The Glutamate Hypothesis: A Pathogenic Pathway from which Pharmacological Interventions have Emerged

Pharmacopsychiatry

For personal use only.
No commercial use, no depositing in repositories.
The Glutamate Hypothesis: A Pathogenic Pathway from which Pharmacological Interventions have Emerged

Authors
S. R. T. Veerman1, P. F. J. Schulte2, L. de Haan3

Affiliations
1 Mental Health Service Organisation North Holland North, Community Mental Health Division, Flexible Assertive Community Treatment, Alkmaar, The Netherlands
2 Mental Health Service Organisation North Holland North, Division for Specialised Treatment, Treatment Centre for Bipolar Disorders, Alkmaar, The Netherlands
3 AMC, Academic Psychiatric Centre, Early Psychosis Department, Amsterdam, The Netherlands

Key words
- schizophrenia
- glutamate
- NMDA receptor
- clozapine
- glycine

Abstract

We discuss the relevance of the glutamate hypothesis in explaining cognitive disturbances and negative symptoms in schizophrenia. 4 lines of evidence support the hypothesis that glutamate deregulation, mainly through dysfunction of the N-methyl-D-aspartate (NMDA) receptor, is an important underlying mechanism of schizophrenia. Glutamate pathways are promising sites for intervention. Glutamate agonists combined with non-clozapine antipsychotics and glutamate antagonists augmented to clozapine show interesting clinical benefits in refractory schizophrenia. We illustrate how unique properties of the NMDA receptor antagonist memantine in addition to clozapine, may cause improvement of positive, negative and cognitive symptoms of schizophrenia.

Introduction

The natural course of schizophrenia is heterogeneous. Negative symptoms contribute to functional impairment, reduced quality of life and predict a worse social outcome [1]. Social and cognitive impairments usually develop many years before the onset of positive symptoms [2]. Various pathophysiological hypotheses have been put forward to explain the development of schizophrenia. Altered receptor functions of several neurotransmitters could be either the primary deficit or secondary to other neuropathology. Dysregulation of the glutamatergic system through hypofunction of the N-methyl-D-aspartate (NMDA) receptor is thought to contribute to the development of schizophrenia [3,4]. The glutamate hypothesis describes the pathophysiology of cognitive disturbances in schizophrenia and elaborates the dopamine hypothesis, which attributes dysregulation of dopaminergic function as a possible cause of negative and positive symptoms in schizophrenia. We explain how both hypotheses are closely entwined through reciprocal relationships between glutamatergic pathways and mesolimbic dopaminergic projections.

If we could fully comprehend the underlying mechanism of schizophrenia, one day we might be able to intervene in the critical or possibly in the prodromal period with neuroprotective medication in order to limit cognitive decline or even prevent the development of negative and positive symptoms. In cases of therapy-resistant schizophrenia, when usual dopamine blockers do not alleviate schizophrenic symptoms sufficiently, clozapine is indicated. Unfortunately 40–70% of patients fail to benefit from clozapine monotherapy or only partially respond [5]. Even after controlling for clozapine plasma level 30% of patients still do not respond [6]. For this subgroup of clozapine-refractory patients the development of innovative pharmacological strategies is imperative. Novel avenues of research are needed for improved drug treatment of schizophrenia. Following functional psychopharmacological principles, the above-mentioned knowledge concerning glutamatergic neurotransmission in schizophrenia results in a novel strategy: modulation of the glutamatergic system [7]. Glutamatergic medication may prevent neurodegenerative changes in early psychosis and subsequently disease progression. Moreover, it is conceivable that glutamatergic modulators are useful in the treatment of patients with refractory schizophrenia, especially with prevailing negative symptoms and invalidating severe cognitive symptoms.

To understand the link between the occupancy of various receptors by glutamate agonists or antagonists and the resulting therapeutic effect of these glutamate modulators, we first describe...
the glutamate system and relationships between different glutamatergic receptors. We summarize evidence for altered glutamatergic neurotransmission in schizophrenia. We clarify the mechanism and function of different glutamate modulators. We review recent clinical findings concerning glutamatergic agents in combination with antipsychotic medication, limited to randomized double-blind placebo-controlled trials. We explain the potency of clozapine, which has glutamate agonistic activity in several ways. We illustrate how the combination of clozapine and memantine, a voltage dependent glutamate antagonist restores imbalanced glutamatergic homeostasis in schizophrenia. Finally we describe the protocol of a proof-of-concept study on memantine augmentation to clozapine in refractory schizophrenia.

Methods

We review the literature on the pathophysiological hypothesis of deregulation of the glutamatergic system in schizophrenia to give a possible explanation of the beneficial effect of glutamatergic modulators. We review randomized double-blind studies on glutamatergic augmentation strategies in patients with clozapine-resistant schizophrenia, which were found after a search of the electronic databases PsycINFO, EMBASE, EBM reviews-Cochrane Database of Systematic Reviews, EBM reviews-Cochrane Central Register of Controlled Trials with the key words “schizophrenia”, “clozapine”, “augmentation/comboination”, “treatment resistant/refractory”, “randomized”, and “glutamate”. Titles, abstracts and related articles were examined. There were no language or year of publication restrictions.

Results

Glutamate system

Glutamate is the primary excitatory neurotransmitter in the brain [8]. Glutamatergic receptors play a pivotal role in regulating neuronal migration, neural growth, synaptogenesis and the pruning of neurons by apoptosis. 2 different types of glutamate receptors can be distinguished. (i) Ionotropic receptors mediate fast excitatory postsynaptic potentials throughout the brain. The kainate receptor and the amino-3-hydroxy-5-methyl-isoxazoloe-4-propionic acid (AMPA) receptor play an important role in excitatory neurotransmission by mediating fast postsynaptic potentials. The N-methyl-D-aspartate (NMDA) receptor is a third ionotropic receptor playing a primary role in long-term potentiation (LTP), which is a major form of use-dependent synaptic plasticity. Each time the NMDA receptor is activated, stimulation of pyramidal neurons becomes easier. Therefore, the synaptic efficacy increases persistently, resulting in LTP. In LTP preferential routes for impulses develop in the brain. This is the physiological foundation of conditioned reaction and thus learning. In the hippocampus these neuroplastic effects play a key role in formation of long-term memory. The NMDA receptor is activated by 2 distinct mechanisms. For the first mechanism several steps are necessary to ensure activation of the NMDA receptor (Fig. 1). At resting membrane potential Mg$^{2+}$ blocks the NMDA channel, prohibiting Ca$^{2+}$ influx. This second messenger causes an increased sensitivity of the synapse, resulting in LTP. A brief period of high-intensity excitatory synaptic activity results in a fall of membrane potential, which removes the Mg$^{2+}$ block of the NMDA receptor. A second mechanism of activation of the NMDA receptor is the occupation of glutamate at 2 binding sites, which occurs in cases of excessive glutamate release [9,10]. After sufficient depolarization of the postsynaptic membrane Mg$^{2+}$ no longer blocks the channel, causing Ca$^{2+}$ influx. However, excessive glutamate in the synapse leads to overstimulation of NMDA receptors. Ca$^{2+}$ influx increases, resulting in elevation of intracellular Ca$^{2+}$ levels. Thus toxic metabolic processes are triggered that may lead to neuronal cell death.

Glutamate metabotropic receptors (mGluRs) affect intracellular metabolic processes [11]. MGlurS are G-protein-coupled receptors with a relatively slow modulating mechanism. After activation of mGluRs, the G-protein is activated, followed by the conversion of ATP to cyclic AMP by adenylyl cyclase. MGlur5 receptors modulate the NMDA receptor, potentiating NMDA receptor function in forebrain regions [12]. MGlur2 and MGlur3 receptors belong to the subgroup II family of mGluRs. High levels of mGlur2 receptors are found in almost all regions of the limbic system, including the prefrontal cortex, thalamus and amygdale [13]. Expression of MGlur3 receptors is also seen in other limbic regions, including the hippocampus. Distribution of MGlur3 receptors is more diffuse than distribution of MGlur2 receptors. Activation of MGlur2 and MGlur3 receptors on presynaptic nerve terminals inhibits presynaptic glutamate release, modulating synaptic plasticity and LTP.

Altered glutamate system in schizophrenia

The hypothesis that an imbalanced dopaminergic neurotransmission is a fundamental underlying mechanism of schizophrenia is supported by the fact that people at high risk of psychosis who subsequently develop psychosis show elevated dopaminergic function in the brainstem region using positron emission tomography [14]. Furthermore, dopamine agonists such as levodopa induce psychosis and all known antipsychotic drugs block dopamine receptors. However, the dopamine hypothesis merely explains the development of positive symptoms and negative symptoms. The glutamate hypothesis elaborates the

Veerman SRT et al. The Glutamate Hypothesis... Pharmacopsychiatry

Fig. 1 Activation of NMDA receptors. Ionotropic receptors gate cation channels, which are permeable to Na$^+$, K$^+$ and Ca$^{2+}$. Glutamate in the synaptic cleft activates the kainate and/or AMPA receptor on the postsynaptic membrane, resulting in substantial neuronal depolarization. After partial depolarization of the postsynaptic membrane both glutamate and glycine or D-serine, which are endogenous co-agonists need to bind simultaneously to the tetrameric structure of the NMDA receptor. The voltage-dependent Mg$^{2+}$ block releases, the NMDA channel opens and Ca$^{2+}$ flows into the postsynaptic neuronal cell.
dopamine hypothesis, describing synaptic relationships between glutamatergic systems and dopaminergic projections. More importantly, the glutamate hypothesis offers an explanation for the pathophysiology of cognitive disturbances in schizophrenia.

There are several lines of evidence which link glutamate and specifically NMDA receptor hypofunction to the pathogenesis of schizophrenia. Firstly, hypofunctional NMDA receptors caused by chronic administration of phenylcyclohexylpiperidine (PCP) and to a lesser extent ketamine lead to transient schizophrenia-like psychosis (including positive, negative and cognitive symptoms) in healthy subjects [3, 15, 16]. Contrary to dopamine agonists like amphetamines and levodopa, PCP and ketamine do not only cause positive symptoms, but also induce prominent emotional blunting, anhedonia and social withdrawal, similar to negative symptoms of schizophrenia. Cognitive disruptions associated with the prefrontal cortex, such as working memory impairments, which are characteristic for schizophrenia, are caused as well by these non-competitive NMDA receptor antagonists. The primary site of action of PCP and ketamine is proposed to be the NMDA receptors on GABA-ergic (gamma-aminobutyric acid) interneurons in the thalamus, the basal forebrain and the hippocampus. Because these drugs block the NMDA channel completely and induce symptoms such as psychosis, social withdrawal and executive function deficits, it is proposed that NMDA receptor agonists could reduce the symptoms of schizophrenia.

The second line of evidence is provided by genetic linkage studies and confirmed by animal models and studies of post-mortem brain tissue of patients with schizophrenia [3]. Highly replicated findings concern several different genes, associated with schizophrenia and linked to hypoactivity of glutamatergic and particularly NMDA-receptor-mediated activity. Examples of candidate genes are G72 and deaminooxidase (DAAO), associated with metabolism of D-serine, which is an agonist of the NMDA receptor. Other candidate genes are involved in glutamate cysteine ligase, which is involved in glutamate metabolism and the synthesis of glutathione, an important anti-oxidant. Recently Japanese researchers examined genetic data from several Asian populations and identified a rare variant in GRIN3A, associated with schizophrenia. GRIN3A is a gene that codes for the GluN3A subunit of NMDA receptor [17].

The third line of evidence that NMDA hypofunction is implicated in the pathogenesis of schizophrenia is the clinical presentation of anti-NMDA-receptor encephalitis, first described in 2007 in women with ovarian teratoma and autoantibodies targeting specifically the NMDA receptor [18]. Generally the clinical course of this severe neuropsychiatric syndrome is a non-specific flu-like prodrome, followed by a psychotic stage with bizarre behaviour, disorientation, confusion, paranoid thought, visual or auditory hallucinations and memory deficits. The following phase is characterized by decreased consciousness, hypoventilation, lethargy, seizures, autonomous instability and the development of dyskinesia. A recent discovery is the presence of increased antibodies against the NMDA receptor in approximately 10% of patients with schizophrenia, which sustains the hypothesis that schizophrenia may be associated with dysfunctional reaction to infectious illness with a genetic predisposition in which the NMDA receptor is a target for the immune system [19].

The fourth line of evidence has been presented recently in a meta-analysis of 241H-MRS (magnetic resonance spectroscopy) studies demonstrating altered frontal glutamate concentration in schizophrenia patients [20]. Glutamatergic metabolites seem to increase between the age of 20 and 30 years in healthy control subjects, followed by a gradual decline. These findings suggest age-related alterations in neuronal and particularly in glutamatergic metabolism in the normal human brain. In early schizophrenia and even in people with prodromal symptoms of psychosis glutamatergic metabolites in frontal brain areas show a higher peak increase than in healthy controls, suggesting an excitotoxic process and neuronal cell death. As described above excessive glutamate levels in the synaptic cleft lead to overstimulation of the NMDA receptors, followed by increased Ca2+ influx and subsequently neuronal cell death. This could explain why negative symptoms and cognitive deficits are the early signs of schizophrenia and cognitive decline is most prominent in this critical period. Thus glutamatergic metabolites seem to peak during the early course of schizophrenia, but glutamate and glutamine levels decrease more progressively thereafter than in healthy control subjects. This decrease of frontonal region glutamate may be a reflection of progressive loss of synaptic activity and brain volume reductions or the effect of antipsychotic medication. Besides changes in membrane metabolism, altered expression of intracellular and extracellular glutamate transporters as well as dysfunction of glutamate transport in the synaptic cleft or inside presynaptic neurons can all be involved in altered glutamatergic levels.

Diminished activation of the NMDA receptor is believed to be an important underlying mechanism of schizophrenia. While the basal activity of pyramidal neurons is not directly regulated by NMDA receptors, the activity of cortical GABA-ergic interneurons is highly sensitive to tonic regulation by NMDA receptors [21] (Fig. 2). Reciprocal synaptic relationships between glutamatergic systems and mesolimbic dopaminergic projections explain how NMDA hypofunction results in dopaminergic hyperfunction in the amygdale, causing positive symptoms [22] (Fig. 2). Studies in rodents have shown that NMDA hypofunction has a disinhibitory effect on glutamatergic transmission in the prefrontal cortex [23]. A sustained firing rate potentiation of prefrontal cortex (PFC) neurons is proposed to lead to an increased number of irregularly discharged single spikes. This increase in spike activity results in cortical noise and transmission of disinformation. Increase in disorganized spike activity also causes significant reduction in organized bursting activity, which reduces transmission efficacy of cortical neurons. Abnormal cortical signal-to-noise patterns cause negative symptoms and impairment of frontal-lobe related cognitive functions. In the beginning NMDA hypofunction results in too little excitation [9, 15]. However, the glutamate concentration in the synaptic cleft increases, resulting in overstimulation of the NMDA receptor. Neurotoxic Ca2+ influx leads to neuronal cell death and subsequently neurodegenerative changes as seen in schizophrenia.

Glutamate modulators

NMDA-receptor based treatment approaches include agonists at the NMDA receptor glycine site (clozapine, glycine, D-serine, D-cycloserine and N-acetylcysteine), a glycine transport inhibitor (sarcosine) and non-competitive open-channel blockers of the NMDA receptor (amantadine and memantine) [24]. Potential sites for intervention also include glutathione synthesis (N-acetylcysteine). An allosteric modulator of the AMPA receptor enhances glutamate-mediated synaptic transmission (amphetamine CX516) [25]. Other pathways regulating glutamate involve...
agents resulting in inhibition of presynaptic glutamate release, which include mixed mGluR2/3 agonists, mGluR2 positive allosteric modulators (PAMs), an antagonist for postsynaptic kainate receptors and AMPA receptors (topiramate) and an antagonist for postsynaptic voltage sensitive sodium channels (lamotrigine) [16,26].

Metabotropic glutamate agonists
Recent animal studies provide strong evidence that specific metabotropic glutamate agonists are effective in the treatment of different symptom domains of schizophrenia [26]. Animal studies of mGluR5 agonists show improvement of positive, negative and cognitive symptoms. MGI5 receptor activation even reverses cognitive dysfunction in preclinical studies. However, this selective agonist has not yet been tested in a clinical trial. Group II mGluR agonists demonstrated efficacy in multiple animal models for schizophrenia and were considered a promising novel approach in the treatment of schizophrenia, selectively targeting downstream glutamate increase due to NMDA receptor hypofunction [13,27].

Pomaglumetad methionil (LY2140023 monohydrate) is a methionine amide prodrug of the active compound LY404039, acting as a selective and potent orbocholic agonist at both mGluR2 and mGluR3 [11,26]. Initially this mixed mGluR2/3 agonist showed promise as potential monotherapy for schizophrenia in the proof-of-concept study, consistent with the predictions from pre-clinical animal studies [28]. In this randomized, 3-armed, double-blind, placebo-controlled trial an intention to treat (ITT) analysis was performed on 97 patients with schizophrenia receiving 40 mg pomaglumetad methionil twice daily, 34 patients receiving 15 mg olanzapine once daily and 62 patients receiving placebo for 4 weeks. Although less efficacious than olanzapine, pomaglumetad methionil showed significant improvement in positive, negative and overall symptoms of schizophrenia without side effects such as weight gain, extrapyramidal symptoms and elevated prolactin. However, antipsychotic properties of pomaglumetad methionil were not confirmed in the second phase 2 clinical trial, which was a 4-week double-blind placebo-controlled dose-ranging study [29]. While in the olanzapine (15 mg/day) treatment group (N = 62) positive symptoms significantly improved, none of the pomaglumetad methionil dosages, varying from twice daily either 5 mg (N = 121), 10 mg (N = 122), 40 mg (N = 120) to 80 mg (N = 122) were found to be superior to placebo (N = 122) for positive, negative and overall symptoms of schizophrenia. The second disappointing result was shown in another dose-ranging study [30]. After 6 weeks of treatment no significant differences in overall symptoms of schizophrenia were found between pomaglumetad methionil and placebo in patients with an acute exacerbation. Risperidone 2 mg twice daily significantly improved Positive and Negative Syndrome Scale (PANSS) total score, while efficacy of pomaglumetad methionil in different dosages (twice daily 40 mg and 80 mg) was similar to placebo. The first long-term open-label phase 2 study without a placebo treatment arm was designed to study safety and tolerability rather than efficacy [31]. Patients with prominent negative symptoms were randomized to either pomaglumetad methionil 40 mg twice daily or a second generation antipsychotic (olanzapine, risperidone or aripirazole). After 24 weeks of treatment a second generation antipsychotic was significantly superior to pomaglumetad methionil for total symptom severity and both treatment groups showed comparable improvement in negative symptoms. In a 24-week double-blind randomized phase 3 study pomaglumetad methionil was found to be significantly inferior to aripiprazole (dosage varying from 10 to 30 mg/day) for total symptom severity [32]. This study was discontinued prematurely, because of disappointing results in the above mentioned phase 2 trials and the early cessation of a double-blind placebo-controlled dose-ranging phase 3 trial in acutely ill patients due to lack of efficacy.

Selective mGluR2 PAMs may be a more preferred approach than a mixed mGluR2/3 agonist like pomaglumetad methionil [13]. PAMs are small molecules, which bind at an alternative site to orbocholic agonists and enhance the agonistic activity in the
presence of the endogenous ligand glutamate. MGLuR2 PAMs modulate excessive synaptic glutamate release in almost all regions of the limbic system. Contrary to orthosteric agonism, positive allosteric modulation does not induce overactivation or desensitization via downregulation of mGlu2 receptors [33]. Therefore, normal or basal glutamate release remains stable.

2 PAMs advanced from animal models of psychosis to clinical trials. The mGluR2 PAM AZD8529 was tested in a phase 1 clinical trial in 2008 [33]. However, AZD8529 did not meet the high expectations of an alternative treatment for schizophrenia. The phase 2 clinical trial in patients with schizophrenia, which started in 2009, discontinued in 2011 without further details. Another mGluR2 PAM, called addex (ADX71149) was studied in a phase 2 clinical trial in 2011 [33, 34]. 15 patients with subacute psychosis, who were not treated with antipsychotic medication, received ADX71149 as monotherapy during 12 weeks with a dose range from 50 mg twice daily titrated up to 150 mg twice daily. Safety and tolerability were confirmed. However, results of this open-label trial, investigating efficacy of addex as mono-therapy in patients with (sub)acute positive symptoms, have not yet been reported.

**Ionotropic glutamate agonists**

Most glutamate agonists are NMDA receptor modulators at the glycine site [10]. The following compounds have glutamate agonistic properties. Glycine and D-serine are full agonists at the glycine site of the postsynaptic NMDA receptor [35]. D-Cycloserine is a partial agonist at the NMDA receptor glycine site with approximately 60% activity [36]. In the presence of low concentrations of glycine, D-cycloserine acts as an agonist, while in the presence of high concentrations of glycine, D-cycloserine acts as an antagonist. N-Acetylcysteine provides cysteine for glutathione synthesis and is a NMDA receptor modulator. Sarcosine acts as an inhibitor of type 1 glycine transporter [37]. Ampakine CX516 is a positive modulator of the postsynaptic AMPA receptor [25].

There seems to be a differential effect of NMDA-receptor based interventions depending on the type of antipsychotic (clozapine or non-clozapine). This may be the result of effects of clozapine on glutamatergic homeostasis and antagonism at GABA receptors.

**Clozapine**

Clozapine is a highly efficacious second-generation agent, which has preferential antagonist activity at 5-HT2 receptors, followed by activity at adrenergic, cholinergic, histamine and muscarinic receptors with high affinity to dopamine 4 receptors in specifically the frontal cortex and amygdala and only modest activity at dopamine 1, dopamine 2 and dopamine 5 receptors [38-40]. The exact mode of action by which clozapine exerts its superior efficacy for both positive and negative symptoms is unknown, but clozapine is hypothesized to interact with GABA as antagonist and to improve the glutamatergic homeostasis in different ways. Clozapine blocks dopamine 4 receptors resulting in upregulation (increase in number) of AMPA receptors [41]. This way clozapine enhances depolarization of the postsynaptic membrane and facilitates NMDA receptor activation. Clozapine activates astrocyte glial cells, star-shaped support cells which are not able to fire like neurons but release, absorb or transport neurotransmitters [42]. Activated astrocyte glial cells release D-serine, followed by NMDA receptor activation. Clozapine first induces release of D-serine, shortly followed by release of glutamate by astrocytes. Glutamate activates metabotropic glutamate receptors, resulting in increased expression of NMDA receptors by increasing brain-derived neurotrophic factor (BDNF).

**Glutamate agonists in combination with antipsychotic medication**

Therapeutic effects of modulation of the glutamate system depend on the type of co-medication. There is limited evidence to support augmentation of non-clozapine antipsychotics with glutamate agonists.

A disappointing result on a mixed mGluR2/3 agonist was shown in a 16-week double-blind placebo-controlled randomized trial in patients with prevailing negative symptoms of schizophrenia [30]. Pomaglumetad methionil as add-on therapy to a second generation antipsychotic did not show any significant change in negative symptoms compared to placebo. The mGluR2 PAM addex seems to be a promising augmentation strategy in patients with residual negative symptoms. In a double-blind phase 2 clinical trial 92 patients received either addex 50 mg twice daily, addex 150 mg twice daily or placebo in addition to antipsychotic medication for 4 weeks. Efficacy was only demonstrated in patients with residual negative symptoms (N = 47) and not in patients with residual positive symptoms (N = 25) or in patients with insufficient response to clozapine (N = 20) [33, 34].

A metaanalysis by Singh and Singh (2011) of 1 253 cases from 29 placebo-controlled, double-blind randomized clinical trials on the efficacy of adjunctive NMDA receptor modulators to antipsychotic treatment confirmed additional therapeutic benefits of glutamate agonists in combination with non-clozapine antipsychotics [24]. Negative symptoms (Positive and Negative Syndrome Scale negative subscale or Scale for the Assessment of Negative Symptoms) improved with D-serine (standardized mean difference = −0.54), N-acetylcysteine (SMD = −0.45) and sarcosine (SMD = −0.39) as adjuncts to non-clozapine antipsychotics. Overall symptoms of schizophrenia (total PANSS score or total Brief Psychiatric Symptom Scale score) improved in combination with D-serine (SMD = −0.45), N-acetylcysteine (SMD = −0.64), sarcosine (SMD = −0.53). Combination therapy of non- clozapine antipsychotics and glycine or NMDA receptor modulators as a group improved positive symptoms (PANSS positive subscale or BPRS positive subscale SMD = −0.54 and −0.14, respectively) and overall symptoms of schizophrenia (total PANSS score or total BPRS score SMD = −1.12 and −0.38 respectively).

As adjuvant to clozapine, these glutamate agonists had no favourable effect on all symptoms of schizophrenia, while glycine even worsened positive symptoms (PANSS SMD = 0.56) [24]. 6 placebo-controlled, double-blind randomized clinical trials on NMDA-receptor agonists showed no beneficial effects in addition to clozapine [43]. A possible explanation for the absence of favourable effects of NMDA-receptor agonists in addition to clozapine is downregulation of NMDA receptors [44]. The glutamate agonist ampakine CX516 with its allosteric agonistic action at the AMPA receptor may be an exception, because ampakine CX516 combined with clozapine does not induce downregulation of NMDA receptors [25]. This hypothesis is supported by a single study on ampakine CX516 combined with clozapine, which showed favourable effects on negative, overall clinical symptoms and cognitive functioning [25].

**Glutamate antagonists**

Lamotrigine is an anticonvulsant drug that acts through voltage sensitive sodium channels antagonism and reduces presynaptic
glutamate release. Lamotrigine is assumed to augment the antipsychotic efficacy of clozapine by antagonizing overactive kainate receptors [35,45]. Topiramate has a mixed profile with both GABA-ergic and glutamatergic actions. This anticonvulsant potentiates GABA-ergic neurotransmission and acts as an antagonist for postsynaptic kainate receptors and AMPA receptors, decreasing the presynaptic release of glutamate [46,47]. Amantadine is an indirect dopaminergic agonist and a non-competitive open-channel blocker of the NMDA receptor with weak antagonist action [48]. Amantadine presumably exerts its neuroprotective effect through reducing the release of pro-inflammatory factors from activated microglia cells (the main cells for immune defence in the brain) and increasing the expression of neurotrophic factors from astrocyte glial cells [49]. Memantine acts as a low to moderate affinity type of uncompetitive, non-selective NMDA receptor antagonist [50]. Whereas PCP and ketamine are non-competitive NMDA receptor antagonists and induce schizoid-like symptoms, memantine is a voltage dependent antagonist. Memantine exerts its subtle effect on the NMDA receptor by binding at or near the Mg$^{2+}$ site within the ion channel. Memantine binds somewhat stronger than Mg$^{2+}$, decreasing Ca$^{2+}$ influx. Through reduction of overstimulation of NMDA receptors in the presence of excessive glutamate in the synaptic cleft, a homeostatic state is restored.

Glutamate antagonists in combination with antipsychotic medication

At the present moment the effect of glutamate antagonists as augmentation of antipsychotics is not clear because of contradictory results. There is one study with lamotrigine augmentation to conventional and atypical antipsychotics (4 patients with clozapine) in patients with treatment-resistant schizophrenia [51]. The last observation carried forward (LOCF) analysis did not show significant differences in all domains at the end of 10 weeks of treatment between 25 inpatients receiving lamotrigine (titrated up to 400 mg/day) and 13 inpatients receiving placebo. Because of the number of early drop-outs in both groups an analysis was performed in which only patients who completed the trial were included. The completer analysis showed significant improvement of positive symptoms (ES = −0.82), general psychopathology symptoms (ES = −0.85) and total PANSS score (ES = −0.92), but no significant improvement of negative symptoms (Scale for the Assessment of Negative Symptoms ES = −0.55), overall clinical symptoms (Brief Psychiatric Rating Scale ES = −0.52) or affective symptoms (21-item Hamilton Rating Scale for Depression ES = −0.05). Because these positive findings were not demonstrated in the LOCF analysis, this study had insufficient power to investigate the efficacy of lamotrigine as adjunctive agent to non-clozapine antipsychotics.

A metaanalysis by Kishi and Iwata (2013) on amantadine and memantine included 8 double-blind placebo-controlled trials across 406 patients (347 patients with schizophrenia related disorder and 59 patients with bipolar disorder) [52]. Amantadine (5 trials with 220 patients) and memantine (3 trials with 186 patients) as adjunctive therapy were not superior to placebo in positive symptoms, negative symptoms, overall symptoms of schizophrenia and symptom severity (Clinical Global Impression Severity scale) (effect sizes were not stated). In 3 cross-over studies on 74 patients with schizophrenia [53–55] amantadine addition to antipsychotics in patients with schizophrenia did not show a significant beneficial effect on overall symptoms of schizophrenia after a very short treatment duration, varying from 2 weeks to 7 weeks. Angus et al. (1997) included 16 patients with schizophrenia, receiving first generation antipsychotics (6 patients fluphenazine decanoate, 3 patients flupentixole decanoate, 3 patients trifluoperazine, 2 patients chlorpromazine, one patient thioridazine and one patient haloperidol) [53]. Amantadine or placebo were administered during 7 weeks (amantadine 100 mg/day in the first week, 200 mg/day in the second week, 300 mg/day during the third, fourth and fifth week, 200 mg/day in the sixth week and 100 mg/day in the seventh week) followed by a washout period of one week before cross-over to placebo or amantadine. This study did not show significant beneficial effect of amantadine on overall symptoms of schizophrenia (Psychiatric Assessment Scale for Rating Chronic Psychiatric Patients).

Silver et al. (2005) included 36 patients (32 patients with schizophrenia and 4 patients with schizoaffective disorder) in a 6-week cross-over trial [54]. A completer analysis was performed on 3 patients receiving clozapine, 9 patients receiving other second-generation antipsychotics and 17 patients receiving first-generation antipsychotics. After 3 weeks amantadine add-on therapy (200 mg/day) showed no significant effect on positive symptoms (Scale for the Assessment of Positive Symptoms), negative symptoms (SANS) and overall symptoms of schizophrenia (PANSS) or cognitive functions (including attention, memory, emotion perception and executive functions). Cognitive assessments were performed with an elaborate test battery. These disappointing results are not surprising because of the short treatment duration of merely 3 weeks. However, in this study, amantadine improved visuomotor coordination and symptom severity (CGI) compared to placebo [52]. Pappa et al. (2010) included 22 patients with schizophrenia, receiving olanzapine with random assignment to either amantadine 100 mg/day or placebo during 2 weeks, followed by a washout period of 4 days before cross-over to placebo or amantadine [55]. Whereas overall symptoms (BPRS) and cognition (Mini-Mental State Examination) did not change compared to placebo, symptom severity (CGI) did improve significantly after merely 2 weeks [40]. Only one of 3 trials on memantine in combination with non-clozapine antipsychotics showed clinical benefits. In a small 8-week placebo-controlled trial 20 patients were randomly assigned to risperidone 6 mg/day combined with memantine (titrated up to 20 mg in 1 week) and 20 patients received risperidone plus placebo [56]. In this trial memantine proved to be an efficacious adjunct for the treatment of primary negative symptoms (ES = −1.5), total symptoms (ES = −1.6) and general psychopathological symptoms of schizophrenia (ES = −1.0) (PANSS), but not for positive symptoms (ES = −0.1) or affective symptoms (ES = 0.0) (Hamilton Depression Rating Scale). Lieberman et al. (2009) compared 70 patients with persistent residual positive symptoms of schizophrenia receiving memantine (titrated up to 20 mg in 3 weeks) and 68 patients receiving placebo as an adjunct to other atypical antipsychotics than clozapine (olanzapine, risperidone, quetiapine, aripiprazole and ziprasidone) during 8 weeks [57]. Memantine showed no therapeutic benefits on total symptoms, positive symptoms, negative symptoms (PANSS), affective symptoms (Calgary Depression Scale for Schizophrenia) or cognitive functioning (Brief Assessment of Cognition in Schizophrenia). Auditory hallucinations occurred only in the memantine group (5.8%), suggesting an increase of psychotic symptoms due to memantine. These contradictory results may be explained by the shorter treatment duration with the maximum memantine dosage in the negative study in com-
parison to the positive study (5 weeks instead of 7 weeks). However, Lee et al. (2012) did not find beneficial effects of adjunctive memantine therapy to non-clozapine antipsychotics either [58]. 15 patients received memantine and 11 patients received placebo during 12 weeks as adjunct to conventional antipsychotic treatment. Results showed no significant differences regarding cognitive functioning (Korean MMSE, Hopkins Verbal Learning Test, Rey Complex Figure Test, Digit Span Forward and Backward Test, Digit Symbol Substitution Test, Stroop test, Trail Making Test (Part A), the Verbal Fluency Test and the Boston Naming Test), general psychopathology, positive, negative symptoms (PANSS) and affective symptoms (Hamilton Rating Scale for Depression). Treatment duration with memantine 20 mg/day was 9 weeks. Accordingly, there is still controversy regarding the role of memantine as adjunctive agent to non-clozapine antipsychotics. When the study by Rezaei et al. (2013) is considered an outlier and is removed from the analysis [56], the trend toward superior effect of memantine over placebo in addition to non-clozapine antipsychotics disappears. Contrary to non-clozapine antipsychotics, clozapine augmentation with glutamate antagonists has therapeutic potential. Combined results of 6 lamotrigine trials show a trend towards reducing positive and negative symptoms [43]. Topiramate add-on treatment to clozapine demonstrated therapeutic benefits in 2 out of 4 small studies, but a meta-analysis showed no significant efficacy nor trend in reducing symptoms [43]. Research on amantadine add-on therapy to clozapine is limited to 3 patients in the study by Silver et al. (2005), in which it is not stated whether these 3 patients were suffering from schizophrenia or schizoaffective disorder [54]. No sub analysis was performed on the efficacy of amantadine addition to clozapine compared with non-clozapine antipsychotics. Memantine addition to clozapine showed substantial beneficial effects on negative symptoms, positive symptoms, overall clinical symptoms (BPRS) and cognitive functioning (MMSE) in clozapine-resistant patients with schizophrenia in a small Brazilian study [50]. Taken together, we conclude that glutamate antagonists may have beneficial effects in combination with clozapine in refractory schizophrenia, but not in combination with non-clozapine antipsychotics.

Discussion

Here, we have reviewed the relevance of the glutamate hypothesis in explaining cognitive disturbances and negative symptoms in schizophrenia and the evidence in support of this hypothesis. Based on this information glutamate pathways are promising sites for intervention. mGlu5 receptors are a potential target for novel drug development, because activation of mGlu5 receptors enhances NMDA receptor function [26]. Highly selective mGluR2 PAMs, inhibiting presynaptic glutamate release, are more promising than mixed mGluR2/3 agonists in clinical studies [30–34]. However, no conclusions regarding efficacy of highly selective mGluR2 PAMs are currently justified based on the preliminary conclusions of one phase 2 clinical trial [33,34]. Medication trials suggest that ionotropic glutamate agonists combined with non-clozapine antipsychotics and ionotropic glutamate antagonists augmented to clozapine show interesting clinical benefits in refractory schizophrenia. Memantine is especially promising as an augmentation strategy to clozapine and perhaps even to non-clozapine antipsychotics, but evidence is limited to 4 trials (one study by Lucena et al., 2009 on augmentation to clozapine shows significant beneficial effects [50] and one out of 3 studies on augmentation to non-clozapine antipsychotics by Rezaei et al., 2013 shows significant beneficial effects [56]).

Preliminary conclusions regarding favourable effects of clozapine augmentation with glutamate antagonists (lamotrigine, topiramate and memantine) are based on a small number of trials with a small sample size (5 out of 12 studies show a significant beneficial effect) [43]. Notably the memantine trial shows impressive effect sizes. Therefore additional studies in larger samples are needed.

The glutamate hypothesis concerning NMDA hypofunction may explain why memantine as an adjunct to clozapine is a logical treatment approach in refractory schizophrenia. Memantine in combination with clozapine contributes to upregulation of NMDA receptors [44]. Moreover, memantine prevents an excitotoxic cascade in the presence of glutamate spill over in the synaptic cleft, which is a result of NMDA hypofunction in schizophrenia. Because memantine is a voltage dependent NMDA receptor antagonist, the NMDA receptor is only activated by a strong stimulus. Thus memantine reduces abnormal cortical signal-to-noise patterns and prefrontal noise in schizophrenia. Glutamatergic transmission efficacy and dopaminergic neurotransmission in the frontal cerebral cortex improves. These 2 mechanisms of action explain why memantine has potential as add-on treatment for primary negative symptoms in combination with non-clozapine antipsychotics as well.

To test this hypothesis we recently started a 26-week randomized, placebo-controlled cross-over study to evaluate the clinical efficacy, tolerability and safety of memantine vs. placebo in combination with ongoing clozapine treatment in 52 outpatients with refractory schizophrenia. Participants failed to achieve the remission criteria, defined as simultaneous ratings of mild or less (≤3 points) on 8 of the PANSS items evaluating the core symptoms of schizophrenia [59] after adequate treatment with clozapine for at least 6 months. Participants were recruited from Flexible Assertive Community Treatment (FACT) teams, specialized in the treatment of patients with severe mental illness. Memantine starts with 10 mg during the first week, builds up in the second week to the maximum dosage of 20 mg daily as add-on therapy. 20 mg are the ordinary study dosage in trials into Alzheimer’s disease and other indications of memantine. To enhance compliance, the dosing regimen in this study is simplified and accelerated. No substantial withdrawal due to adverse events is anticipated, since memantine tends to cause a similar range of side effects as placebo [60,61]. Expectations are that poor-outcome patients, who suffer from debilitating cognitive disturbances and negative symptoms as well as persistent positive symptoms will have clinical relevant advantages with memantine as add-on therapy to clozapine, reflected in improvement of daily functioning and quality of life. To improve the original proof-of-concept study by Lucena et al. (2009) cognitive functioning will be assessed by the Cambridge Neuropsychological Test Automated Battery (CANTAB), a sensitive and extensively validated cognitive testing battery [62]. Severity of psychopathology (measured with Clinical Global Impression Severity Scale [63] and PANSS [64]) is an additional primary parameter. Secondary study parameters are social cognition (Emotion Recognition Task of the CANTAB and Reading the Mind in the Eyes test [65]), psychosocial functioning (Health of the National Outcome Scales [66]) and quality of life (Manchester...
Short Assessment of Quality of Life [67]). Depressive and obsessive-compulsive symptoms are frequent in schizophrenia. Since memantine may have a beneficial effect on these symptoms [68–70] they are measured as additional secondary parameters (Calgary Obsessive-Compulsive Scale for Schizophrenia [71] and Yale-Brown Obsessive-Compulsive Scale [72]). Safety measures are laboratory tests (fasting plasma glucose, triglycerides, LDL, HDL and total cholesterol, liver enzymes, renal function, white blood cell count and differentiation, plasma clozapine level), blood pressure and waist circumference. Neuroleptic induced side-effects are assessed by the Liverpool University Neuroleptic Side-Effect Rating Scale [73]. All parameters are assessed at baseline, week 12, week 14 (after a wash-out period of 2 weeks) and week 26 (or drop-out). If this second proof-of-concept study replicates and extends findings concerning relevant clinical improvement in patients with clozapine-resistant schizophrenia, clinical investigation should be the follow-up for this augmentation strategy.

### Conclusion

Glutamate agonists are hypothesized to restore glutamatergic neurotransmission in schizophrenia, which is imbalanced due to NMDA receptor hypofunction. Focusing on the early diagnosis and treatment of psychosis, glutamatergic modulators may prevent neurodegenerative changes in the early stage of schizophrenia and subsequent cognitive decline. Inhibition of mesolimbic dopaminergic neurotransmission by activation of GABA interneurons may ameliorate positive symptoms. Negative symptoms improve by reduction of cortical noise and enhancement of efficacy of neuronal transmission in the prefrontal cortex. Cognitive deficits ameliorate due to this decrease of prefrontal noise and improvement of organized bursting activity, as well as reduction of neuronal excitotoxicity. Drug development based on positive allosteric modulation of mGlur5 receptors, potentiating NMDA receptor function, has not yet advanced from animal studies to clinical studies. Evidence regarding efficacy and tolerability of glutamate modulators in addition to antipsychotics is limited. A novel MGlur2 PAM (addex) shows potential as the first non-dopaminergic drug that may address negative symptoms of schizophrenia, based on a single double-blind placebo-controlled augmentation study. Ionotropic glutamate agonists in addition to non-clozapine antipsychotics appear to render clinically meaningful benefits for patients with residual positive symptoms and debilitating negative symptoms. The addition of a NMDA receptor agonist to clozapine does not improve therapeutic response, possibly due to downregulation of NMDA receptors. Ampakine CX516 does not induce downregulation of NMDA receptors and seems to be the only glutamate agonist to have favourable effects combined with clozapine. Glutamate antagonists seem to have therapeutic potential as add-on treatment for patients, who respond unsatisfactory to clozapine. Perhaps lamotrigine and memantine have clinical value in addition to non-clozapine antipsychotics as well, but evidence regarding efficacy is limited and not convincing at the present moment.

The superior efficacy of clozapine is probably due to its glutamate agonistic actions. Clozapine restores glutamate dysfunction in schizophrenia, especially when combined with the voltage dependent NMDA receptor antagonist memantine through upregulation of NMDA receptors. Memantine blocks the effects of excessive glutamate in schizophrenia, while preserving physiological activation of NMDA receptors required for long term potentiation. The combination of clozapine and memantine is believed to create a specific glutamatergic environment, in which neurotransmission is improved and clinical improvement is sustained. Based on the glutamate hypothesis, functional psychopharmacological characteristics of memantine and evidence from one proof-of-concept study, memantine as an add-on treatment to clozapine is expected to have favourable effects on cognitive functioning, negative and positive symptoms, reflected in improvement of daily functioning and quality of life in patients with refractory schizophrenia. If a second proof-of-concept study replicates and extends the findings of the earlier study memantine is a promising novel augmentation strategy in patients with clozapine-refractory schizophrenia.

### Conflict of Interest

The authors declare no conflicts of interest.

### References

2. Heaton RK, Gladsjo JA, Palmer BW et al. Stability and course of neuropsychological deficits in schizophrenia. Arch Gen Psychiatry 2001; 58: 24–32
11. Harrison HJ, Lyon L, Sartorius IJ et al. The group II metabotropic glutamate receptor 3 (mGlur3, mGlur3, GRM3): expression, function and involvement in schizophrenia. J Psychopharmacol 2008; 22: 308–322


