

Received: 2011.02.07  
Accepted: 2011.05.11  
Published: 2011.06.07

## Glutamate NMDA receptors in pathophysiology and pharmacotherapy of selected nervous system diseases

### Rola receptorów NMDA w patofizjologii i farmakoterapii wybranych chorób układu nerwowego

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#### Summary

Glutamate is the basic excitatory neurotransmitter acting via N-methyl-D-aspartate receptors (NMDARs). It co-regulates many important physiological functions, including learning, memory, and behaviour. An excess of glutamate, as well as NMDAR over-activity, produce pathological effects. Glutamate-related neurotoxicity is involved in the pathogenesis of many neurological conditions. This article briefly describes the role of the glutamate system in the pathophysiology of brain ischemia, selected neurodegenerative disorders, and schizophrenia. It also reviews the current and potential future status of agents targeting NMDARs in neuropsychopharmacology.

**Key words:**

glutamate • NMDA receptors (NMDARs) • excitotoxicity • NMDAR antagonists

#### Streszczenie

Glutaminian jest podstawowym neuroprzekaźnikiem pobudzającym, który działa na receptory NMDA. Związek ten jest współodpowiedzialny za regulowanie wielu ważnych fizjologicznych funkcji, wliczając w to uczenie się, pamięć i zachowanie. Nadmiar glutaminianu i nadaktywność receptorów NMDARs wywołuje patologiczne zmiany. Zjawisko neurotoksyczności zależnej od glutaminianu bierze udział w patogenezie wielu zaburzeń neurologicznych. Artykuł pokrótce opisuje rolę glutaminianu w patofizjologii udaru niedokrwienego mózgu, wybranych chorób neurodegeneracyjnych i schizofrenii oraz omawia obecne i potencjalne znaczenie leków działających na receptory glutaminergiczne w neuropsychofarmakologii.

**Słowa kluczowe:**

glutaminian • receptory NMDA • neurotoksyczność glutaminianu • antagoniści receptora NMDA

**Full-text PDF:**

<http://www.phmd.pl/fulltxt.php?ICID=946637>

**Word count:**

4925

**Tables:**

1

**Figures:**

1

**References:**

28

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**Abbreviations:** **AD** – Alzheimer’s disease; **ALS** – Amyotrophic lateral sclerosis; **AMPA** –  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor; **CJD** – Creutzfeldt-Jacob disease; **EAATs** – Excitatory aminoacids transporters; **GABA** –  $\gamma$ -aminobutyric acid; **glyT1** – glycine transporter 1; **HD** – Huntington disease; **KAR** – Kainate receptor; **LTD** – Long term depression; **LTP** – Long term potentiation; **MAPK** – Mitogen activated protein kinase; **NMDAR** – N-methyl-D-aspartate receptor; **PD** – Parkinson disease; **PSD** – Postsynaptic density; **TBI** – Traumatic brain injury.

## 1. GLUTAMATE RECEPTORS

In mammalian central nervous system synapses, glutamate is the major neurotransmitter mediating excitatory neurotransmission. It is released from presynaptic vesicles, diffuses across the synaptic cleft, and acts on both metabotropic and ionotropic glutamate receptors located in the presynaptic terminals and postsynaptic membranes of the brain and spinal neurons. Three ionotropic glutamate receptor subtypes can be distinguished, and they are named according to their agonists: NMDAR (N-methyl-D-aspartate receptor), AMPAR ( $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor) and KAR (kainate receptor). NMDARs are composed of protein complexes which form an intrinsic ion channel, permeable to monovalent cations (including  $\text{Na}^+$  and  $\text{K}^+$ ), and bivalent ones (mostly  $\text{Ca}^{2+}$ ). We also know of a metabotropic glutamate receptor coupled to a G intrinsic membrane protein [12,13].

NMDARs contain four subunits which are a combination of: NR1, NR2, and NR3, (encoded by genes GRIN1, GRIN2A-D, GRIN3A-B, respectively). There is consensus that NMDARs are tetramers composed of two NR1 subunits and two NR2 subunits, less commonly including NR3 subunits. NR1 subunits exhibit basic NMDAR features and disruption of NR1 genes abolishes NMDAR responses. This demonstrates that NR1 subunits are rather essential. There are eight different NR1 elements, generated by alternative splicing of a single gene. Four (A-D) NR2 compounds can be distinguished, located in various brain regions and playing a modulatory role in regards to NMDARs. It has been shown that the combination of NR1 with different NR2 subunits results in diverse electrophysiological and pharmacological responses. NR1 and NR2A are ubiquitous, NR2B occurs in the fore-brain, NR2C in the cerebellum, with NR2D being the rarest. There is a binding place in the channel pore for  $\text{Mg}^{2+}$ , and at resting membrane potential,  $\text{Mg}^{2+}$  is attached to this binding site, blocking ion flow through the channel [3,12,22].

Generally, NMDARs occur in many but not all cerebral cortex neurons and some cortical astrocytes. They are mostly located on dendrites. Immunocytochemical studies have revealed that NMDARs are less frequently found in the 4<sup>th</sup> layer than in layers 2 to 3 and 5 to 6, where they are preferentially expressed by pyramidal neurons. This is in agreement with the notion that afferent glutaminergic input reaches the cerebral cortex through the thalamocortical pathway, formed by axons of the 4<sup>th</sup> layer. NMDA receptors are localised in the postsynaptic membrane, organised by a multi-protein structure called the postsynaptic density (PSD). These receptors, however, are mobile and move between the synaptic and extra-synaptic pools. The PSD is defined as a type of postsynaptic membrane that contains high concentrations of glutamate receptors, ion channels, kinases, phosphatases and associated proteins [2,13].

Considering the NMDAR molecular structure, one may surmise that they have a common membrane topology with a large extracellular N-terminus, a membrane region composed of three transmembrane segments, and differ in the cytoplasmic C-terminus, depending on the various subunits, which interacts with numerous intracellular proteins [3,22].

Opening of the channel pore by NMDAR requires the simultaneous binding of the major agonist – glutamate (which play a neurotransmitter role) and the co-agonist – glycine (or D-serine, which act as modulators). The glutamate binding site is located on the NR2 subunit, whereas the glycine binding site is located on the NR1 subunit. After presynaptic glutamate release, and with sufficient glycine concentration in the synaptic cleft, NMDAR activation takes place. When the membrane is depolarised, the voltage-dependent  $\text{Mg}^{2+}$  block is removed from the channel interior, allowing for ion to enter. By contrast, AMPARs, composed of various combinations of four subunits (GluR1-GluR4) are permeable to  $\text{Ca}^{2+}$  only in the absence of the GluR2 element [3,12,13,22].

The general scheme of NMDA receptor structure together with the potential sites for pharmacological action described below presents figure 1.

NMDARs can be divided into two classes according to their conductance properties: high conductance channels (built from NR2A or NR2B) and low conductance ones (formed by NR2C and NR2D) with reduced sensitivity to  $\text{Mg}^{2+}$  block. An influx of extracellular calcium initiates complex signalling pathways compromised of the mitogen-activated protein kinase (MAPK) superfamily that transduces excitatory signals across the neuron. Taking into consideration *in vitro* substances, MAPKs have been also called microtubule associated protein-2 kinase (MAP-2 kinase), myelin basic protein kinase (MBP kinase), ribosomal S6 protein kinase (RSK-kinase) and epidermal growth factor (EGF) receptor threonine kinase (ERT kinase). MAPK activation was observed in response to NMDARs stimulation, starting with tyrosine phosphorylation of MAPKs. An additional class of kinases, named MAP kinase kinases and MAPK kinase kinases (MAPKK kinases), are then excited, through an intermediate step involving MAPK stimulation. Usually, three MAPKs are distinguished: extracellular signal-regulated kinase-1 (ERK1 and 2), the Jun N-terminal kinases (JNKs) activating Jun transcription factor, and the p38 MAPKs. The two last ones are called stress-activated kinases (SAPKs), because they are stimulated by stressful conditions. Consequently, the transcription factor cAMP- $\text{Ca}^{2+}$  response element-binding protein (CREB) causes the expression of genes that encode brain-derived neurotrophic factor (BDNF) and other factors promoting neuronal survival and activity [5,9,24,27]. Detailed information

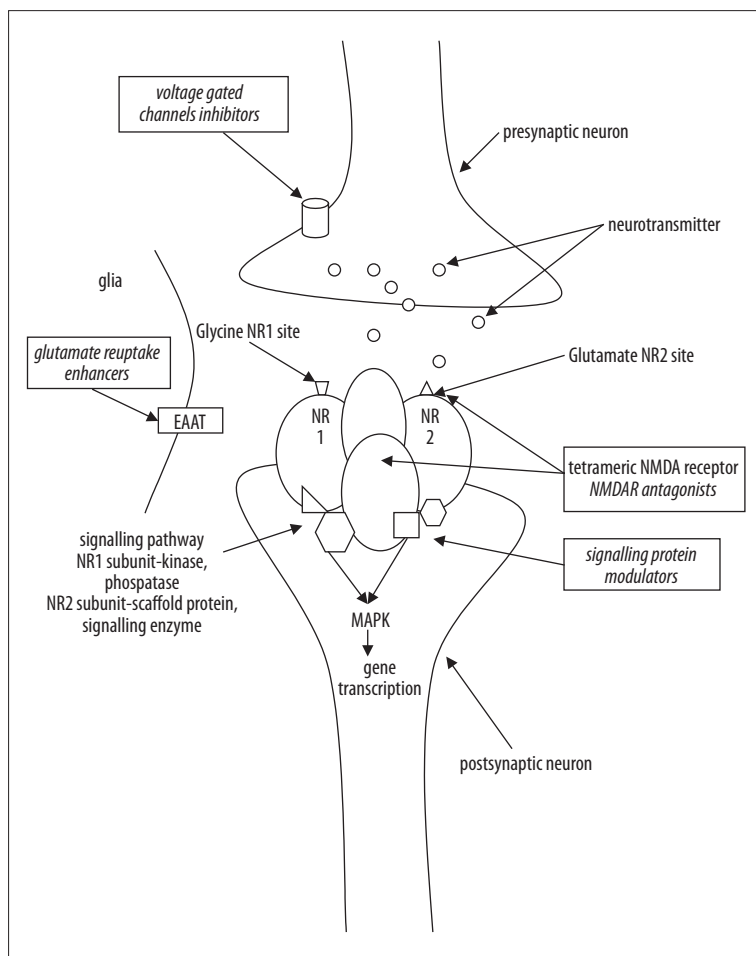


Fig. 1. NMDA receptor scheme and selected NMDAR-based pharmacological strategies for treatment [12,22]

concerning NMDAR and MAPK signalling is given in an excellent review prepared by Haddad [9].

AMPA receptors, similar to NMDARs, couple to the MAPK pathways through similar sets of signalling systems and with  $Ca^{2+}$  entrance resulting in the synthesis of CREB and other transcription factors. However, AMPARs also can activate MAPK through  $Ca^{2+}$ -independent mechanisms through the SRC-family tyrosine kinase. In summary, at the molecular level NMDARs interact with MAPKs, creating a neurochemical axis that regulates neuronal functions [6].

As a side note, it should be mentioned that the glutamate system changes together with development and growth. GABA ( $\gamma$ -aminobutyric acid), the main inhibitory neurotransmitter in the adult central nervous system, acts as an excitatory neurotransmitter in the early postnatal state, influencing its GABA-A receptors. It has been revealed that GABA-A receptor activation depolarizes neuroblasts and immature neurons in all brain regions. It is unknown why GABA operates as an excitatory agent in neonatal neurons while being an inhibitory one in later stages. Factors responsible for the shift from this excitatory to inhibitory action have not been determined so far. Moreover, glutaminergic transmission is initially purely mediated by NMDARs, without any contribution of AMPA receptors. Thus, these three receptors exhibit a sequential participation in neuronal excitation [1].

## 2. GLUTAMATE AND SYNAPTIC PLASTICITY

The glutamate system is thought to be involved in learning and memory processes. It is associated with the phenomenon of synaptic plasticity – the variable efficacy of neurotransmission which enables the brain to store memories and experiences. This phenomenon requires gene transcription and protein synthesis to stabilize synaptic changes over time. Both NMDA and AMPA receptors are suspected in regulating synaptic plasticity, however the consensus exists that AMPARs are responsible for short-term changes in synaptic strength, while NMDARs affect genes that are necessary for long-term maintenance of these changes. In short, AMPAR depolarisation of the postsynaptic membrane facilitates NMDAR activation, which in turn modulates surface AMPAR presence. Thus, postsynaptic changes develop changes in the synaptic strength which is characterised by long-term potentiation (LTP) or long-term depression (LTD) [16,25].

LTP is defined as a strengthening of synaptic transmission that is long lasting (at least more than an hour), commonly induced by brief, high-frequency stimulation. LTD is regarded as being long-lasting suppression of synaptic strength that is elicited by low frequency stimulation, typically resulting from NMDAR activation. LTP is believed to be a key molecular element involved in learning and memory, while LTD is said to be the means by which we obtain information storage and consolidation in the brain [16].

Both LTP and LTD are forms of associative plasticity based on positive feedback, counteracting both the maximum and minimum synaptic strength changes in an effort to normalise neuronal excitability. Decreased neuronal activity leads to a homeostatic increase in the strength of excitatory synapses, while increased neuronal activity has the opposite effect. This preserves the balance between both excitatory and inhibitory synapses, termed synaptic scaling. Under basal conditions, the ratio of the relative activities of NMDARs and AMPARs is about 1:1. During “pre-LTP”, the ratio between AMPARs and NMDARs is disturbed because of AMPAR postsynaptic expression. This decreases the NMDAR: AMPAR ratio (1:3) and triggers postsynaptic removal, a decrease in the number of AMPA receptors, and proportional NMDAR potentiation. The result of these processes is a LTP state. While the ratio between NMDARs and AMPARs is restored (1:1), the synapses become twice as strong, with twice as many AMPARs and NMDARs as in the “pre-LTP” state [25].

### **3. GLUTAMATE EXCITOTOXICITY IN PATHOPHYSIOLOGY OF SELECTED NEUROLOGICAL DISORDERS. THE POTENTIAL ROLE OF NMDAR ANTAGONISTS IN PHARMACOTHERAPY**

As summarised above, glutamate and glutamate receptors are engaged in cognition and behaviour control. However, an excessive amount of glutamate and over-activation of glutamate receptors leads to neuronal cell injury. Thus, the same processes which are essential and critical for normal neuronal functioning, in excess lead to excitotoxic cell death. This phenomenon is termed glutamate-related excitotoxicity and was first used by John Olney in 1970 [16,21].

The mechanisms of excitotoxicity arise from many factors. In pathological conditions, an excessive  $\text{Ca}^{2+}$  influx into the neuron promotes various processes resulting in dendritic and/or synaptic damage and cell necrosis or apoptosis. This is the consequence of  $\text{Ca}^{2+}$  mitochondrial overload, causing oxygen free radical formation, caspase activation, and intracellular protein degradation. This calcium overload also causes Ca-dependent activation of neuronal NOS which in turn causes overproduction of toxic peroxynitrite ion (ONOO<sup>-</sup>). An important element of excitotoxicity is the stimulation of mitogen-activated protein kinase p38 (MAPK p38) which activates transcription factors affecting neuronal apoptosis [13,17].

The phenomenon of excitotoxicity is a target of both neuroprotective efforts and modern pharmacotherapy since it is implicated in the pathophysiology of many acute and chronic neurological diseases mentioned below.

#### **3.1. Traumatic brain injury**

Many factors may lead to an excessive glutamate presence. One of them is mechanical insult – head or spinal cord injuries lead to a sudden, large glutamate release from injured neurons. This “fast” excitotoxicity in traumatic brain injury (TBI) has been confirmed in both animal models and human studies. It points to the neuro-protective potential of NMDAR antagonists in TBI treatment. Some experimental evidence supports this hypothesis, although several clinical programmes were terminated prematurely because of failure to find any benefits in using the same

agents as in clinical trials dealing with stroke (see below). Selfotel and dexanabolol, some of the agents studied in TBI, were found to have no impact on mortality or clinical outcomes; however the number of participants was inadequate to determine unambiguous benefits and risks. Data from other studied agents (aptiganel and eliprodil) remain inadequately reported [13,19].

#### **3.2. Ischemic stroke**

The next important set of entities manifesting in a sudden and high glutamate delivery are neuronal ischemic events. During an ischemic stroke, energy deprivation of membrane protein pumps causes many neurons to lose their ability to maintain ionic homeostasis. This causes their depolarization, lysis, and/or autodestruction, similar to that observed in traumatic injury. Energy failure causes abnormal glutamate accumulation and prolonged synapse activation, mostly because of impaired glutamate re-uptake into astrocytes [11,17,19].

Among all studied entities, the preclinical rationale for acute brain ischemia treatment with non-selective NMDAR antagonists was possibly the strongest. Nevertheless, clinical studies with these agents have failed so far. The reasons for this failure are multifactorial. First, there have been many difficulties regarding the methodology of conducted clinical trials: patient selection, problems with blinding, random allocation of treatment dose, and interactions of drugs with anaesthetic agents. Moreover, experimental studies suggest that NMDAR antagonists are most effective when given in the pre-ischemia phase or up to two hours after induction of ischemia – patients were often diagnosed and treated at a later time, beyond the short therapeutic time window. Moreover, the levels of the studied agents cannot be easily measured in the brain, and plasma concentrations determined in previous studies were consistently below values needed for maximal protection as reported in animal models. On the other hand, higher doses of NMDAR antagonists were reported to induce serious side effects: psychomimetic effects (hallucinations, agitations, peripheral sensory disturbances), a centrally mediated increase in blood pressure, nausea, vomiting and catatonia. These disadvantages could have been circumscribed if NR2B selective NMDAR antagonists – ifenprodil or traxoprodil were administered since they produce minimal side effects. However, the enthusiasm for these approaches has diminished because of their variable neuroprotective efficacy. This may be associated with reduced affinity and unbinding from inactivated NMDARs, leading to transient activation of unaffected receptors. Thus, further studies are required, particularly in order to determine the receptor mechanism. There are also efforts to introduce another NMDAR antagonist – gavestinel – in stroke pharmacotherapy [13,19].

At present, the basic treatment for stroke is still thrombolytic therapy.

#### **3.3. Neurodegenerative disorders**

A high glutamate level is also involved in the pathophysiology of many slowly progressive neuronal disorders. The concept of “slow excitotoxicity” was developed as a

pathogenetic factor explaining the gradual neuronal loss in neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), multiple sclerosis, HIV-associated dementia, and amyotrophic lateral sclerosis (ALS). In these diseases, it is suspected that long-term exposure to moderate levels of glutamate causes NMDARs hyperactivity, resulting in the apoptotic-like cell death of neurons [17,19].

For many years, NMDAR antagonists have been known to be effective in animal models of Parkinson's disease (PD) which could be explained by the pathophysiological premises of PD. The depletion of nigrostriatal dopamine produces over-activation of glutaminergic pathways to the striatum, which contributes to dyskinesia. Amantadine, a low-affinity NMDAR blocker, is used as an adjuvant therapy in PD (especially because of its additional ability to enhance dopamine release and impair dopamine re-uptake). It improves levodopa-induced dyskinesias (see further). Some preclinical studies have shown that non-selective NMDAR antagonists act in a synergistic way with levodopa and dopamine agonists. Remacemide is a sodium channel blocker and its principal metabolite – remacemide desglycine – is a low affinity uncompetitive NMDAR antagonist. The reports of some clinical trials suggest improved overall motor function, however these results were not statistically significant [10,13,19]. This drug was also tested for treatment of refractory focal seizures, showing its efficacy after 14 to 15 weeks of administration in three clinical trials including over 500 patients. Dizziness was a notable side effect. These agents also have pharmacokinetic interactions with several conventional anti-epileptic drugs. The exact mechanism of remacemide action in epilepsy is unknown, but a general agreement exists amongst researchers that NMDAR participation in the pathogenesis of epilepsy is without any doubts. A strong argument supporting this hypothesis is the efficacy of felbamate therapy [19,26].

A low-affinity uncompetitive antagonist – memantine – has also been studied as a treatment for dementias including Alzheimer's disease (AD, see the last chapter). Considering the role of NMDARs in cognition, it seems irrational that NMDAR antagonists may improve AD symptomatology [13,19]. On the other hand, there are links between excitotoxicity and AD development. Misfolded mutant proteins are thought to be implicated in the pathogenesis of this disease, especially soluble oligomers of  $\beta$ -amyloid ( $A\beta$ ) peptide and hyperphosphorylated tau proteins. Oxidative stress and intracellular  $Ca^{2+}$  influx both enhance synthesis of these proteins. Moreover,  $A\beta$  increases the NMDAR response and inhibits glutamate re-uptake. Thus, NMDAR disturbances are secondarily co-responsible for AD development, also diminishing learning and memory functioning [17]. Preclinical studies have reported that a small antagonism of NMDARs improves learning of certain tasks, although the precise mechanism underlying this observation remains unknown.

There are also reports indicating that NMDAR disturbances participate in neurodegeneration associated with Creutzfeldt-Jacob disease (CJD). The endogenous cellular prion protein PrPC protects from excitotoxicity by down-regulating the NMDAR subpopulation. CJD is characterised

by a progressive misfolding of the PrPC form into a pathological PrPSc form, that may contribute to a neuroprotective function loss, leading subsequently to excitotoxicity and neurodegeneration [12].

### 3.4. Schizophrenia

Contrary to previous findings, there is evidence indicating that potentiating NMDARs seems to be beneficial for schizophrenia treatment. Schizophrenia is a chronic psychiatric condition characterized by positive, negative and cognitive symptoms. Positive symptoms include hallucinations, delusions, disorganised speech and behaviour. Negative symptoms include flattened or restricted affect and lack of motivation. Cognitive symptoms include progressive memory and learning disturbances and symptoms associated with cortical processing [14].

The pathophysiology of schizophrenia is associated with several neurotransmitter systems. A common theory exists implicating altered dopaminergic transmission in schizophrenia development. However, a different hypothesis implicates glutamate in the pathogenesis of schizophrenia. This second hypothesis arises from studies revealing that administration of non-competitive NMDAR antagonists (such as phencyclidine or ketamine) disrupts cognitive and behaviour functions, producing a schizophrenia-like syndrome, recapitulating both positive and negative symptoms. When administered to schizophrenic patients, these antagonists can worsen symptoms. These findings indicate that schizophrenia is also characterized by diminished glutamate system and NMDAR activity. Thus, achieving an increase in the activity of the glutamate system through the administration of dopaminolytic agents seem to be a logical therapeutic option [7,14,15,20].

### 3.5. NMDARs in pain

There are findings supporting a hypothesis that NMDARs are co-responsible for nociception. Animal studies revealed that NMDARs are located in unmyelinated and myelinated axons of peripheral tissues. It has been demonstrated that peripheral nociceptive fibres express NR2B and NR2D subunits of NMDARs, while NR2A subunits appear to be absent from afferent terminals. Consistent with this, local glutamate injection results in nociceptive behaviour. Use of NR2B-selective antagonists potentiates NMDAR inhibition and should alleviate pain esthesia. NMDAR numbers increase in inflammatory changes, contributing to allodynia and hyperalgesia that both have a peripheral and a central component. Allodynia results from low-intensity stimuli acting via low-threshold afferents, generating pain. The phenomenon of hyperalgesia develops from noxious input that generates a pain response but with augmented amplitude and duration. The central sensitisation of the spinal cord is mediated by presynaptic NMDAR activation. Many primary afferents terminating in the dorsal horn express NMDARs and activation of presynaptic NMDARs results in the release of substance P (SP), calcitonin gene related peptide (CGRP), and glutamate from primary afferents. This facilitates and prolongs nociceptive transmission to the central nervous system, resulting in an elevation of dorsal horn excitability. The NMDAR-induced increased nociception seems also

to be related to a disinhibition phenomenon – a blockade of inhibitory mechanisms which should suppress hyperesthesia. Disinhibition may be a consequence of a reduction in inhibitory neurotransmitters such as GABA ( $\gamma$ -aminobutyric acid), disturbed or diminished GABA receptors or loss of inhibitory neurons. In physiological conditions, the excessive participation of NMDARs in synaptic transmission is prevented by GABA-A receptor mediated hyperpolarizing currents. Lack of this inhibition after GABA system deprivation and low-intensity stimulation begins to induce central sensitization which never takes place under physiological states [4,19,23].

At present, glutamate antagonists are proposed to be effective in postoperative pain. Postoperative hyperalgesia is a complex modulated by peripheral, spinal and supra-spinal level perception, caused by several physiopathologic mechanisms. Postoperative pain involves many neurotransmitter systems that either facilitate or inhibit nociception of somatic, neuropathic, inflammatory or visceral origins. Ketamine is a well-known NMDAR antagonist agent with a confirmed efficacy in reducing surgery-induced hyperalgesia. This drug was developed as an anaesthetic agent and has been demonstrated to cause “dissociative anaesthetics.” Many small trials confirm the adjuvant role of ketamine in subanaesthetic doses during analgesia. On the other hand, ketamine must be administered via a systemic route to produce its analgesic effect, excluding the suspected direct neuronal NMDAR blocking. The mechanism of action of systemic ketamine may be related to different elements – this drug produces multiple pharmacologic effects and interacts with many systems that mediate analgesic effects unrelated to NMDAR antagonism. Ketamine is an agonist of opioid receptors, exhibits inhibition of neuronal nicotinic receptors and activates the mono-aminergic descending inhibitory system that modulates nociception in the dorsal horn. Ketamine also suppresses the production of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6) and exerts a direct anti-inflammatory effect on macrophages. Thus, these other ketamine actions, rather than pure NMDAR antagonism, may account for the efficacy of this drug in postoperative pain management [4,23,28].

Apart from ketamine, several other drugs possess antagonistic NMDAR properties. Dextrometorphan, a D-isomer of the codeine analogue levorphanol, was shown to inhibit central sensitization in experimental studies. However, clinical use has been disappointing. Patients receiving dextrometorphan parenterally reported less pain in the early postoperative period, although the results were inconclusive. As a side note, dextrometorphan is being tested in clinical trials involving children suffering from Rett's syndrome – a neurodevelopmental disease affecting mostly females, characterized by the development of autistic features, stereotypic hand movements and epileptic attacks [4,23,28]. Other opioid analgesics, such as methadone or buprenorphine, also have anti-NMDAR properties at lower doses than those needed to induce complete analgesia. Anticonvulsant drugs such as gabapentin also display anti-hyperalgesic properties and have the ability to modulate glutaminergic neurotransmission. Nefopam, a centrally acting analgesic, also diminishes glutamate receptor activity and prevents postoperative opioid overconsumption. Selected non-steroidal anti-inflammatory drugs such as

ketorolac, demonstrate a central analgesic effect involving modulation of NMDAR activity [4,23,28].

In summary the inhibition of the glutaminergic system is suspected to be involved in the pain-relief mechanism of many drugs. Ketamine may also have a role in pre-emptive analgesia for surgical procedures, however, this comes at the expense of a small increase in the risk of psychomimetic effects. At present, there is no evidence supporting an unambiguous rationale for other NMDAR antagonists in both acute and chronic pain. This area needs further study in the future.

#### 4. NMDAR-TARGETING AGENTS – CURRENT THERAPIES AND FUTURE PERSPECTIVES

Early preclinical and clinical trials of glutamate targeting drugs concentrated on NMDARs and three main types of antagonists have been studied: competitive NMDAR antagonists (glutamate – e.g. selfotel or glycine binding site – e.g. gavestinel), non-competitive allosteric drugs, and/or NMDAR channel blockers. As was mentioned before, these agents were not effective for the following indications: ischemic stroke, and TBI, because of the restrictions discussed above. Further development of these NMDAR antagonists is unlikely and the initial enthusiasm for this kind of therapy has been abolished [12,13,19].

Lack of success caused a diminished interest in the glutamate system as a potential target of modern pharmacotherapy. It was surprising to find that well-tolerated drugs with multiple mechanism of action, demonstrating benefits for some neurological conditions, also exhibit anti-NMDA properties. These were mentioned above: felbamate, riluzole, amantadine and memantine.

Felbamate was initially screened for anticonvulsant activity in experimental models and showed a broad anticonvulsant profile similar to valproic acid but with less neurotoxicity. The complex pharmacodynamic mechanism of felbamate involves inhibition of voltage-dependent Na<sup>+</sup> and Ca<sup>2+</sup> channels as well as NMDAR antagonism. These drugs seem to be a non-competitive allosteric inhibitors with some selectivity for NR2B-containing receptors. During clinical trials, felbamate produced no adverse side effects specific to other anti-epileptic drugs or antagonists tested in TBI and ischemic stroke. The most common reported side effects of felbamate were nausea, anorexia and insomnia. However, pharmacovigilance studies published after the introduction of this agent to common clinical practice revealed two rare but of special importance reactions related to felbamate: aplastic anaemia (with incidence of about 1:8000) and hepatotoxicity (1:26000). These unexpected adverse events have limited felbamate clinical use – nowadays this drug is recommended only in intractable partial seizures and in Lennox-Gastaut syndrome that is refractory to primary therapy [12].

Riluzol was also developed as an anticonvulsant agent, but findings demonstrating its modulatory effects in NMDAR-mediated neuronal death caused that further development of riluzol was geared towards its neuroprotective functions. It was discovered that riluzol is a Na<sup>+</sup> channel blocker in NMDAR containing neurons and that it prevents the

entrance of neuronal  $\text{Ca}^{2+}$ , stabilising NMDARs and protecting them from depolarization. This drug also enhanced glutamate clearance from the synaptic cleft by increasing glutamate re-uptake. The most promising results of riluzol were in clinical trials obtained for amyotrophic lateral sclerosis (ALS). The drug showed a statistically significant survival advantage and a 9% absolute risk reduction compared to placebo. Patients receiving riluzol reported small beneficial effects on limb function. The drug was well tolerated with asthenia, nausea and elevation of liver enzymes being the most frequent side-effects. Although the efficacy of riluzol in treating ALS was not acceptable, the drug was still approved in many countries for ALS treatment [12].

Amantadine was introduced into clinical practice for the prophylaxis of respiratory infections due to influenza. It was discovered that this drug had anti-Parkinsonian properties through direct dopaminergic activity. Further studies revealed that amantadine is also an NMDAR antagonist acting as an open-channel blocker. Further clinical trials are still needed to establish this drug's value in PD [10,12,19].

Memantine was synthesised as a potential hypoglycaemic agent, however it was ineffective in lowering glucose blood level. On the other hand it was observed that cognitive functions of diabetic patients treated with memantine were improved, thus this drug was used in the treatment of dementia. Memantine, similar to amantadine, was discovered to be an open-channel NMDARs blocker but it was shown to have a relatively low affinity for NMDARs, allowing fast binding but also rapid dissociation from the receptors. Moreover, the action of memantine is voltage-dependent and therefore, this agent did not block NMDAR channels upon strong postsynaptic depolarization (which is specific during physiological conditions). The NMDARs were blocked during moderate, long-lasting depolarization, observed in pathological, excitotoxic conditions. Memantine was also shown to inhibit nicotinic cholinergic receptors that, when activated, also contributes to amyloid- $\beta$  – induced tau protein phosphorylation and AD development. This drug was well-tolerated with nausea, diarrhoea, headache, insomnia, and dizziness being the most serious adverse effects. It was approved for moderate and severe AD treatment in most of the European countries, the USA and Canada. Clinical trials were carried out on memantine in the treatment of frontotemporal lobar degeneration, PD dementia, cognitive symptoms related to Huntington's disease, and cognitive dysfunction in the course of TBI; this drug may also be neuroprotective in these entities [12,19].

It should be mentioned that novel agents focused on AD treatment are being developed. There are two other NMDAR targeting agents currently being studied: neramexane and dimebon. Neramexane, similarly to memantine, is an open-channel NMDAR blocker and displays a similar pharmacokinetic and comparable clinical tolerability. Dimebon, which was initially classified as an antihistaminic drug, is being studied as a potential agent for AD and HD because it also blocks NMDARs [12].

Prospective NMDAR antagonists and other agents affecting the glutamate system are based on two major strategies, which do not target the extracellular part of NMDARs, as other medications have so far.

The first novel strategy is based on the transporter systems regulating the glutamate amount in the synaptic cleft and the second one targets intracellular proteins which are involved in NMDAR signalling pathways.

Glutamate released into the synaptic cleft is then removed using a family of excitatory amino acids transporters (EAATs). The idea of developing glutamate re-uptake activators arose thanks in part to the theory behind selective serotonin re-uptake inhibitors. There are five types of EAATs (EAAT1 – EAAT5) with EAAT2 responsible for most of the glutamate turnover in the brain. Agents increasing EAAT2 activity or its membrane expression on both neurons and glia might provide new a therapeutic approach in reducing glutamate-mediated excitotoxicity because of glutamate clearance enhancement. It was found that ceftriaxone, a beta-lactam antibiotic, increases EAAT2 activity in mouse models of ALS. It is currently going through clinical trials in regards to this. There are also reports that riluzol, with its complex mechanism of action, also increases the activity of EAAT2 [12].

Among signalling proteins, there are many potential enzymes (such as protein kinases and phosphatases) which may act as modulatory sites by interfering in signalling downstream to the nucleus. The idea behind such agents already exists in modern oncology. Several molecules driving neoplastic transformation have been identified. This has allowed us to develop designed cancer therapeutics which can inhibit intracellular neoplastic signal transduction. An example of this are monoclonal antibodies (imatinib or trastuzumab). One molecular target that regulates NMDAR activity is protein kinase C. In animal studies, it was demonstrated that inhibition of this enzyme impairs spatial memory. Consequently, artificial elevation of protein kinase C activity might be especially effective in neurological disturbances with memory impairment (AD and other dementias). Studies concerning protein kinase C activators are ongoing, with the most advanced studies involving bryostatin-1 and nefiracetam [12].

As mentioned above, there are attempts to increase glutamate system function and NMDAR activity in schizophrenia. Already some opportunities exist when it comes to enhancing NMDAR function. Some drugs have already been studied in pre-clinical and clinical trials. Direct glutamate agonists may be used, although their application is limited because of possible overdosing and excitotoxicity. Other therapeutic opportunities involve the use of NMDAR modulatory site agonists, especially agonists acting on the glycine/D-serine site. When administrated together with classic neuroleptic agents, they significantly improved symptoms. The antibiotic D-cycloserine is a partial glycine site agonist that improves glutaminergic activity in schizophrenia, however, further studies are required to establish its usefulness in the treatment of this disease. Pilot studies suggesting that the core symptoms of social impairment in patients with autism may be improved when the patients are treated with D-cycloserine are under way.

An interesting approach in the development of antipsychotic agents involves the glutamate system. This approach increases extracellular glycine levels through re-uptake inhibition (by blocking the glyT1-transporter), similar

Table 1. NMDAR-targeting agents [12,19]

Drug	Pharmacological profile	Studied therapeutic area	Remarks
<b>NMDAR antagonists</b>			
Selfotel	Competitive NMDA antagonist at glutamate binding site	Stroke TBI	Most clinical trials were terminated prematurely with trends towards worse functional outcome or higher mortality – however, they were methodologically controversial
Gavestinel	Competitive NMDA antagonist at glycine binding site	Stroke	
Eliprodil Traxoprodil	Competitive NMDA antagonist at NR2B binding subunit	Stroke TBI	
Aptiganel	High affinity uncompetitive ion channel blocker	Stroke TBI	
Amantadine	Low affinity NMDA antagonist	PD	
Memantine	Low affinity NMDA antagonist	AD PD	Complex action; also inhibits nicotinic Ach receptors (nAChRs)
Felbamate	Uncompetitive NMDA antagonist	Intractable partial seizures Lennox-Gastaut Syndrome	Complex action, also inhibits voltage gated Na <sup>+</sup> and Ca <sup>2+</sup> channels
Riluzol	Uncompetitive NMDA antagonist	ALS	Complex action, also inhibits voltage gated Na <sup>+</sup> and Ca <sup>2+</sup> channels
Remacemide	Uncompetitive NMDA antagonist	Epilepsy, PD	Complex action, also inhibits Na <sup>+</sup> channels
Dexanabinol	Uncompetitive NMDA antagonist	TBI	Synthetic cannabinoid with antioxidant and anti-inflammatory properties
Ketamine	Low affinity NMDA antagonist	Analgesia	Complex action, also affects opioid receptors, activates descending monoamine antinociceptive system and displays anti-inflammatory properties
Dextrometorphan	Low affinity NMDA antagonist	Analgesia Rett's Syndrome	Complex action, also affects opioid receptors
Neramexane	NMDA open channel blocker	AD	
Dimebon	NMDA blocker at a site distinct from memantine	AD	Complex action, also inhibits H1 receptors
<b>EAATs upregulators – glutamate reuptake enhancers</b>			
Ceftriaxon	Increases EAAT2 activity and glutamate clearance	ALS	
Riluzol	Increases EAAT2 activity and glutamate clearance	ALS	
<b>Signalling proteins modulators</b>			
Bryostatatin-1	Protein kinase C activators	AD	
Nefiracetam	Protein kinase C activators	AD	
<b>Glycine agonists</b>			
D-cycloserine	NMDA agonist at glycine site	Schizophrenia	
<b>glyT1 upregulators – glycine reuptake inhibitors</b>			
Sarcosine	Decreases glyT1 activity and glycine clearance	Schizophrenia	

NMDA – N-methyl-D-aspartate, TBI – Traumatic Brain Injury, PD – Parkinson Disease, AD – Alzheimer Disease, ALS – amyotrophic lateral sclerosis, EAATs – Excitatory Amino Acids Transporters, EAAT1 – Excitatory Amino Acid Transporter 1, glyT1- Glycine Transporter 1



to serotonin re-uptake inhibitors in depression pharmacotherapy. One example of these blockers is sarcosine. Sarcosine was more effective in ameliorating both positive and negative schizophrenic symptoms when compared to D-serine. This finding suggests that the central glycine level in schizophrenia may be insufficient to saturate NMDARs [8,12,14,18].

The summary of NMDAR – targeting agents discussed is presented in table 1.

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The authors have no potential conflicts of interest to declare.