NMDA-gated ion channel research and its therapeutic potentials in neurodegenerative diseases: a review

Maryam Majdi1
Huei-Sheng Vincent Chen1,2
1Center for Neuroscience, Aging and Stem Cell Research, Burnham Institute for Medical Research, La Jolla, California, USA; 2Division of Cardiology, University of California, San Diego, San Diego, California, USA

Abstract: The N-methyl-D-aspartate (NMDA) subtype of glutamate receptor (NMDAR) is essential for normal function of the central nervous system (CNS). Classical NMDARs, activated by glycine and glutamate, are heteromultimers comprising NR1 and NR2 subunits. Nonetheless, excessive activation of NMDARs by excitatory amino acids such as glutamate is thought to mediate neuronal damage in many neurological disorders. The dual role of NMDARs in normal and abnormal functioning of the CNS imposes significant constraints on possible therapeutic strategies aimed at ameliorating neurodegenerative diseases. To create safe NMDAR-based therapies, blockade of excessive NMDAR activity must therefore be achieved with minimal interference on its normal neuronal function. In general, NMDAR antagonists can be classified pharmacologically according to the site of action on the receptor-channel complex. These include drugs acting at the agonist sites (NMDA and glycine), channel pore, and modulatory sites. Both competitive NMDA and glycine antagonists result in generalized inhibition of NMDAR activities and have, thus, failed in clinical trials. Open-channel blockers with uncompetitive antagonism and drugs modulating NMDAR activities are appealing therapeutic strategies because, in theory, these properties could decrease neurotoxicity due to excessive levels of glutamate while sparing physiological neurotransmission. We review here NMDAR-related research that may lead to future therapeutic intervention against neurotoxicity.

Keywords: excitotoxicity, open-channel block, uncompetitive antagonism, Alzheimer disease, memantine

Introduction
Glutamate receptors are essential for normal function of the central nervous system (CNS), such as long-term potentiation (LTP) responsible for memory.1 However, excessive activation of NMDA subtype of glutamate receptor (NMDAR) is thought to mediate neuronal damage during pathological conditions such as stroke, Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), and amyotrophic lateral sclerosis.2,3 The fine line between the physiological function and toxic reactions elicited by NMDARs is a major concern for developing safe therapeutic interventions. NMDAR antagonists can be categorized pharmacologically into 4 major groups according to the site of action on the receptor channel complex: Drugs acting at the 1) NMDA (agonist) recognition site, 2) glycine (co-agonist) site, 3) channel pore, and 4) modulatory sites such as the redox modulatory site, proton sensitive site, high-affinity Zn2+ site, and polyamine site.4 NMDARs are found in most regions of the brain;1 therefore, both competitive NMDA and noncompetitive glycine antagonists, although effective in preventing glutamate-mediated neurotoxicity, will cause generalized...
inhibition of glutamate receptor function. Such a side effect clearly limits their potential for future clinical applications. Therapeutic strategies involving modulatory agents acting at ligand-binding or modulatory sites of NMDARs may provide a better therapeutic index. Furthermore, open-channel block with uncompetitive antagonism is currently the most appealing strategy for therapeutic intervention during excessive NMDAR activation. This property, in theory, leads to a higher degree of channel blockade in the presence of excessive levels of glutamate and little blockade at relatively lower levels, for example, during physiological neurotransmission. Utilizing this pharmacological strategy of action, we helped develop memantine as the first clinically tolerated, yet effective agent against NMDAR-mediated neurotoxicity. Memantine has been clinically demonstrated to be effective in treating moderate-to-severe AD, while being well tolerated. Other strategies of creating NMDAR modulatory agents to combat neurodegenerative diseases will also be briefly discussed.

**NMDA receptor structure**

The glutamatergic neurotransmitter system comprises the majority of excitatory synapses in the neocortex. Based on the pharmacology of agonist sites, there are three classes of glutamate-gated ion (or i onotropic) channels, known as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate, and NMDA receptors. Three gene families are known to encode for NMDA receptor families, termed NMDAR1 (NR1), NMDAR2 (NR2), and NMDAR3 (NR3). In contrast to non-NMDA (AMPA and kainate)-type glutamate receptors, all functional NMDARs are heteromultimers. A common structural scheme for glutamate receptors contains an extracellular amino-terminal domain (ATD) for various modulatory functions, extracellular S1S2 domains for agonist binding, an ion channel domain with four transmembrane segments (M1–4) for gating and ion permeation, and a carboxy-terminal domain for communicating with intracellular milieu (Figure 1A–B). Conventional NMDARs composed of NR1 and NR2A-D subunits require dual agonists, glutamate and glycine, for activation (Figure 1). The activity of NMDAR-associated channels is modulated by voltage-dependent block of magnesium (Mg²⁺) and the channel manifests high permeability to calcium (Ca²⁺). Depolarization of the postsynaptic membrane through activation of adjacent non-NMDA glutamate receptors (eg, AMPA receptors) in synaptic spines removes Mg²⁺ blockade and enables NMDA channel activation. Amino acid residues at or adjacent to the so called N-site or “Q/R/N” (glutamine/arginine/asparagine)-site in the second membrane region (M2) of NMDAR subunits control the permeability and block of NMDAR-activated channels by Ca²⁺ and Mg²⁺. Mutation of the N-site asparagine in NR1 or NR2 subunits also dramatically decreases the potency of antagonism by organic open-channel blockers, eg, MK-801, ketamine, amantadine, and memantine. Included within the NMDAR structure are various modulatory sites, such as the polyamine, redox, Zn²⁺, and proton sites (Figure 1C), which regulate NMDA function.

Structurally, NMDARs are likely composed of a tetramer of NR1 and NR2 subunits. The subunit composition determines the properties of receptor-ion channel complex. NR2 subunits dictate overall pharmacological and biophysical properties of the NMDAR complex, and determine whether NMDARs will be involved in induction of LTP or synaptic plasticity. Alternative splicing of NR1 subunits further contributes to the diversity of pharmacological properties of NMDARs. NMDAR subunits are differentially expressed both regionally in the brain and temporally during development. For example, NR2B-containing NMDARs with slow-decaying currents are predominantly found in the early postnatal brain; NR2A outnumber NR2B subunits as the brain matures. This developmental switch of NR2 subunits results in differential properties of synaptic NMDARs, which may contribute to synaptic development or plasticity. Additionally, co-expression of the novel NR3 family of NMDAR subunits decreases the magnitude of NR1/NR2 receptor-mediated currents or forms glycine-activated channels with the NR1 subunit alone. Physiological function of NR3-containing receptors remains to be determined.

**NMDA receptors and normal synaptic neural function**

Most neurons (and also glia) in brain tissue contain high intracellular concentrations of glutamate (~10 mM). Upon accumulation into synaptic vesicles, glutamate is released for a very brief period (on the order of milliseconds) during normal glutamatergic neurotransmission in order to communicate with other neurons through synaptic terminals (Figure 2). It is well established that activation of NMDARs is required for synaptic plasticity in normal neural function, such as LTP and long-term depression (LTD). LTP (or LTD) is an activity-dependent form of increased (or decreased) transmission efficacy at synapses which is considered to represent the cellular basis for learning and memory. Many neurodegenerative diseases and brain aging, which are associated with cognitive decline, display a decrease in LTP and
reduced synaptic plasticity. While both LTP and LTD require NMDAR activation, the level of postsynaptic Ca\(^{2+}\) increases after differential NMDAR activation may determine the types of synaptic modification. Importantly, NR2A- or NR2B-containing NMDARs may play differential roles in the induction and polarity of synaptic plasticity, that is LTP versus LTD. Moreover, proper synaptic localization of NR2B-containing NMDARs might be important for the strength of physiological induction of LTP. Therefore, synaptic versus extrasynaptic NMDARs and their subunit compositions further contribute to their differential and diverse roles in neural plasticity and neuronal survival.

**NMDA receptor and excitotoxicity**

During normal synaptic transmission, glutamate is released into the synaptic cleft and is available for activation of NMDARs for a very brief period of time (Figure 2), yet excess levels of glutamate or its presence for prolonged periods of time may elicit neuronal insults ultimately leading to cell death (Figure 3), also known as “excitotoxicity”. In many areas of the CNS, the predominant form of neurotoxicity appears to be mediated by overactivation of NMDARs and subsequent influx or release of excessive Ca\(^{2+}\). Ca\(^{2+}\) overload could consequently lead to mitochondrial dysfunction, production of reactive oxygen species (ROS) and nitric oxide (NO) radicals, activation of protein kinases, phosphatases and pro-apoptotic pathways through second messenger cascades, resulting in cell death due to oxidative stress and excitotoxicity (also see legend for Figure 3).

Various insults can lead to excessive or prolonged release of glutamate within the nervous system resulting in excitotoxicity. For instance, copious levels of glutamate are released from damaged cells at the focus of insults during traumatic brain injury or cerebral ischemia following stroke.
The resulting high concentrations of glutamate cause neighboring cells to depolarize, swell, lyse, and die by necrosis due to overactivation of NMDARs. Damaged cells at penumbral regions of the insult further release glutamate, resulting in a vicious cycle of auto-destructive events leading to progressive cell death that can continue for hours or even days following original injury. Furthermore, during ischemic insults many neurons are deprived of essential energy needed to maintain ionic homeostasis; as a result, these neurons depolarize and propagate the same type of auto-destructive events seen in traumatic injury.3,7,40

A subtler form of excitotoxicity has been implicated in many chronic and slowly progressive neurodegenerative disorders. Neurological diseases such as AD, HD, PD, multiple sclerosis, and amyotrophic lateral sclerosis are caused by various mechanisms but may share a final common pathway as a result of chronic, prolonged exposure to moderately elevated levels of glutamate relative to that occurring during normal neurotransmission, which ultimately leads to perturbed Ca\(^{2+}\) homeostasis, activation of apoptotic mechanisms and cell death (Figure 3).36–38 The extent of excitotoxicity from these chronic, subtle insults may also depend on activation of extrasynaptic NMDARs\(^{39}\) or NMDARs of different subunit compositions.3,8,33,39,40

Moreover, excitotoxicity can occur with normal levels of glutamate if the activity of NMDARs is increased, for example, when neurons are injured and become depolarized. As a result, the normal block of NMDAR-associated ion channels by Mg\(^{2+}\) is relieved; thereby enabling increased activity of NMDARs.7,40 In addition, increased activity of the enzyme NO synthase (NOS) is associated with excitotoxic cell death.38 The neuronal isoform of the enzyme is physically tethered to the NMDAR and activated by Ca\(^{2+}\) influx via...
Increased levels of NO have been detected in animal models of stroke and several neurodegenerative diseases.\(^3\),\(^7\)

Additionally, recent research suggested that induction of NMDAR-mediated neurotoxicity might depend on the NR2 subunit composition of NMDARs involved and their subcellular localization (synaptic versus extrasynaptic).\(^3\),\(^9\),\(^40\)

Overactivation of extrasynaptic NR2B-containing NMDARs appears to be associated with activation of many neurotoxic pathways and subsequent cell death; yet synaptic activation of NMDAR seems to be neuro-protective, supporting neuronal survival. Overactivation of NR2A- and/or NR2B-containing NMDARs have also been linked to mitochondrial dysfunction and elevated production of ROS and NO radicals in neurons, resulting in excitotoxicity.\(^3\),\(^9\),\(^40\)

Other Ca\(^{2+}\)-permeable channels and routes of Ca\(^{2+}\) entry, such as transient receptor potential (Trp), acid-sensing and Ca\(^{2+}\)-permeable AMPA channels are also known to contribute to excitotoxicity.\(^39\),\(^41\)–\(^44\)

Apart from postsynaptic receptors, changes in various postsynaptic proteins, such as components of cytoskeletal actin-regulatory machinery and postsynaptic scaffold proteins (e.g., PSD95/SAP90), are also thought to underlie the cognitive impairments involved with neurodegenerative disorders.\(^43\),\(^45\)–\(^50\)

It is also known that in the aged brain there is a progressive accumulation of neurotoxic compounds.\(^31\)–\(^54\)

This neurochemical change in the aged, cognitively impaired cerebral cortex could alter NMDARs and their corresponding neurotransmission. Furthermore, abnormal dendritic spine morphology and subsequent impaired function has been implicated in many neurological
disorders associated with cognitive impairments, such as fragile X syndrome, AD, and Down syndrome.55

**NMDA receptors and age-related cognitive decline**

Formerly, age- and AD-related cognitive impairments had been thought to occur due to a loss or decline of cholinergic neurons and functional integrity of the forebrain cholinergic systems.56,57 However, other neurotransmitter systems, which may contribute to the wide and complex range of cognitive deficits observed in the pathology of brain aging and various neurodegenerative diseases, have not been as extensively studied.58–60

In addition to the cholinergic system, the most consistent modification in the aging brain is a loss of glutamate receptors.51,62 Among the various excitatory glutamate receptors, NMDARs appear to be preferentially altered in the cerebral cortex during the aging process.63 A reduction of NMDAR density in the hippocampus and cortex of aging monkeys and rodents has been reported.62–65 Also, expression of various NMDAR subunits undergoes significant age-related alterations, which may ultimately affect the composition of NMDAR complexes and lead to changes in the binding properties, kinetics and physiological properties of NMDARs during brain aging.66–69 Thus, of the various age-related modifications, changes in postsynaptic NMDAR sites may be a major contributor to the behaviorally observed cognitive impairments associated with the aging process.

**NMDA receptors and pathogenesis of AD**

There are several potential links between excitotoxic damage and the primary insults of AD, which, based on rare familial forms of the disease, are believed to involve toxicity from misfolded mutant proteins.70,71 These proteins include soluble oligomers of β-amyloid peptide (Aβ) and hyperphosphorylated tau proteins.71 For example, oxidative stress and increased intracellular Ca2+ generated by Aβ have been reported to enhance glutamate-mediated neurotoxicity in vitro. Additional experiments suggest that Aβ can increase NMDA responses and thus excitotoxicity.72–74 Another potential link comes from recent evidence that glutamate transporters are down-regulated in AD and that Aβ can inhibit glutamate reuptake or even enhance its release.75,76 Moreover, excessive NMDAR activity has been reported to increase hyperphosphorylation of tau, which contributes to neurofibrillary tangles and is involved in NMDA-mediated neurotoxicity.77 The NMDAR antagonist, memantine, has been found to offer protection from these neurotoxic processes, as discussed below.

**NMDA receptors and other neurodegenerative diseases**

NMDARs play a key role in a variety of physiological processes. Either disruption in NMDAR activity or NMDAR overactivation has been implicated in the pathophysiology of a number of neurodegenerative disorders apart from AD. However, the role of NMDARs and the exact pathophysiological pathways involved in other neurodegenerative diseases, such as HD, remain to be determined.

HD is caused by degeneration of striatal medium spiny neurons. Striatal medium spiny neurons consist of a plethora of predominantly NR2B-containing NMDARs.78 Experimental evidence implicates NMDAR-mediated excitotoxicity as the underlying mechanism of neuronal loss and degeneration in HD (see19,40,78–86 for details of experimental evidence); whereby an up-regulation of NR2B subunits occurs in HD.30 It has been demonstrated that NMDAR-mediated excitotoxicity of striatal neurons in transgenic mice could be ameliorated through specific antagonism of NR2B receptor subunits alone;81,82 however, a consensus regarding the primary mechanism of neuronal loss in HD is still lacking.83–85 Importantly, a NMDAR open-channel blocker, memantine has demonstrated moderate success with respect to the amelioration and delay in progression of HD in clinical trials.86

PD occurs as a result of degenerating nigral dopaminergic neurons and depletion of nigrostriatal dopamine. The dopaminergic deficit could lead to relative NMDAR overactivity and an increase of glutamatergic projections to the striatum and basal ganglia, resulting in further progressive neurodegeneration and clinical symptoms associated with PD.87,88 As such, many studies have demonstrated that NMDAR antagonists could protect nigral neurons from excitotoxicity, ameliorate symptoms and slow disease progression of PD.87–91 Among various NMDAR antagonists, low affinity NMDAR antagonists, amantadine and memantine have demonstrated efficacy and tolerability towards alleviating PD-related symptoms and dementia.89,90 NR2B-subunit specific antagonist, CP-101,606 have also demonstrated efficacy in reducing PD-related symptoms with mild undesirable cognitive effects.87,91

Other than neurodegenerative diseases, a disruption or overactivation in NMDAR activity is also implicated in a number of neurological disorders, such as epilepsy, major
depression, chronic pain, ischemic and traumatic brain injury. We refer readers to several excellent review articles for an overview of NMDAR-mediated neurotoxicity in these diseases.\textsuperscript{3,7,87,88}

**Drug design of NMDA receptor antagonists**

Excitotoxicity from overactivation of NMDARs has been implicated in a large number of acute or chronic neurological disorders; consequently, devising therapeutic strategies towards neuroprotection through combating excitotoxicity has drawn intense research interest.\textsuperscript{3,7,36,40} However, the major concern for potential therapeutic intervention at NMDARs is the fact that these receptors are involved in both normal neurotransmission and, if excessively activated, excitotoxic pathways. For clinical purposes, neuroprotective agents must block overactivation of NMDARs while preserving normal neurotransmission to avoid adverse effects due to generalized inhibition of normal NMDAR activity. Drugs that act as competitive antagonists at either glutamate or glycine agonist binding sites, block normal neurotransmission mediated by low levels of NMDAR activation more than overactivated NMDARs due to their competitive nature, thus depicting undesirable side effects or an unfavorable risk-benefit ratio for applications in clinical therapies.\textsuperscript{36,92} Furthermore, under excitotoxic conditions where high levels of glutamate exist, competitive antagonists at agonist-binding sites are likely displaced from NMDARs by competing levels of glutamate and will be less effective in preventing excitotoxicity. As such, lack of clinical benefit of these types of NMDAR antagonist in ameliorating stroke has been demonstrated.\textsuperscript{36,92}

Nonetheless, partial agonists acting at the NMDAR glycine (agonist) site have been suggested with therapeutic potential as cognitive enhancers to combat age-related cognitive decline due to NMDAR loss.\textsuperscript{93,94} The long-term effects and clinical utility of these NMDAR-dependent cognitive enhancers remain to be investigated.\textsuperscript{95} Allosteric modulators and uncompetitive open-channel blockers of NMDAR appear to have better clinical safety profiles and therapeutic potentials in combating neurodegenerative diseases.

**Uncompetitive antagonism and open-channel block**

When a drug is considered an open-channel blocker of NMDARs, it only indicates that this drug enters and blocks the ion channel when NMDARs are activated by dual agonists and in an “open-channel” conformation. However, it is the mode of “unbinding” from the NMDAR channel pore of open-channel blockers that determines their mechanisms of action (Figure 4). If an open-channel blocker can leave the channel pore in a closed or trapped conformation regardless of agonists binding, it would behave as a non-competitive antagonist, and would not distinguish low from high levels of NMDAR activation (Figure 4A).\textsuperscript{96} On the other hand, an open-channel blocker that could only leave NMDAR channel pore after it re-opens by agonists would act as an “uncompetitive” antagonist (Figure 4B).\textsuperscript{96} Such “uncompetitive” antagonism, in theory, leads to a higher degree of channel blockade in the presence of excessive activation of NMDARs and little blockade at relatively lower levels of NMDAR activation (Figure 4C and see below). Pure uncompetitive antagonism through open-channel block is, therefore, an ideal approach for therapeutic purposes during excessive NMDAR activation, as a greater number of channels will be in the open-channel state and available for blocking while normal neurotransmission with lower levels of NMDAR activation would be relatively spared.\textsuperscript{96–98} Based upon these premises, an open-channel blocker would protect against more severe excitotoxic conditions as opposed to less severe conditions. Therefore, moderate-to-severe dementia that is associated with overactivation of NMDARs and subsequent cell injury and death, theoretically, would be more effectively treated by uncompetitive open-channel blockers relative to milder conditions.

**NR2 subunit-selective antagonism and extrasynaptic NMDA receptor blockade**

A new therapeutic strategy using NR2B-selective antagonists to target excitotoxicity has recently emerged as extrasynaptic NR2B-containing NMDARs appear to be associated with glutamate-mediated excitotoxicity more than other NMDAR subtypes. Most NR2B-selective antagonists act as allosteric modulators on the ATD domain of NR2B subunits, displaying a higher affinity towards activated and desensitized conformations of NMDARs with a noncompetitive mode of action.\textsuperscript{99} However, NR2B-selective antagonists could display phencyclidine (PCP)-like behavioral effects and abuse potentials, as well as block the human \textit{ether-à-go-go} (hERG) K$^+$ channel, which could lead to long QT-related lethal arrhythmias. Further development and clinical trials of these NR2B-selective compounds are needed to explore their therapeutic efficacy and potential adverse side effects in treating neurological diseases. Another potential strategy is targeting extrasynaptic NMDARs, since synaptic activation of NMDARs supports normal neurotransmission and neuronal survival while in pathological conditions, abnormal
excitation of extrasynaptic NMDARs is frequently linked to excitotoxicity. However, further study is required to support the extrasynaptic targeting approach as extrasynaptic glutamate receptors are also rich in NR2B-containing NMDARs.

**Memantine as an NMDAR uncompetitive open-channel blocker**

To date, few NMDAR-targeted pharmacological agents have succeeded with adequate efficacy and acceptable side effects in randomized clinical trials for the treatment of neurological disorders. Memantine (MEM) and its analogues are the most well-tolerated and efficacious agents in this regard. MEM (1-amino-3,5-dimethyl-adamantane, Figure 4D) is a derivative of amantadine, an anti-influenza agent and has been used clinically with an excellent safety record for over 20 years in Europe to treat PD, spasticity, and AD. At clinically relevant concentrations (1–12 μM), memantine acts as an open-channel, uncompetitive blocker of the NMDAR-coupled channel pore. We first reported that the extent to which a fixed, low-micromolar concentration of memantine blocked NMDA activity actually increased as the NMDA concentrations increased during pathological situations (Figure 4C). As a result of this uncompetitive antagonism during therapeutic treatment, memantine exerts stronger blocking effects under pathological conditions due to excessive or prolonged glutamate-mediated excitotoxicity; while normal synaptic transmission, LTP and physiological responses to behavioral tests such as the Morris water maze are preserved. Memantine is currently the only nonacetyl cholinesterase inhibitor that is therapeutically approved for the treatment of patients with moderate to severe AD. The clinical tolerability and therapeutic potential of memantine is supported by safety and efficacy profiles in recent clinical trials for AD treatment.

Additionally, at <12 μM, memantine is relatively specific for NMDA-antagonistic action with a 50% inhibition constant (IC50) of ~1 μM at ~60 mV, but is far below the effective level of memantine at most other receptor or ligand-gated channels (but see). The therapeutic benefits of memantine in PD, and possibly cerebral ischemia, dementia, and epilepsy are, therefore, thought to occur via its antagonistic action on NMDARs.

Neuroprotective agents that work by high-affinity binding to the NMDAR result in inhibition of the majority of all receptor activity, thereby yielding intolerable clinical side effects. Thus, open channel blockers with uncompetitive antagonism appear to be the best available strategy to combat excitotoxicity under pathological conditions while sparing physiological neurotransmission.

**Other possible advantages of memantine action for its efficacy and safety profile**

One of most intriguing characteristics of memantine is its clinical safety profile accompanying its therapeutic efficacy when compared to other open channel blockers. A number of factors have been suggested to explain memantine’s clinical tolerability and efficacy, including moderate-to-low affinity, moderate voltage dependence, fast blocking and unblocking kinetics, and partial trapping in the NMDAR-associated channel. The proposed explanations are based on the assumption that memantine and other open-channel blockers bind at the same site as extracellular Mg in the channel selectivity filter. We showed that this assumption is incorrect. Mg, a physiological open-channel blocker for NMDARs interacts differently on the N-site residues of NR1 and NR2 subunits when applied from the intracellular versus extracellular surface. We reported that the specific memantine blocking site is the intracellular Mg blocking site, which is located at the N-site asparagine of the NR1 subunit and is slightly deeper than the extracellular Mg blocking site. The N and N + 1 sites of NR2A subunits are the extracellular Mg blocking site and provide the major electrostatic interaction with memantine upon binding to this deep, specific site (Figure 5). The distinct patterns of interaction of memantine with the channel selectivity filter may confer upon memantine unique kinetic features leading to the drug’s excellent clinical tolerability. In accordance with these observations, in the absence of extracellular Mg, memantine displays minimal differences in blocking NMDARs containing various NR2 subunits.

Furthermore, several studies have also reported a second binding site for memantine in NMDA-gated channels. We recently demonstrated that the second, superficial site of memantine action is nonspecific and may explain the noncompetitive (or nontrapping) component of memantine at near millimolar concentrations (Figure 4C). Occupancy by memantine of this shallow site would allow dissociation of the drug in either the open or closed conformation, resulting in a form of noncompetitive antagonism that would not confer advantages in clinical safety. Lipophilic leak of memantine from its blocking site cannot explain this noncompetitive component. Importantly, what renders
memantine different from other so-called “low-affinity” NMDA open channel blockers is that the affinities of the two sites of memantine blockade are sufficiently distinct so that the pharmacological properties of memantine specific sites may account for its lack of side effects.\textsuperscript{15} In our hands, memantine, at therapeutic concentrations, displays minimal closed-channel block or egress (minimal lipophilic leak), and therefore behaves as a perfect uncompetitive blocker (Figure 5, see\textsuperscript{96} for details). Furthermore, 6 µM memantine blocked 80% to 85% of extrasynaptic NMDAR-gated current (Figure 4E),\textsuperscript{6,15} but only 35% to 40% of NMDAR-mediated excitatory postsynaptic currents (EPSCs),\textsuperscript{97} indicating memantine exerts a preferential blockade of extrasynaptic NMDARs that are activated only during pathological insults. These additional blocking properties of memantine and its uncompetitive antagonism most likely account for its clinical tolerability and efficacy at low micromolar concentrations.

**Efficacy of memantine in animal models of neurological disorders**

The neuroprotective potential of memantine has been demonstrated in a large number of in vitro and in vivo animal
models by many laboratories (reviewed in\textsuperscript{108}). For example, memantine has been shown to protect cerebrocortical, cerebellar and retinal neurons from NMDA-mediated neurotoxicity.\textsuperscript{6,97,121} In a rat stroke model, memantine reduced hypoxic-ischemic brain injuries by 30% to 50% when it was provided up to 2 hours following the ischemic event.\textsuperscript{6,97} Also, memantine treatment has been shown to decrease the loss of cholinergic neurons induced by NMDA-mediated toxicity or mitochondrial toxins in rat models.\textsuperscript{70} Furthermore, chronic infusion of memantine attenuated neuronal loss, improved short-term memory impairment, reduced learning deficits and neurotoxicity caused by quinolinic acid-induced cortical lesions in rat models.\textsuperscript{108}

In terms of AD treatment, memantine was found to reduce neurotoxicity by preventing hippocampal neuronal loss and apoptosis instigated by intrahippocampal injection of Aβ,\textsuperscript{122} as well as by enhancing the processing of nonamyloidogenic β-amyloid precursor proteins.\textsuperscript{70} Although exact mechanisms by which memantine offers neuroprotection in in vivo or in vitro models of AD may be complex and remain to be determined, a mechanism associated with its NMDAR antagonism is favored. Nonetheless, memantine treatment significantly protected cultured rat cortical neurons against Aβ-induced toxicity by attenuating activation of caspase-3, hyper-phosphorylation of tau proteins and its associated signaling mechanisms.\textsuperscript{123} Memantine also improved performance on spatial behavioral tests in a transgenic mouse

Figure 5 An atomic model illustrating two memantine (MEM) binding sites in the channel permeation pathway of the NMDAR. Locations of memantine binding sites in the channel permeation pathway are shown at the level of the channel selectivity filter (the specific and uncompetitive site) and at the L651 residue of the NR1 subunit (the nonspecific and noncompetitive site). Permeant ions, Na\textsuperscript{+}, Ca\textsuperscript{2+} and K\textsuperscript{+} as well as nonpermeant blocker, Mg\textsuperscript{2+}, can compete or interact with exogenously applied MEM for binding, and MEM binding interacts with the intracellular Mg\textsuperscript{2+} blocking site.\textsuperscript{15,96} Reproduced with Permission from Chen HSV; Lipton SA. Pharmacological implications of two distinct mechanisms of interaction of memantine with N-methyl-D-aspartate-gated channels. J Pharmacol Exp Ther. 2005;314:961–971.\textsuperscript{15} Copyright © 2005 American Society for Pharmacology and Experimental Therapeutics.
model of familial AD consisting of a mutant form of amyloid precursor protein and presenilin 1.\textsuperscript{124}

Although exact mechanisms by which memantine offers neuroprotection and cognitive improvement in animal or culture models of AD remain to be determined and could be complex, a mechanism related to its NMDAR antagonism is favored. Several hypothetical and beneficial outcomes following memantine treatment have been suggested, including the resumption of optimal “signal to noise” ratio in synaptic activities,\textsuperscript{111} re-establishing the balance between inhibitory and excitatory neural networks and changing the balance of synaptic and extrasynaptic NMDAR activation for neuronal survival.\textsuperscript{125}

**Therapeutic potentials of memantine in human clinical trials**

A number of human clinical trials are completed or in progress to investigate the efficacy of memantine for the treatment of neuropathologies related to AD, vascular dementia, HD, PD, traumatic brain injury, ataxic NMDA channel and related therapeutics

125

Although the results of memantine trials are quite promising, it is imperative to continue exploring pharmacotherapies targeted towards various modulatory sites on NMDARs. Further investigation in this area may lead to future opportunities towards developing NMDAR

**Conclusions and future perspective**

Glutamate receptor-mediated excitotoxicity is implicated in the pathogenesis of several neurological diseases and may be a common final pathway shared by many neurodegenerative disorders. This type of excitotoxicity is caused, at least in part, by excessive activation of NMDARs. However, NMDAR activity is also required for physiological neurotransmission. Many drugs that showed promise as inhibitors of excitotoxicity also blocked normal neuronal function and consequently depicted unacceptable side effects in clinical trials.\textsuperscript{7,36,111} In contrast, clinical trials in AD have demonstrated excellent safety profiles of memantine with minimal adverse side effects. We and others have shown that memantine is a relatively low-affinity open-channel blocker of NMDARs with uncompetitive antagonism at therapeutic concentrations. Memantine also exerts more blocking activity on extrasynaptic NMDARs, binds at the “intracellular” Mg\textsuperscript{2+} site in the channel pore and displays differential affinity for specific and nonspecific binding sites on the NMDAR. Due to its uncompetitive antagonism and unique interaction with permeant ions and Mg\textsuperscript{2+} in the channel pore, memantine prevents neurotoxicity from excessive NMDAR activation, yet spares low (physiological) levels of synaptic NMDAR activation during normal neurotransmission. These molecular interactions confer upon memantine favorable biophysical and pharmacological properties that contribute to the drug’s clinical tolerability as well as its neuroprotective profile.\textsuperscript{7} Results from clinical studies have supported our hypothesis that low-affinity/uncompetitive memantine is a NMDAR-based therapeutic agent which exhibits promising efficacy in treating moderate-to-severe AD with an excellent safety profile.

Although the results of memantine trials are quite promising, it is imperative to continue exploring pharmacotherapies targeted towards various modulatory sites on NMDARs. Further investigation in this area may lead to future opportunities towards developing NMDAR.

...
subtype-specific modifying agents or extrasynaptic NMDAR blocking drugs that might inhibit excitotoxicity even more effectively and safely than memantine alone.

Acknowledgments
We thank Dr Rongsheng Jin for the NMDAR modeling shown in Figure 1C. This work was supported by an Early Career Development Award from American College of Cardiology Foundation, Bechtel Trusts and Foundation Grant, and California Institute of Regenerative Medicine (CIRM) Grants (RS1-00171-1 and RTI-01143) to H-S.V.C. Due to quickly expanding literature in basic research and clinical trials of therapies against NMDAR-related neurotoxicity, we could not cover every paper published and we express our sincere regret if we missed any important papers or research works.

Disclosures
The authors declare no conflicts of interest.

References
31. Li R, Huang FS, Abbas AK, Wigstrom H. Role of NMDA receptor subtypes in different forms of NMDA-dependent synaptic plasticity. BMC Neurosci. 2007;8:55.


110. Rogawski MA. Low affinity channel blocking (uncompetitive) NMDA receptor antagonists as therapeutic agents – toward an understanding of their favorable tolerability. Amino Acids. 2000;19:133–149.

111. Parsons CG, Stöffer A, Danyasz W. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system – too little activation is bad, too much is even worse. Neuropharmacology. 2007;53:699–723.

112. Wollmuth L, Kuner T, Sakmann B. Adjacent asparagines in the NR2-subunit of the NMDA receptor channel control the voltage-dependent block by extracellular Mg2+. J Physiol. 1998;506:13–32.

113. Wollmuth LP, Kuner T, Sakmann B. Intracellular Mg2+ interacts with structural determinants of the narrow constriction contributed by the NR1-subunit in the NMDA receptor channel. J Physiol. 1998;506:33–52.


