

## Structure, Function, and Pharmacology of NMDA Receptor Channels

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### Summary

NMDA receptors have received much attention over the last few decades, due to their role in many types of neural plasticity on the one hand, and their involvement in excitotoxicity on the other hand. There is great interest in developing clinically relevant NMDA receptor antagonists that would block excitotoxic NMDA receptor activation, without interfering with NMDA receptor function needed for normal synaptic transmission and plasticity. This review summarizes current understanding of the structure of NMDA receptors and the mechanisms of NMDA receptor activation and modulation, with special attention given to data describing the properties of various types of NMDA receptor inhibition. Our recent analyses point to certain neurosteroids as NMDA receptor inhibitors with desirable properties. Specifically, these compounds show use-dependent but voltage-independent block, that is predicted to preferentially target excessive tonic NMDA receptor activation. Importantly, neurosteroids are also characterized by use-independent unblock, compatible with minimal disruption of normal synaptic transmission. Thus, neurosteroids are a promising class of NMDA receptor modulators that may lead to the development of neuroprotective drugs with optimal therapeutic profiles.

### Key words

Glutamate receptor • NMDA receptor • Ion channel • Channel blocker • Pharmacology • Synaptic transmission

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### Introduction

Synaptic transmission is central to the ability of the nervous system to process and store information. Synapses are specialized contacts between neurons, where the release of neurotransmitter by the presynaptic neuron activates neurotransmitter receptors on the membrane of the postsynaptic neuron. Excitatory synaptic transmission in the mammalian brain is mediated primarily by the amino acid glutamate, activating two different groups of glutamate receptors: ionotropic and metabotropic. Ionotropic glutamate receptors are ligand-gated ion channels further divided with respect to their pharmacological properties into the following sub-groups: GluA (AMPA, 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid), GluK (kainate), GluN (NMDA, N-Methyl-D-aspartic acid), and GluD ( $\delta$ ) receptors. Properties of all ionotropic glutamate receptors have been recently reviewed in detail by Traynelis *et al.* (2010).

NMDA receptors in particular have received much attention over the last few decades, due to their key role in many types of neural plasticity on the one hand, and their involvement in neuronal excitotoxicity on the other hand. An important goal of much recent research is to identify clinically relevant NMDA receptor antagonists capable of preferentially blocking excitotoxic NMDA receptor activation without interfering with NMDA receptor function needed for normal synaptic transmission and plasticity. Here we summarize current understanding of the structure of NMDA receptors and

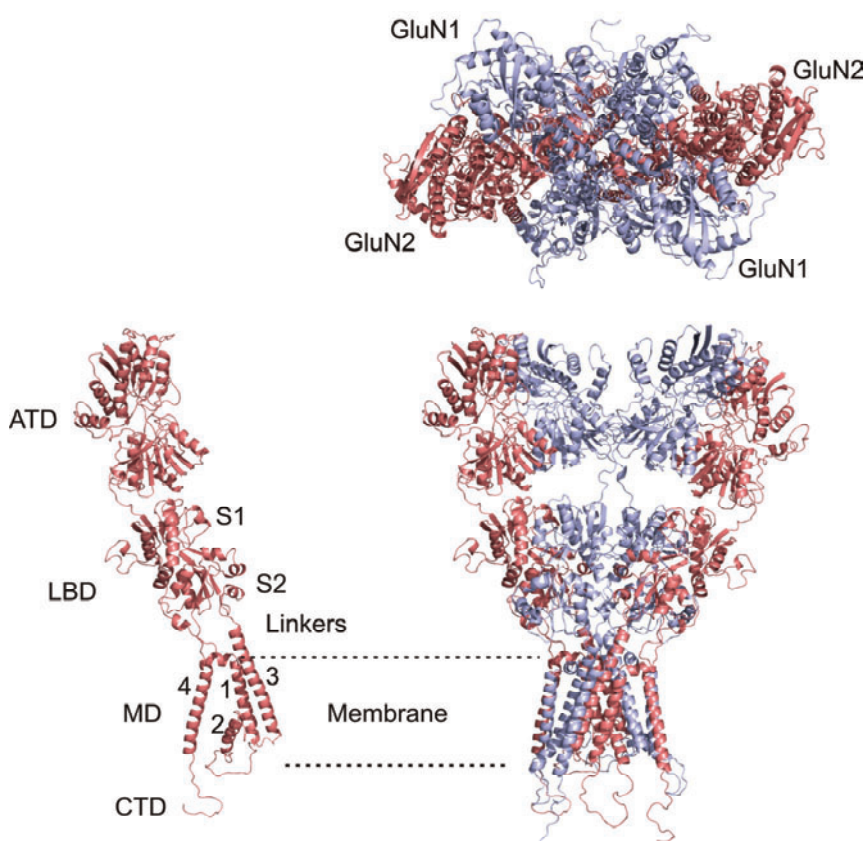
the mechanisms of NMDA receptor activation and modulation by various endogenous and exogenous agents. Special attention is given to data describing the properties of various types of NMDA receptor inhibition, aiming to characterize NMDA receptor blockers with optimal therapeutic profiles.

### Structure and gating of NMDA receptors

The family of NMDA receptors consists of three different subunits termed GluN1-3. Posttranscriptional RNA processing of GluN1 subunits gives rise up to eight different splice variants (Moriyoshi *et al.* 1991, Sugihara *et al.* 1992, Zukin and Bennett 1995). GluN2 and N3 subunits are encoded by four (GluN2A-D) and two

(GluN3A and B) genes respectively (Monyer *et al.* 1992).

Functional NMDA receptors are heterotetramers containing two obligatory GluN1 subunits in combination with two GluN2 and/or GluN3 subunits (Monyer *et al.* 1992, Ulbrich and Isacoff 2008). All subunits have a significant level of homology and are highly related in structure, with a conserved domain organization. An extracellular amino-terminal domain (ATD) is linked to an extracellular ligand binding domain (LBD), which in turn is connected to a transmembrane domain (MD) forming the ion channel. The transmembrane helices in turn communicate with an intracellular carboxy-terminal domain (CTD) (Fig. 1) – reviewed in Traynelis *et al.* (2010).



**Fig. 1. Right.** Homology model of tetrameric GluN1/GluN2 receptor. Top view illustrates currently predicted GluN1 (in gray) - GluN2 (in red) heterodimeric arrangement of subunits. **Left.** Domain organization of a single glutamate receptor subunit. Two extracellular clamshell-like domains – the amino-terminal (ATD) and the ligand binding domain (LBD) composed of two lobes (upper S1 and lower S2) – are connected to the ion channel formed by four membrane helices (MD) which communicate with the intracellular carboxy-terminal domain (CTD).

Specific functional properties determining the role of the receptor in signal transduction are determined by the mechanism of gating and by the structural features that form the ion channel. The majority of crystallographic and functional data were acquired on the AMPA and kainate types of glutamate receptors (Armstrong and Gouaux 2000, Mayer 2005). These data suggest how the ligand binding domain communicates through short linkers to the ion channel-forming helices.

The current model of channel gating proposes three sequential steps: a) ligand binding into its pocket, b) conformational changes resulting in the closing of the clamshell-like LBD, c) the propagation of these changes leading to channel opening (Mayer and Armstrong 2004, Mayer 2006). These gating principles have been corroborated for NMDA receptors, where the simultaneous presence of two different ligands is required for the activation of a typical GluN1/GluN2 receptor

(Furukawa *et al.* 2005). The LBD is composed of two domains. The upper domain S1 forms a tight interface in contact with the second upper domain in a dimer of two LBDs. This arrangement keeps both S1 domains fairly rigid. In contrast, the bottom domains S2 are without a visible dimerization contact and are relatively mobile (Sobolevsky *et al.* 2009). This mobility feature became apparent in the comparison of AMPA LBD dimer crystal structures binding different full and partial agonists or antagonists, in which the binding pocket closure induced movement of the bottom domains whereas the interface and the position of the upper domains was constant (Madden 2002, Midgett *et al.* 2012). The ligand-induced movement of the bottom domains immediately impacts on the linker regions connected to the ion channel-forming helices. The structural determinants and flexibility of the LBD-MD linkers have a significant role in shaping the mode of gating. Their function is especially fascinating with respect to the change in the symmetry in this region where the LBD 2-fold symmetry is switched to the 4-fold symmetry of the ion channel (Sobolevsky *et al.* 2009).

In the membrane, three helices (M1, 3 and 4) are linked to the LBD. M3 and the pore forming re-entrant loop M2 are central to channel gating. M3 contains the most conserved region (SYTANLAAF) among glutamate-gated ion channels (Chiu *et al.* 1999). Many studies employing mutagenesis have described the importance of this region for channel function. Particularly clear evidence came from the lurcher mutant of glutamate receptor delta (GluD2) where a mutation of the third alanine to threonine in the SYTANLAAF motif created a constitutively active mutant (Zuo *et al.* 1997). Additional experiments based on substituted cysteines accessible during the channel opening revealed considerable rearrangement of M3 during channel gating (Sobolevsky *et al.* 2002, 2007). Despite recent progress in transmembrane protein crystallography, we still do not have accurate data to fully delineate the mode of gating. However, structural similarity between the glutamate receptor channel and the potassium channel, together with the availability of potassium channel structures in open and closed states (Jiang *et al.* 2002, Long *et al.* 2007) allow us to propose that the MD gate is open through the rotation and side movement of M3 away from the axis of the pore formed by the re-entrant loop M2 (Sobolevsky *et al.* 2009). The function of pre-M1, M1 and M4 helices is not well understood. Mutation cycle studies show modulating effects of these structures

on the mode of gating (Villaruel *et al.* 1998, Ogden and Traynelis 2013).

In the process of gating, the rearrangement of M3 opens access to the central cavity. The central channel cavity terminates with the ion selective pore, which determines the fundamental properties of the channel – ion permeation and unitary conductance. The tip of the M2 loop contains a critical QRN site that primarily determines the calcium permeability of the channel. AMPA and kainate receptors genetically encoding the amino acid glutamine are calcium permeable (Burnashev *et al.* 1992a, Kohler *et al.* 1993). In GluA2 and GluK1/2 subunit mRNAs, posttranscriptional AtoI RNA editing results in the glutamine residue being replaced with arginine, forming calcium impermeable ion channels (Lomeli *et al.* 1994). In contrast, this site is occupied by asparagine in all GluN subunits leading to high calcium permeability, a hallmark feature of NMDA receptors (Burnashev *et al.* 1992b).

### Activation of NMDA receptors

The activation of NMDA receptors composed of GluN1/GluN2 subunits requires two molecules of coagonist glycine (Johnson and Ascher 1987, Kleckner and Dingledine 1988) and two molecules of agonist glutamate (Watkins and Evans 1981, Patneau and Mayer 1990, Clements and Westbrook 1991). NMDA receptors composed of GluN1/GluN3 require only glycine for activation (Chatterton *et al.* 2002, Pachernegg *et al.* 2012). Since GluN1/GluN2 receptors appear to be physiologically more important, GluN1/GluN3 receptors will not be described further. GluN3 subunit-containing receptor properties are reviewed in Pachernegg *et al.* (2012).

Glycine is naturally occurring in the extracellular environment of the nervous system (cerebrospinal fluid contains  $4.2 \pm 1.6 \mu\text{M}$  of glycine; Iijima *et al.* 1978). Since the EC<sub>50</sub> for glycine acting as a coagonist at GluN1/GluN2 receptors is  $\sim 1 \mu\text{M}$ , most of its binding sites are naturally occupied. Several other molecules can activate NMDA receptors as coagonists e.g. D-serine (0.65), L-serine (77), D-alanine (0.89), L-alanine (36). Numbers in parentheses show the EC<sub>50</sub> value in  $\mu\text{M}$  for each coagonist as determined for GluN1/GluN2B NMDA receptors (Chen *et al.* 2008). Recently, D-serine has been described as the main NMDA receptor coagonist at synapses while glycine is the coagonist of extrasynaptic NMDA receptors (Papouin

*et al.* 2012).

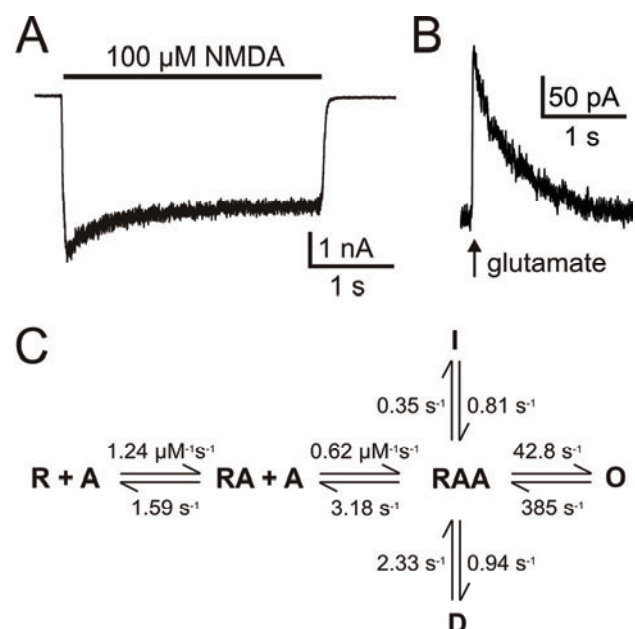
Glutamate as the major excitatory neurotransmitter in central nervous system (CNS) is released from presynaptic terminals of glutamatergic synapses and activates NMDA receptors by occupying two binding sites localized at the LBD of GluN2 subunits. NMDA receptor agonists and partial agonists include: L-glutamate (2.9), D-glutamate (160), NMDA (30), N-methyl-L-aspartate (127), D-aspartate (10), L-aspartate (14) and many others. Numbers in parentheses show the  $EC_{50}$  value in  $\mu\text{M}$  for each agonist as determined for GluN1/GluN2B NMDA receptors (Erreger *et al.* 2007).

The spatiotemporal profile of extracellular glutamate concentration determines the specific pattern of NMDA receptor activation. Synaptically released glutamate reaches a peak cleft concentration of  $\sim 1$  mM and then it is rapidly removed by diffusion and uptake, with a time constant of  $\sim 1$  ms (reviewed by Bergles *et al.* 1999). This brief glutamate concentration transient results in phasic activation of NMDA receptors, contributing to normal synaptic transmission. The highly efficient glutamate uptake system normally maintains baseline extracellular glutamate concentration in the low nM range (Herman and Jahr 2007, Herman *et al.* 2011), preventing substantial NMDA receptor activation at rest. Importantly, many acute and chronic neurological diseases are associated with dysregulated glutamate release/uptake (Mattson 2003, Nyitrai *et al.* 2006), resulting in prolonged elevation of extracellular glutamate concentration, likely reaching  $\mu\text{M}$  values (Phillis and O'Regan 2003, Heinrich *et al.* 2012). Such high levels of ambient glutamate cause excessive tonic activation of receptors, inducing excitotoxicity.

Upon coagonist and agonist binding, ion channel opens selectively for cations (especially  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$ ). The extracellular concentration of  $\text{Na}^+$  is typically 145-150 mM while that of  $\text{Ca}^{2+}$  is 1-1.5 mM (Vander *et al.* 2001). Since relative permeability of ion channel for  $\text{Ca}^{2+}$  is 10 times higher compared to  $\text{Na}^+$  (Mayer and Westbrook 1987), significant amount of calcium gets into neurons. Calcium is a key intracellular signaling molecule involved in many types of neural plasticity (Malenka and Nicoll 1999), but it is also a key mediator of excitotoxic cell death if present in excess (Mattson 2003).

Figure 2 panels A and B illustrate tonic and phasic NMDA receptor activation, respectively. Figure 2C presents a basic kinetic scheme of NMDA

receptor activation, showing the receptor (R) binding the agonist (A), as well as receptor opening (O), desensitization (D), and calcium-dependent desensitization (I). The entry to the I state is induced by  $\text{Ca}^{2+}$  ions entering neurons through the activated NMDA receptors. As a result, the extent of this calcium-dependent desensitization depends on the extracellular calcium concentration (Clark *et al.* 1990, Vyklicky 1993). NMDA receptor responses may also exhibit glycine-dependent desensitization, caused by a lowered affinity of glycine for its binding site when agonist molecules are bound to the receptor (Mayer *et al.* 1989, Benveniste *et al.* 1990). Saturating concentrations of glycine eliminate this effect.



**Fig. 2.** Figure 2A illustrates tonic NMDA receptor activation by NMDA applied for 3 s. After a fast onset, this response exhibits desensitization which typically has a double-exponential time course. Deactivation occurs after NMDA is washed off and its time course also has a fast and a slow component. Figure 2B illustrates phasic NMDA receptor activation by synaptically released glutamate. Following a rapid onset, the NMDA receptor response deactivates with double-exponential kinetics (Korinek *et al.* 2010). Figure 2C presents a basic kinetic scheme of NMDA receptor activation (Lester and Jahr 1992). The rate constants shown were determined for HEK cells expressing recombinant GluN1-1a/GluN2B receptors activated by glutamate at 25 °C with 0.5 mM extracellular calcium (Cais *et al.* 2008). Their values are different for other agonists, temperatures,  $\text{Ca}^{2+}$  concentrations and subunit compositions of NMDA receptors.

Even though the kinetic scheme in Figure 2C is sufficient to describe whole-cell NMDA receptor responses (to either phasic or tonic activation), a more complicated situation occurs when single channel

openings in excised patches are to be described. Millions of ion channel openings are typically analyzed for open time and close time histograms. These analyses have not yet produced a unanimous kinetic scheme of single channel gating. They revealed that ion channel opening does not occur directly from the RAA state but rather there is a fine structure of 3 to 4 closed states and one or two open states behind the RAA state (Banke and Traynelis 2003, Popescu and Auerbach 2003, Erreger *et al.* 2005, Dravid *et al.* 2008, Amico-Ruvio and Popescu 2010).

### **NMDA receptor pharmacology**

NMDA receptor activity can be modulated both positively and negatively. Positive modulators increase the maximal response or the affinity for the agonist, but have a binding site that is different from the agonist binding site. Some endogenous compounds such as polyamines can potentiate the activity of GluN2B-containing receptors (Williams *et al.* 1990) by increasing the glycine sensitivity of the receptor (Traynelis *et al.* 1995). It is assumed that polyamines bind at the interface between ATDs of GluN1 and GluN2B subunits (Mony *et al.* 2011). Furthermore, certain neurosteroids may positively modulate the function of NMDA receptors. For example, pregnenolone sulfate (PS) potentiates GluN2A or GluN2B-containing receptors (Wu *et al.* 1991, Malayev *et al.* 2002, Horak *et al.* 2006) by increasing the open channel probability (Horak *et al.* 2004), with the potentiating ability of PS dependent on receptor phosphorylation (Petrovic *et al.* 2009). Recently, Mullasseril *et al.* (2010) identified a highly selective allosteric potentiator (3-chlorophenyl)(6,7-dimethoxy-1-((4-methoxyphenoxy)methyl)3,4-dihydroisoquinolin-2(1H)-yl)methanone (CIQ), that enhances the activity of GluN2C or GluN2D subunit-containing recombinant receptors by increasing channel opening frequency.

Antagonists may be sorted according to their place of action: at the agonist binding site (competitive), within the ion channel pore (channel blockers), or at specific modulation sites (noncompetitive). Competitive antagonists compete with the agonist for the binding site, but do not activate the receptor. One of the first described competitive antagonists at the glutamate binding site was D-2-amino-5-phosphonovalerate (D-APV), which is highly selective for NMDA receptors over AMPA and kainate receptors (Watkins and Evans

1981). However, D-APV displayed a low selectivity (less than 10-fold) for different GluN2 subunits (Feng *et al.* 2005). Although some antagonists with a bulky hydrophobic substituent, such as (2R\*,3S\*)-1-(phenanthrene-3-carbonyl) piperazine-2,3-dicarboxylic acid (UBP141) or (2R\*,3S\*)-1-(9-bromophenanthrene-3-carbonyl)piperazine-2,3-dicarboxylic acid (UBP145) show an improved selectivity for GluN2D subunit (Morley *et al.* 2005, Costa *et al.* 2009), in general, subunit selectivity for competitive antagonists is difficult to achieve due to the high homology among the LBDs of the different GluN2 subunits (Kinarsky *et al.* 2005).

Some dissociative anesthetics (e.g. phencyclidine or ketamine), as well as a widely-used inhibitor dizocilpine maleate (MK-801), act as potent channel blockers (Wong *et al.* 1986, Anis *et al.* 1990, Lodge and Johnson 1990). Blockade of the ion channel is voltage- and use-dependent, which means that the block of the channel requires previous receptor activation. Because of this, the inhibition by channel blockers is slow in onset and increases with the probability of channel opening. A low-affinity channel blocker memantine (Kalia *et al.* 2008), is better tolerated clinically, probably due to its fast dissociation out of the channel upon receptor inactivation, resulting in minimal interference with normal synaptic transmission (Chen and Lipton 2005). The ion channel region is highly conserved across the different receptor subunits, which is likely the reason for the low subtype selectivity (less than 10-fold) of the various channel blockers (Dravid *et al.* 2007), but it was shown that physiological levels of  $Mg^{2+}$  decrease memantine inhibition of GluN2A or GluN2B-containing receptors nearly 20-fold, so the selectivity for NMDA receptors containing GluN2C and GluN2D subunits increases up to 10-fold (Kotermanski and Johnson 2009).

The first subunit-specific NMDA receptor antagonist discovered was ifenprodil, representing a class of noncompetitive, voltage- and use-independent inhibitors of NMDA receptors (Legendre and Westbrook 1991). Ifenprodil inhibits GluN1/GluN2B receptors with a 200- to 400-fold higher affinity than any other combination of GluN1/GluN2 subunits (Williams 1993). Crystallographic studies revealed that the binding site for ifenprodil is at the interface between ATDs of the GluN1/GluN2B heterodimers (Karakas *et al.* 2011). In recent years, novel noncompetitive antagonists with distinctive GluN2

subunit selectivity have been described. From a series of sulfonamide derivatives, 3-chloro-4-fluoro-*N*-[(4-[(2-(phenylcarbonyl)hydrazino)carbonyl]phenyl)methyl]-benzenesulfonamide (TCN-201) shows a high selectivity for GluN2A-containing receptors (Bettini *et al.* 2010). TCN-201 acts as a negative allosteric modulator of glycine binding and the binding site of TCN-201 is presumed to be located at the interface between LBDs of GluN1 and GluN2A subunits (Hansen *et al.* 2012).

A novel class of compounds selective for GluN2C or GluN2D subunit-containing receptors has recently been identified. (E)-4-(6-methoxy-2-(3-nitrostyryl)-4-oxoquinazolin-3(4H)-yl)-benzoic acid (QNZ46) inhibits NMDA receptor activity in a noncompetitive, voltage-independent manner (Mosley *et al.* 2010). However, the mechanism of QNZ46 action is unusual in that the inhibition by QNZ46 is use-dependent and requires the presence of glutamate (Hansen and Traynelis 2011). The study of chimeric GluN2A/GluN2D receptors and site-directed mutagenesis revealed that specific amino acid residues in the S2 portion of the GluN2 subunit LBD are critical for antagonist activity (Hansen and Traynelis 2011). In addition, the S2 region has been implicated in the modulation of NMDA receptors by structurally unrelated compounds such as DQP1105 (Acker *et al.* 2011), phenanthrene and carboxylated naphthalene derivatives (Costa *et al.* 2010, 2012), as well as endogenous sulfated neurosteroids (Jang *et al.* 2004, Petrovic *et al.* 2005, Horak *et al.* 2006), that can display both potentiating and inhibiting activity with various degrees of subunit selectivity. These facts indicate that the S2 region plays an important role in controlling channel gating.

Endogenous neurosteroid pregnanolone sulfate ( $3\alpha,5\beta$ S) inhibits NMDA receptor activity in a voltage-independent but a use-dependent manner by reducing the open channel probability, and it has a two times lower effect on receptors containing the GluN2A or GluN2B subunits compared to the GluN2C or GluN2D subunits (Petrovic *et al.* 2005). It has been shown that  $3\alpha,5\beta$ S acts solely from the extracellular side of the NMDA receptor (Park-Chung *et al.* 1997, Petrovic *et al.* 2005), and our recent results indicate the importance of membrane for the inhibitory effects of neurosteroids (Borovska *et al.* 2012). However, the identification of the binding site for steroids with the inhibitory action on NMDA receptors has so far failed.

## Inhibition of tonically and phasically activated NMDA receptors

As mentioned above, excessive activation of NMDA receptors leads to excitotoxic cell death and plays a crucial role in many acute and chronic neurological disorders. Extensive effort has been made in the last 20 years to develop NMDAR antagonists which could be used as neuroprotective drugs. However, most of the compounds tested so far have failed in clinical trials (Kalia *et al.* 2008). A common side effect of NMDAR antagonists is their psychomimetic effect caused by the distortion of normal synaptic transmission. How to fulfill these two demands – block the excessive NMDA receptor activation and, simultaneously, leave the normal NMDA receptor-mediated synaptic transmission intact? The answer could be hidden in the pattern of channel activation by synaptically released versus pathologically elevated glutamate. While during synaptic transmission NMDA receptors are briefly activated by a saturating  $\sim 1$  mM concentration of glutamate (phasic activation, see above), excitotoxicity is induced by prolonged activation of NMDA receptors by  $\mu$ M concentrations of glutamate that may range widely depending on the type of the pathology (tonic activation).

We have tested an endogenous neurosteroid  $3\alpha,5\beta$ S for its ability to preferentially block tonically over phasically activated recombinant GluN1-1a/GluN2B receptors and we found that the  $EC_{50}$  for tonically activated receptors is approximately 2 times lower than the  $EC_{50}$  for peak inhibition of phasically activated receptors (unpublished data). These data are comparable with the published results for memantine, a drug approved for treatment of Alzheimer's disease, which does not exert any psychomimetic side effects (Kornhuber and Weller 1997, Xia *et al.* 2010).

To determine which features of NMDA receptor antagonism are crucial for a high ratio of tonic over phasic inhibition, we used Gepasi 3.30 software to simulate inhibitory effects of some common types of NMDA receptor inhibitors for different channel activation patterns. The types of inhibition considered were: competitive inhibition, non-competitive use-independent inhibition, open-channel blockade, and neurosteroid inhibition. For the open-channel blockade, the “trapping” type means, that the channel may close and consequently allow the agonist to dissociate while

the blocker is bound inside the channel. The term “foot in the door” is used for open channel blockers which prevent channel closing and agonist dissociation while bound inside the channel. A simplified kinetic model activated by two molecules of glutamate without any desensitized states was used. The rate constants for GluN1-1a/GluN2B at 25 °C were used, based on our previous data (Cais *et al.* 2008). For the rate constants of inhibitor binding and unbinding to the receptor, we used the data for D-APV (a competitive inhibitor, Benveniste and Mayer 1991), ifenprodil (a noncompetitive use-independent inhibitor, Amico-Ruvio *et al.* 2012), memantine (an open-channel blocker, the same rate constants were used for both the “trapping” and the “foot-in-the-door” types of open channel blockade, Johnson and Kotermanski 2006) and the neurosteroid 3 $\alpha$ 5 $\beta$ S (a noncompetitive use-dependent inhibitor, Papouin *et al.* 2012).

Tonic inhibition in Figure 3 shows the degree of inhibition for various combinations of glutamate and inhibitor concentrations. The effect of glutamate concentration on the inhibitory effect varies from the competitive inhibitor which has no inhibitory effect for high glutamate concentrations, to the noncompetitive use-independent inhibitor and the open-channel blocker “trapping” where there is almost no effect of glutamate concentration on the degree of inhibition, to the open-channel blocker “foot in the door” and the neurosteroid, where the degree of inhibition is higher for higher glutamate concentrations. Phasic inhibition in Figure 3 displays the time course of simulated synaptic transmission responses during the ongoing presence of an inhibitor. The insets show the dose-response curves for synaptic transmission, calculated from the peak and from the area (charge transfer) of the synaptic current.  $\Delta$  Inhibition in Figure 3 shows the difference between the degree of tonic and phasic inhibition. Since the goal is to leave phasically activated receptors unaffected while inhibiting tonically activated receptors as much as possible, neither the competitive inhibitor nor the use-independent inhibitor is useful. The best results are obtained for both types of open channel blockers and the neurosteroid – note that these three models represent use-dependent blockers.

The tonic/phasic ratio, however, is not the only requirement for an inhibitor with an expected positive therapeutic profile. Due to the fact that the inhibitor is present in the brain throughout the treatment, it is

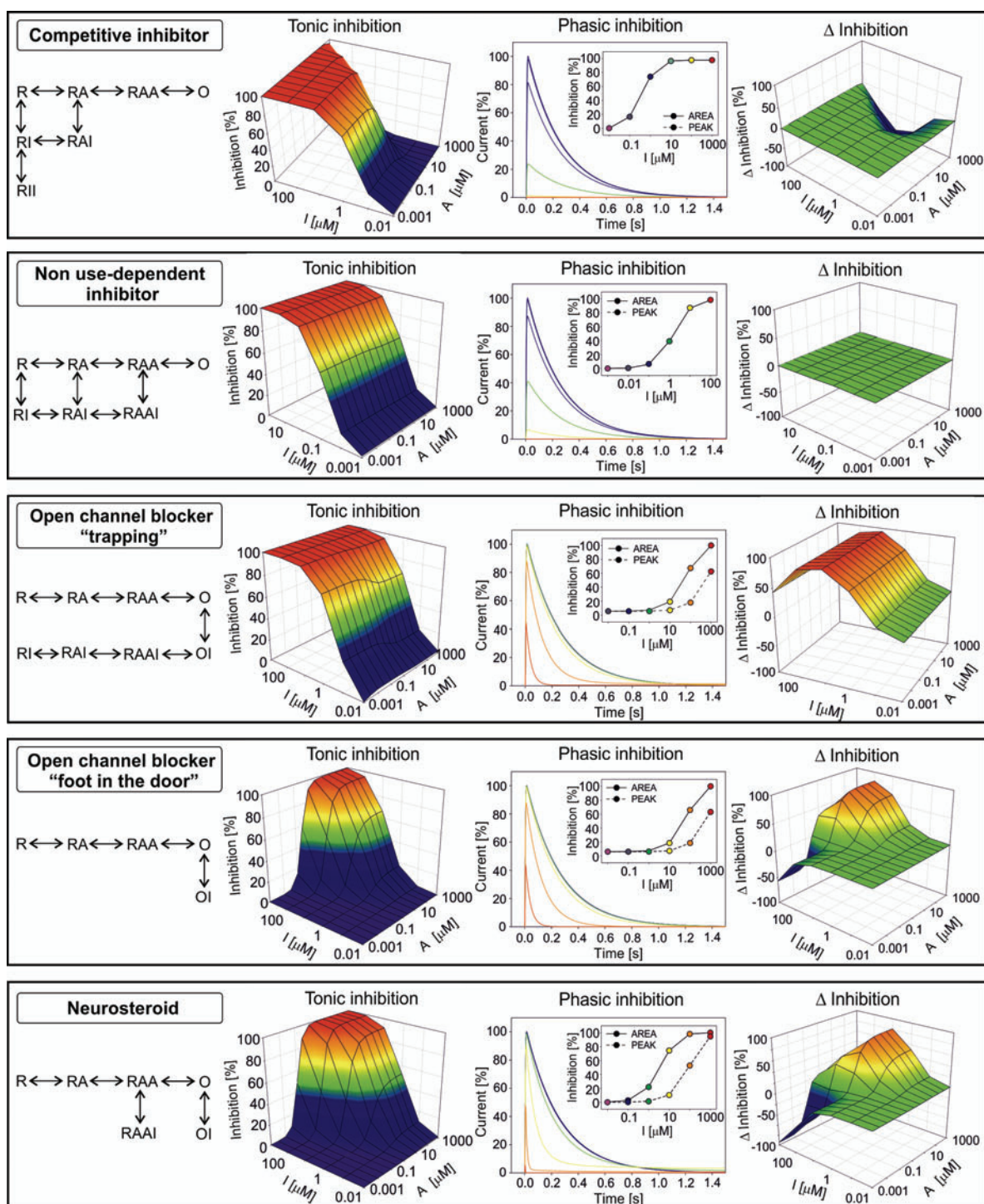
necessary to use an inhibitor which has a use-independent unblock or must be able to escape the channel during cell depolarization due to its fast unblocking rate and its voltage-dependency (Gilling *et al.* 2009). This would provide a chance to a synaptic receptor to be activated again if it is “accidentally” blocked, e.g. during a fast train of action potentials. For this reason, a “trapping” open-channel blocker is not a good choice for an ideal NMDAR blocker. The best choice appears to be a “foot in the door” open-channel blocker or a neurosteroid. But there is an additional difficulty: a typical feature of open-channel blockers is that the inhibition is voltage-dependent. This means that they lose their inhibitory effect on long-term depolarized cells – such as tonically excited cells. In contrast, neurosteroids are voltage-independent NMDAR blockers and act to the same extent at all physiological membrane potentials (Papouin *et al.* 2012).

The results of our modeling indicate, that the neurosteroid 3 $\alpha$ 5 $\beta$ S or its synthetic analogs with use-dependent but voltage-independent block and use-independent unblock are a promising class of NMDAR modulators and could lead to the prospective development of new neuroprotective drugs with desirable therapeutic profiles.

## Conclusion

Results of recent work using advanced experimental and computational techniques greatly improve our understanding of how the NMDA receptor is organized and inform the search for modulators with specific positive or negative effects. One important challenge is to identify substances capable of differential modulation of tonically vs. phasically activated receptors, reducing neurodegeneration caused by pathologically elevated extracellular glutamate, while leaving normal synaptic transmission intact.

Our recent analysis suggests that certain neurosteroids are NMDA receptor inhibitors with the desired properties, showing use-dependent but voltage-independent block and use-independent unblock, in contrast to open channel blockers that are voltage dependent. Thus, neurosteroids are a promising class of NMDA receptor modulators that may lead to the development of neuroprotective drugs with optimal therapeutic profiles.



**Fig. 3.** Simulation of inhibitory effect of some common types of NMDA receptor inhibitors. Different ion channel activation patterns are compared. For each type of inhibitor, the kinetic scheme of inhibitory action of inhibitor "I" on the receptor "R" activated by two agonists "A" (in this case glutamate) is shown first. Note that desensitization states were omitted and no membrane potential changes were applied during simulations. Next to the kinetic scheme is a plot of the degree of steady-state inhibition induced by the inhibitor acting on the receptor activated by various concentrations of the agonist ("Tonic inhibition"). The "Phasic inhibition" graph shows the kinetic profile of a simulated postsynaptic current (1 mM Glutamate for 5 ms) in the ongoing presence of various concentrations of the inhibitor. The inset shows the dose response curves for postsynaptic current inhibition calculated from the peak (maximum current) and the area (total charge transfer) of the current. The graph " $\Delta$  Inhibition" refers to the difference in the degree of inhibition between tonically and phasically activated receptors ( $\Delta$  Inhibition = Tonic inhibition - Phasic inhibition (peak)). That means that ideal inhibitor should have maximal tonic inhibition and simultaneously minimal phasic inhibition, thus we are looking for maximal  $\Delta$  Inhibition – indicated by red color. Rate constants were as follows [in  $s^{-1}$  and  $\mu M^{-1} \cdot s^{-1}$ ]: R-RA:  $k_{on}=0.616$ ,  $k_{off}=2.166$ ; RAA-O:  $k_{on}=42.77$ ,  $k_{off}=384.9$ ; competitive inhibitor,  $k_{on}=22.2$ ,  $k_{off}=19.4$ ; noncompetitive use-independent inhibitor:  $k_{on}=1400$ ,  $k_{off}=90$ ; open-channel blocker:  $k_{on}=0.5$ ,  $k_{off}=0.25$ ; neurosteroid: RAA-RAAI:  $k_{on}=0.98$ ,  $k_{off}=0.82$ , O-OI:  $k_{on}=1.6$ ,  $k_{off}=7.0$ . Except for the neurosteroid which has different binding constants to different states of the receptor, the rest of the inhibitors were taken to bind to all states in the kinetic scheme with the same rate constants.



## Conflict of Interest

There is no conflict of interest.

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