channel blockers. Due to its uncompetitive antagonism and relatively fast off-rate, memantine blocks excessive NMDA receptor activation but spares lower (physiological) levels of NMDAR activity. Importantly, memantine binds at the ‘intracellular’ Mg²⁺ site in the channel pore and displays differential affinity for specific and nonspecific binding sites on the NMDA receptor. These molecular interactions confer upon memantine favorable kinetic properties that contribute to the clinical tolerability of the drug, in addition to its neuroprotective profile [35]. The discovery that memantine is potentially neuroprotective yet clinically tolerated is triggering a paradigm shift in drug development by the pharmaceutical industry, in that low-affinity agents can prove to be effective. Clinical studies have borne out our hypothesis that the UFO mechanism of memantine action yields a safe NMDAR antagonist in humans, and one that is also beneficial in the treatment of neurological disorders such as moderate-to-severe Alzheimer’s disease, apparently mediated, at least, in part by excitotoxicity. As alluded to earlier, the possibility that other diseases primarily affecting glia can be restrained by memantine has been hinted at in animal studies of EAE showing protection [42,46] but remains to be proven in human clinical studies of multiple sclerosis or periventricular leukomalacia.

**Conclusion**

Preserving normal function of the mammalian CNS after injurious stimuli, either acute or chronic, categorically requires sparing of neurons and their connections concentrated in the white matter. Recent advances in our understanding of the pathophysiology of white matter injury [47,48] have now laid the foundation for rational drug design aimed at specifically identified molecular targets. Perhaps one of the more unexpected targets is the recent identification of NMDARs as prominent players in the genesis of damage to oligodendrocytes and myelin, suggesting that antagonists or gentler modulators of these receptors might have an important role in protecting the CNS in disorders where demyelination is a prominent pathological manifestation. However, complex heterogeneity is appearing even in this tissue: whereas oligodendrocytes and their myelin might be strongly protected by NMDAR antagonism, the ensheathed axon will probably not be spared by such treatments [14], This emphasizes the approach of targeting several signaling pathways to mitigate the deleterious effects of multifarious assaults on the CNS. Therefore, the concept of modulating a single pathway selectively in diseases exhibiting complex parallel mechanisms might have been doomed from the start. Nonetheless, the NMDAR seems to be acquiring a prominent, albeit not a unique, position for rational therapeutic design.

**Conflict of interest statement**

Dr. Lipton is the named inventor on issued patents for the use of the drug memantine (Namenda®) in the treatment of neurodegenerative diseases. Subsequently, memantine was approved for clinical use in the treatment of moderate-to-severe Alzheimer’s disease by the European Union and by the Food and Drug Administration (FDA) of the USA. Dr. Lipton has no direct ownership in memantine, but under the rules of the institution where this work was performed, Harvard University, he participates in a royalty-sharing plan administered by Harvard Medical School and Children’s Hospital, Boston, to whom the patents are assigned.

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**References**

17. Chatterton, J.E. et al. (2002) Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. Nature 415, 793–798
18. Das, S. et al. (1998) Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3B. Nature 393, 377–381
33 Chen, H.S. et al. (1992) Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. J. Neurosci. 12, 4427–4436