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## **Title Page**

Glutamatergic (N-methyl-D-aspartate receptor) hypofrontality in schizophrenia: Too little juice or a miswired brain

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**Running Title:** Glutamatergic approaches for miswired schizophrenic brains

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**Abbreviations**

ADX47273, [S-(4-fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]-oxadiazol-5-yl]-piperidin-1-yl}-methanone]; AMPA,  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionate; CDPPB, 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide; DOI, ( $\pm$ )-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; DTI, diffusion tensor imaging; GABA,  $\gamma$ -aminobutyric acid; GAD67, glutamic acid decarboxylase-67 isoform; GluN1, NMDA NR1 subunit; GluN2A-D, NMDA NR2A-D subunits; GlyT1, glycine transporter subtype 1; GRM3, mGlu3 receptor gene; 5-HT<sub>2A</sub>, 5-hydroxytryptamine<sub>2A</sub>; LSD, lysergic acid diethylamide; LY354740, (1S,2S,5R,6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid ; LY404039, (-)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]-hexane-4,6-dicarboxylic acid; LY544344, (1S,2S,5R,6S)-2[(2'S)-(2'-amino)-propionyl]aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid ; LY2140023, methionine prodrug of LY404039; mGlu, metabotropic glutamate; MK-801, dizocilpine; MRI, magnetic resonance imaging; NAMs, negative allosteric modulators; NMDA, N-methyl-D-aspartate; NFPS, N[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine; PAMs, positive allosteric modulators; PANSS; positive and negative symptom score; PCP, PFC, prefrontal cortex; phenylcyclohexylpiperidine; PSD95, postsynaptic density 95 protein; SSR 504734, (2-chloro-N-[(S)-phenyl[(2S)]-piperidin-2-yl]methyl)-3-trifluoromethyl benzamide

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## ABSTRACT

Dopamine D2 receptor blockade has been an obligate mechanism of action present in all medications which effectively treat positive symptoms of schizophrenia (e.g., delusions and hallucinations) and have been approved by regulatory agencies since the 1950's. Blockade of 5-hydroxytryptamine<sub>2A</sub> (5-HT<sub>2A</sub>) receptors plays a contributory role in the actions of the second generation of antipsychotic drugs, the so-called atypical antipsychotics. Nevertheless, substantial unmet medical needs remain for the treatment of negative symptoms and cognitive dysfunction. Recognition that dissociative anesthetics block the N-methyl-D-aspartate (NMDA) receptor channel has inspired a search for glutamatergic therapeutic mechanisms since ketamine and phencyclidine are known to induce psychotic-like symptoms in healthy volunteers and exacerbate the symptoms of schizophrenic patients. Current pathophysiological theories of schizophrenia emphasize that hypofunction of NMDA receptors at critical sites in local circuits modulate the function of a given brain region or control projections from one region to another (e.g., hippocampal-cortical or thalamocortical projections). The demonstration that a metabotropic glutamate<sub>2/3</sub> (mGlu<sub>2/3</sub>) receptor agonist prodrug decreased both positive and negative symptoms of schizophrenia raised hopes that glutamatergic mechanisms may provide therapeutic advantages. In addition to discussing activation of mGlu<sub>2</sub> receptors with mGlu<sub>2/3</sub> receptor agonists or mGlu<sub>2</sub> receptor positive allosteric modulators (PAMs), we will also discuss other methods that may potentially modulate circuits with hypofunctional NMDA receptors such as glycine transporter (GlyT1) inhibitors and mGlu<sub>5</sub> receptor PAMs. The hope is that by modulating glutamatergic neurotransmission, the dysfunctional circuitry of the schizophrenic brain (both local circuits and long-loop pathways) will be improved.

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Dopamine and serotonin, as opposed to emerging interest in glutamate, played a dominant role during the formative years of modern psychopharmacology when thinking about the pathophysiology and or treatment of schizophrenia. Models derived from studying the behavioral effects of amphetamine and serotonergic hallucinogens were emphasized (see Table 1). The seminal observations by Arvid Carlsson and his colleagues in the late early 1960's which first postulated functional dopamine receptor blockade as a necessary therapeutic action by the phenothiazine and butyrophenone structural classes set the stage for our current mechanistic understanding of all antipsychotic drugs currently used today (Carlsson and Lindquist, 1963). The emergence of *in vitro* receptor binding and PET receptor occupancy studies in healthy volunteers and patients identified dopamine D2 receptor blockade as a critical feature for antipsychotic drugs in the clinic (Creese et al., 1976; Farde et al., 1988; Seeman et al., 1976). The early identification of dopamine D2 receptor binding sites by Seeman and Snyder in the 1970s and correlating antipsychotic efficacy to dopamine D2 receptor binding, prior to cloning of the D2-like family of dopamine receptors, further enriched the understanding for the molecular target which all antipsychotic drugs share in common (Creese et al., 1976; Seeman et al., 1976). Blockade of 5-hydroxytryptamine<sub>2A</sub> (5-HT<sub>2A</sub>) receptors was an important molecular target complimenting blockade of dopamine D2 receptors for the development of atypical antipsychotic drugs (Kapur et al., 1999; Meltzer et al., 1989).

Clinical observations that channel-blocking N-methyl-D-aspartate (NMDA) receptor blockers induced psychotic symptoms in healthy volunteers or exacerbated the positive, negative and cognitive dysfunction of schizophrenic patients has been the most salient driving force behind the glutamatergic hypotheses regarding the pathophysiology

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and treatment of schizophrenia (Krystal et al., 1999; Luby et al., 1959; Tamminga, 1998). The initial hypothesis of glutamatergic hypofunction was strongly influenced by neuroimaging studies suggesting cortical hypofunction during rest or the performance of cognitive tasks. Later, the non-competitive NMDA receptor antagonists were found preferentially increase metabolism and extracellular glutamate levels in defined limbic circuits rather than over the entire cortical manner (Duncan et al., 1999; Holcomb et al., 2005; Lahti et al., 1995; Moghaddam et al., 1997).

Thus, these studies suggested that subanesthetic doses of non-competitive NMDA receptor antagonists were activating glutamatergic neurons in the cerebral cortex and hippocampus; the glutamate released by these neurons could activate other ionotropic glutamate receptors (AMPA and kainate) or metabotropic glutamate (mGlu) receptors. While these studies do not support a general notion that glutamate function or NMDA receptor function is necessarily decreased everywhere in the schizophrenic brain, the hypothesis remains that impaired NMDA function in important cellular compartments of the limbic forebrain may be a critical feature underlying the pathophysiology of schizophrenia. The variability from neuroimaging studies describing hyperactivation as well as hypoactivation in the brains of schizophrenic patients compared to healthy volunteers suggest that the brain from a schizophrenic patient is more aptly characterized by dysfunctional circuitry rather than simply increased or decreased activity (Callicott et al., 2003).

Concurrently with the notion of a dysfunctional circuitry, the hypothesis that schizophrenia might be a neurodevelopmental disorder gained prominence as a variety of neuroimaging modalities indicated that substantial changes may be present even prior to

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initial treatment. The magnetic resonance imaging (MRI) diffusion tensor imaging (DTI) studies (Ellison-Wright and Bullmore, 2009) were especially important as they highlighted substantial abnormalities in long-loop tracts such as thalamocortical pathways that were consistent with histopathological studies defining a diminished neuropil in the prefrontal cortex and loss of cells in a number, though not all, thalamic stereology studies. In addition to suspected developmental defects in these long-loop pathways connecting the prefrontal cortex, thalamus, striatum, hippocampus, amygdala and dopamine-containing brainstem neurons, a better understanding a local circuit defects in schizophrenia has also emerged. Thus, currently investigators work within the framework that schizophrenia involves dysfunctional local and long-loop network activity rather than there being simply decreased glutamatergic NMDA receptor tone in the brain (Tan et al., 2007).

How does this new view explain the earlier data that channel blocking NMDA receptor antagonist induce psychotomimetic effects in animals and humans? The effects of the NMDA receptor antagonists could occur locally in cortical circuits in the prefrontal cortex or the hippocampus. GABAergic interneurons are known to spend a greater proportion of time in relatively depolarized states compared to pyramidal neurons and would experience a relaxation of  $Mg^{2+}$ -induced block allowing for cation flux through the channel. Several classes of GABAergic interneurons (e.g., chandelier and basket cells) provide a major inhibitory drive on cortical output cells, the pyramidal cells. Thus, systemic administration of non-competitive NMDA receptor antagonists would preferentially block active NMDA channels in interneurons rather than pyramidal cells, resulting in a dysinhibition of circuits. For schizophrenia, hypofunction of NMDA

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receptors on local GABAergic interneurons could play a key role in dysfunction of neuron ensembles leading to disturbances of gamma oscillations that may be the foundation for optimizing cortical function (Ford and Mathalon, 2008; Gonzalez-Burgos and Lewis, 2008). Alternately, dysinhibition of local circuits in areas projecting to crucial limbic circuitry such as hippocampal-prefrontal cortical pathways could account for an increase in activity in the PFC following the acute administration of non-competitive NMDA receptor antagonists. For schizophrenia again, hypofunction of NMDA receptors at various nodes within these micro- and macrocircuits may contribute to disturbances of gamma oscillations leading to disturbances of cognition, affect and bizarre delusions and hallucinations (Roopun et al., 2008). The importance of glutamate and NMDA receptors during normal CNS development is likely to play an important mechanistic role in the neurodevelopmental hypothesis of schizophrenia.

The clinical report that a mGlu2/3 receptor agonist prodrug effectively treated both positive and negative symptoms of schizophrenia patients from a multi-center, double-blind, placebo-controlled study (Patil et al., 2007) is significant for at least several reasons. First, this is the first non-dopamine D2 receptor based treatment that has produced an apparently robust antipsychotic effect. Secondly, the primary effect of activating mGlu2 receptors involves stimulation of an autoreceptor with a limbic localization in many of the regions suspected to be involved in the pathophysiology of schizophrenia (prefrontal cortex, dorsal and ventral striatum, thalamus, hippocampus, amygdale, see Fig. 1, (Ghose and Tamminga, 2007)). Thus, activation of mGlu2 receptors would be expected to decrease glutamate release from excitatory amino acid containing axons/nerve terminals as shown in Fig. 2 (Schoepp, 2001).

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In this minireview we will review evidence that modulating glutamate in dysfunctional limbic circuits may be an important strategy opening up new molecular targets for the treatment of schizophrenia. The discussion first will center around orthosteric mGlu2/3 receptor agonists or mGlu2 receptor positive allosteric modulators (PAMs). The last two targets that will be exemplified will be those that might address hypofunctional NMDA receptor in the miswired dysfunctional limbic circuits, inhibitors of the glycine transporter 1 subtype and mGlu5 receptor PAMs.

### **mGlu2/3 receptor agonists**

It has been more than ten years ago that the first studies using selective mGlu2/3 receptor agonists convincingly demonstrated antipsychotic-like potential of such agents (Cartmell et al., 1999; Moghaddam and Adams, 1998). One of the most publicized pharmacological effects of mGlu2/3 receptor stimulation is the reversal of various effects induced by psychotomimetic drugs such as PCP. For instance, in full accordance with the predominantly presynaptic function of mGlu2 receptors and their role in the negative feedback regulation of excess release of glutamate, LY354740 was shown to attenuate PCP-induced increase in glutamate release in the prefrontal cortex in rats (Moghaddam and Adams, 1998).

Cortical glutamate release is also under control of 5-HT<sub>2A</sub> receptors. 5-HT<sub>2A</sub> receptor activation induces excitatory postsynaptic currents at apical dendrites of pyramidal neurons in the prefrontal cortex. 5-HT<sub>2A</sub> receptor-mediated increases in glutamate release have been linked to cognitive, perceptual, and affective distortions produced by hallucinogenic 5-HT<sub>2A</sub> agonists. Importantly, these effects can be blocked



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not only by 5-HT<sub>2A</sub> receptor antagonists but by mGlu2/3 receptor agonists as well (Marek et al., 2000). These data implicate mGlu2/3 receptors in the common mechanisms underlying actions of different types of psychotomimetic drugs such as PCP-like NMDA receptor channel blockers as well as LSD-like 5-HT<sub>2A</sub> receptor agonists. Physiological significance of such interactions is supported by emerging evidence on co-localization of 5HT<sub>2A</sub> and mGlu2/3 receptors in the cortex (Gonzalez-Maeso et al., 2008).

In full agreement with the results of neurochemical and electrophysiological studies, one of the best documented behavioral effects of mGlu2/3 receptor stimulation is reversal of behaviors induced by PCP-like agents and 5-HT<sub>2</sub> receptor agonists. Reversal of PCP-induced motor hyperactivity was demonstrated for one of the first selective mGlu2/3 receptor agonists LY354740 (Moghaddam and Adams, 1998) and was repeatedly confirmed for this and other mGlu2/3 receptor agonists in several laboratories (e.g., Cartmell et al., 1999). Various motor stereotypies induced by PCP-like drugs and 5-HT<sub>2A</sub> agonists (DOI) are also readily inhibited by mGlu2/3 receptor stimulation (Gewirtz and Marek, 2000; Homayoun and Moghaddam, 2008).

Increased cortical glutamate release is likely to account for the ability of systemic administration of PCP-like agents (such as MK-801) to increase the spontaneous firing rate of prefrontal cortical neurons, to decrease the variability of spike trains, and disrupt patterns of spontaneous bursting. Stimulation of mGlu2/3 receptors was shown to reverse these effects (Homayoun et al., 2005), a finding that is well in line with the results of neurochemical studies.

At the first glance, enhanced glutamatergic activity and increased firing rate of cortical neurons after acute NMDA receptor channel blockade do not fit well with the

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views on hypofrontality in schizophrenia. Indeed, there are several reports on enhanced activity of brain areas involved in pathophysiology of schizophrenia under conditions of limited cognitive load. For example, one study found greater activation in the hippocampus, thalamus, and dorsolateral prefrontal cortex in patients with schizophrenia during the sensory gating task as well as in response to urban noise (Tregellas et al., 2007; Tregellas et al., 2009) while another group reported on elevated hippocampal and amygdala activity during the passive viewing of human faces (Holt et al., 2006). However, when the behavioral performance sets additional demands on these networks, activity will increase further and such above-physiological increase will now be accompanied by abnormally high or lost synchronization, phenomena also observed after acute application of sufficiently high doses of PCP-like drugs (Pinault, 2008; Sebban et al., 2002). Such synchronization is seen as a potential facilitator of neural communication and synaptic plasticity. For the tasks, commonly used to study hypofrontality (e.g., working memory tasks), synchronized network activity is very critical as it favors information retention via increased activity of certain ensembles of neurons. Decorrelated or desynchronized activity may functionally be expressed as hypofrontality.

Another hypothetical approach to look at the relationships between neuronal activity and functional performance is best represented by the analysis of dopaminergic modulation of working memory and prefrontal cortical activity. It is currently believed that there is a certain level of dopamine receptor stimulation (more specifically, D1 receptors) that is required for optimum performance (Arnsten, 1998). Both reduced and increased dopamine receptor stimulation result in impaired cognitive performance. In

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other words, there is an inverted U-shaped relationship between cortical activity and cognitive performance. These studies led further to explain existence of both hyper- and hypofrontality states in schizophrenia patients. Callicott and colleagues (2003) suggested that schizophrenia is associated with the leftward shift in the inverted U function compared to healthy control subjects. At low working memory load, patients are thus expected to appear hyperfrontal. However, their working memory capacity is eventually breached that results in a hypofrontal pattern.

Interestingly enough, cognitive effects of mGlu2/3 receptor agonists may also follow a biphasic dose-effect function. There were reports that acute systemic application of selective mGlu2/3 receptor agonists such as LY354740 impairs performance in cognitive tasks in laboratory animals (Higgins et al., 2004). Following the inverted U-shaped activity-performance function hypothesis outlined above by Callicott and colleagues (2003), one could speculate that the impairing effects of mGlu2/3 receptor agonists are more likely to be observed under somewhat challenging conditions that require a significant increase in cortical activity but still result in proper cognitive performance. In contrast, one may expect then that lower-demand cognitive tasks may fail to detect impairing effects of mGlu2/3 receptor agonists. Perhaps, this explains why no cognitive-impairing effects of LY354740 were observed in humans where tasks may have been optimized to study cognitive impairments produced by ketamine (Krystal et al., 2005).

Within the inverted U hypothesis, it presents no difficulty to understand how a compound that may impair cognition when given alone, is able to reverse cognitive deficits induced by another agent. Moghaddam and Adams (1998) demonstrated that

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LY354740 counteracted the detrimental effects of PCP on working memory performance in the T-maze task. It should be noted that there are negative results as well. More specifically, mGlu2/3 receptor agonists were shown not to be able to antagonize cognition-impairing effects of PCP in other studies where experimental protocols may have been optimized to detect cognition-impairing effects of mGlu2/3 agents (Schlumberger et al., 2009). Further, failures to reverse certain cognitive effects of PCP may be related to the fact that PCP-like agents are able to impair performance in behavioral tasks via a number of different mechanisms including motor activation, behavioral disinhibition, induction of state-dependence, etc. Meanwhile, relationships between excessive glutamate release, synchronization loss and hypofrontality induced by psychotomimetic drugs and/or observed in patients with schizophrenia were studied using mainly working memory tasks and it is unclear to what extent these phenomena contribute to effects of PCP-like agents observed in various other behavioral cognitive tasks.

A study by Moghaddam and Adams (1998) explicitly linked reversal of PCP-induced cognitive impairment with the ability of LY354740 to attenuate PCP-induced cortical glutamate release. Taken together with other work from this and other groups, these data suggest that mGlu2/3 receptor stimulation may reverse this excessive release of glutamate, restore normal neuronal activity and thereby improve cognitive performance.

The only known clinical laboratory study on cognitive effects of mGlu2/3 receptor stimulation in humans directly supports these expectations. Krystal and co-workers (2005) reported that LY354740 induced a dose-dependent reversal of ketamine-

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induced impairment of working memory. These data are supporting the view that mGlu2/3 receptor stimulation should have beneficial effects on cognition in schizophrenia patients and these effects are localized specifically in the domain of working memory, one of the key domains implicated in the pathophysiology of schizophrenia. Given that treatment with LY354740 did not modulate other effects induced by ketamine infusion (e.g., reduced vigilance, increased distractibility, impaired performance on a verbal learning test, increased PANSS scores), it remains to be established whether reversal of working memory impairments (and more general, reversal of schizophrenia-associated hypofrontality) is predictive of clinical efficacy. Further regarding the effects of mGlu2/3 receptor agonists on cognition in humans, LY354740 and the prodrug LY544344 did not induce adverse events suggesting cognitive impairment in over 450 patients with generalized anxiety disorder where this mGlu2/3 receptor agonist and prodrug were found to produce therapeutic effects (Dunayevich et al., 2008; Michelson et al., 2005).

First support for these predictions relative to schizophrenic patients is provided by the results of a recent clinical study where the mGlu2/3 receptor agonist LY404039 (administered as a prodrug known as LY2140023) reduced PANSS total, positive and negative scores in patients with schizophrenia (Patil et al., 2007). Effects of LY2140023 on cognition were not assessed in this study. However, one may want to note that, in the Patil study, there were two cognition-related PANSS items (difficulty in abstract thinking and poor attention) that were reportedly positively affected by mGluR2/3 agonist treatment (communicated by A. Breier at 46<sup>th</sup> ACNP meeting, 9-13 December 2007). While effects of mGlu2/3 receptor agonists on cognition in schizophrenia patients await

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more formal evaluation, these preliminary data may be taken as the first evidence confirming the expectations generated by the results of preclinical studies (Table 1). Further confirmatory studies will be required to understand the therapeutic efficacy effect size for LY2140023, especially compared to aripiprazole, quetiapine, and ziprasidone. These atypical antipsychotic drugs, unlike olanzapine and risperidone, have not been found to provide a clinically significant benefit with respect to treating the positive and negative symptoms of schizophrenia over the first generation of antipsychotic drugs when compared in the CATIE studies or meta-analyses.

One important question of relevance for ongoing and future drug discovery efforts is the mGlu receptor subtype mediating the therapeutic effects of LY2140023 in patients. Genetic association analyses have been consistently pointing at single-nucleotide polymorphisms in the mGlu3 receptor gene (GRM3) in several populations of schizophrenia patients. This GRM3 polymorphism is associated with a non-coding region that does not appear to affect mGlu3 receptor expression outside of a single report suggesting altered levels of mGlu3 receptor homodimers (Corti et al., 2007). However, detailed studies conducted by Weinberger and colleagues suggested a specific molecular pathway by which GRM3 genotype alters glutamate neurotransmission, prefrontal and hippocampal physiology and cognition, and thereby increased risk for schizophrenia (Egan et al., 2004). More specifically, these studies revealed a link between a high-risk GRM3 allele and reduced expression of glial glutamate transporter. While there is not much further evidence in support of this hypothesis, a genetic silencing of glial transporter expression in mice resulted in the behavioral abnormalities that are typically observed after the treatment with psychotomimetic drugs (Karlsson et al., 2008). The

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most intriguing is perhaps that these behaviors were effectively antagonized by stimulation of mGlu2/3 receptors.

However, a series of studies conducted in mGlu2 and mGlu3 receptor knockout mice clearly indicated the role of mGlu2 receptors in the antipsychotic-like effects of mGlu2/3 receptor agonists. Indeed, mGlu2/3 receptor agonists, including the mGlu2/3 agonist with demonstrated antipsychotic activity in the clinic, lose the ability to attenuate behaviors induced by psychotomimetic drugs when tested in mGlu2, but not mGlu3, knockout mice (Fell et al., 2008; Spooren et al., 2000; Woolley et al., 2008).

### **mGlu2 receptor potentiators (e.g., PAMs)**

Another growing body of evidence supporting the significance of mGlu2 receptors in the antipsychotic effects of orthosteric mGlu2/3 receptor agonists comes from a number of studies that demonstrated comparable efficacy of mGlu2 receptor PAMs (Galici et al., 2005; Johnson et al., 2005). The latter class of drugs presents considerable interest for a number of reasons. For instance, orthosteric receptor agonists act at the glutamate binding site and are themselves structurally constrained glutamate analogues that sets several serious limitations (e.g., restricted chemical space, lower receptor selectivity). Given the predominantly extrasynaptic location of mGlu2 receptors that is likely to control the release of glutamate by detecting excess amounts of glutamate that escape the reuptake mechanisms, the ability to selectively target these receptors offers significant advantages. Most importantly, mGlu2 receptor PAMs may not induce any appreciable effects under normal conditions but will enhance negative feedback control under the conditions of abnormally enhanced glutamate release as reflected in

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frequency-dependent modulation of synaptic transmission in the striatum (Johnson et al., 2005). Such ability to “recognize” pathological state(s) and/or networks could result in improved therapeutic efficacy and safety.

Available evidence appears to support such a profile of mGlu2 receptor PAMs. On the one hand, there are no signs that these drugs produce any cognitive impairment in normal subjects. This is an important phenomenon to consider because of the knockout mouse data that specifically implicated mGlu2 receptors in cognitive-impairing effects of mGlu2/3 receptor agonists (Higgins et al., 2004). On the other hand, mGlu2 receptor potentiators reproduce both antipsychotic-like (Table 1) and anxiolytic-like effects of mGlu2/3 receptor agonists (Galici et al., 2005; Johnson et al., 2005). Currently, there is much less information available to claim that positive allosteric modulation of mGlu2 receptors would be sufficient to overcome cognitive impairments associated with acute NMDA receptor channel blockade by PCP-like drugs or with glutamatergic dysbalance associated with schizophrenia. With several research groups actively working in this field and a number of fairly potent compounds already known, characterization of the cognitive effects of selective mGlu2 receptor potentiators will certainly be completed soon.

### **GlyT1 inhibitors**

Among the approaches aiming at the reversal of a suspected NMDA receptor hypofunction in schizophrenia activation of the glycine-site represents the most direct one. Several small clinical trials usually restricted to one or two centers have been performed with either glycine and with D-serine, the natural (full) agonists, with D-



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cycloserine, a partial agonist of the glycine site, and with sarcosine, a naturally occurring low affinity glycine transport inhibitor. Across studies the full agonists were more effective than the partial agonist D-cycloserine and the tested compounds were more efficacious in conjunction with typical and newer atypical antipsychotics than with clozapine (Javitt, 2008). The latter outcome has been attributed to a putative intrinsic component of clozapine enhancing glutamatergic neurotransmission (Coyle, 2006). The most prominent effect across all trials was an improvement of negative symptoms by 15 % (Javitt, 2008). Positive and cognitive symptoms were significantly improved in some but not all trials. Due to the small number of patients recruited in these trials and the negative outcome of one large multi-center trial, the CONSIST study (Buchanan et al., 2007), the trials do not allow a final conclusion about the clinical effectiveness of NMDA glycine-site activation. A key problem in the interpretation of the results is the uncertainty whether appropriate brain concentrations, and more importantly, synaptic levels of the glycine site agonists have been achieved. To obtain a more efficient increase of synaptic glycine and hence NMDA receptor activation, high affinity inhibitors of the glycine transporter 1 (GlyT1) are currently being developed by several pharmaceutical companies.

GlyT1 shows a widespread distribution throughout the brain. In the cerebellum and caudal parts of the CNS GlyT1 appears to be predominantly localized on glial cells and to be involved in the regulation of inhibitory glycinergic neurotransmission (Zafra and Gimenez, 2008), while in the forebrain it has been localized pre- and postsynaptically at glutamatergic synapses. At the molecular level, physical association with syntaxin 1A and with PSD95, an NMDA receptor-associated protein indicates a synaptic localization

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close to NMDA receptors. The vicinity to NMDA receptors makes GlyT1 ideally suited to modulate their activity. A number of companies have GlyT1 inhibitor programs at different stages (preclinical to clinical phase 2) and have published preclinical profiles of some of the compounds. To demonstrate expected target-related effects the compounds were shown to increase extracellular glycine levels in the cortex (Boulay et al., 2008; Depoortere et al., 2005) and hippocampus (Martina et al., 2004) of freely moving rats and to enhance NMDA receptor activity (Boulay et al., 2008; Depoortere et al., 2005; Martina et al., 2004). The results correspond to increases of NMDA receptor-mediated currents in heterozygous GlyT1 knockout mice as well as forebrain selective homozygous GlyT1 knockout mice (Tsai et al., 2004; Yee et al., 2006). Apart from enhancing synaptic activity GlyT1 inhibition also increased LTP in line with the role NMDA receptors in synaptic plasticity (Martina et al., 2004). Together the data support the validity of the concept to enhance NMDA receptor activity by GlyT1 inhibition.

Preclinical antipsychotic-like effects of GlyT1 inhibitors are described in an increasing number of publications which can only in part be summarized in this review (Table 1). Several compounds have been reported to antagonize the hyperlocomotion induced by PCP or MK-801 (Boulay et al., 2008; Depoortere et al., 2005; Harsing et al., 2003; Singer et al., 2009). In adult rats treated with PCP at neonatal age the enhanced locomotor response to amphetamine could be reversed (Depoortere et al., 2005). The attenuation of locomotor responses may, at least in part, be mediated via an effect on dopaminergic neurotransmission. GlyT1 inhibitors have been reported to inhibit PCP and methamphetamine-induced (Drescher et al., 2006) (Drescher, personal communication) and ethanol-induced (Molander et al., 2007) dopamine release in the nucleus accumbens,

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whereas basal dopamine in the PFC was increased (Depoortere et al., 2005). In chronically PCP-treated rats the increased sensitivity of the DA release as well as the locomotor response to amphetamine could be reversed by GlyT1 inhibition (Javitt et al., 2004). These results indicate the capability to reverse evoked or sensitized mesolimbic hyperdopaminergic states and to increase basal dopamine in the PFC suggesting a beneficial effect on positive symptoms associated with imbalanced dopaminergic activity. Support for efficacy against positive symptoms comes also from the reported reversal of amphetamine-disrupted latent inhibition (Black et al., 2009), a model thought to reflect increased salience and distractibility associated with psychotic symptoms.

By increasing dopamine in the PFC in conjunction with an expected enhancement of NMDA receptor activity, GlyT1 inhibitors are believed to reverse dysfunctional PFC-subcortical circuitry associated with negative symptoms. In support of potential efficacy against negative symptoms we have observed the attenuation of PCP-induced social interaction deficits by different GlyT1 inhibitors (data not shown). Furthermore the two GlyT1 inhibitors SSR103800 and SSR504734 were found to reverse abnormally persistent latent inhibition induced by MK801 (Black et al., 2009) which has been linked to impaired set shifting associated with negative symptoms (Weiner, 2003).

Since the latter model is also thought to reflect cognitive inflexibility the results point to the ability of GlyT1 inhibition to re-instate cognitive flexibility. Several other effects of GlyT1 inhibitors in the cognitive domain have been reported. SSR103800 (Boulay et al., 2008) and NFPS (Karasawa et al., 2008) reversed object recognition impaired by acute or chronic NMDA receptor antagonists; SSR504734 reversed impaired social discrimination in rats treated with PCP at neonatal age (Harich et al., 2007) and

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SSR504734 enhanced working memory performance in a continuous delayed alternation task in C57BL/6 mice (Singer et al., 2009). These effects are of relevance for improving cognition in schizophrenia since these models reflect particular schizophrenia-related cognitive deficits such as impaired visual object memory, face recognition memory (Calkins et al., 2005) and working memory. Altogether, available data describe a broad antipsychotic-like profile of GlyT1 inhibitors promising efficacy across different symptoms in schizophrenia. Little is known about the cells or circuits mediating the reported effects. Numerous examples of GlyT1 inhibitors antagonizing effects of NMDA receptor antagonists suggest that GlyT1 inhibitors may affect cells which exhibit a particular sensitivity for NMDA receptor antagonists. An intriguingly selective PCP antagonistic effect was recently reported for SSR504734, which completely and selectively reversed the pattern of brain activation by PCP in a pHMRI study (Gozzi et al., 2008). The most plausible explanation for this observation is the reversal of reduced NMDA receptor activity at GABAergic interneurons suspected to have a particular sensitivity for a blockade by non-competitive NMDA receptor antagonists (Coyle, 2004).

The re-instatement of impaired NMDA receptor activity at interneurons (but not necessarily selectively at interneurons) may be fundamental for the antipsychotic activity of GlyT1 inhibitors. It was shown in a recent study that the expression of the GluN2A (NMDA NR2A subunit) in GAD67 positive cells in layer 2 and 5 of the cingulate cortex of subjects with schizophrenia is dramatically reduced by more than 50% (Woo et al., 2008). The reduced GluN2A expression is likely due to a reduced NMDA receptor activity at the affected interneurons since it has been shown in other studies that GluN2A expression appears to be downregulated by reduced glutamatergic input and vice versa.

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In line with this assumption subchronic treatment with MK-801 has been shown to almost completely downregulate the expression of NMDA receptor subunits and parvalbumin in microdissected parvalbumin positive interneuron in the rat frontal cortex (Xi et al., 2009). Whether GlyT1 inhibition is able to enhance NMDA receptor expression in GABAergic interneurons needs to be demonstrated.

There have been concerns that GlyT1 inhibition may be associated with unwanted side effects, in particular with motor impairments and respiratory effects mediated via activation of glycine A receptors in the cerebellum and in the brainstem. It is important to note that these concerns are based on effects of a subclass of GlyT1 inhibitors, which contain a sarcosine moiety in their structure. It has been shown that sarcosine-derived inhibitors exhibit an allosteric and irreversible inhibition while non-sarcosine-derived structures were found to be reversible competitive inhibitors (Mezler et al., 2008). Consistent with an irreversible inhibition, sarcosine-derived inhibitors produce slowly increasing and sustained locomotor impairments (Perry et al., 2008) pointing to cumulating binding associated with a poorly controllable degree of inhibition. Non-sarcosine-derived structures may exhibit a more appropriate safety margin, not only because of their reversible mode of action, but also because of the competitive and surmountable transport inhibition. Different glycine concentrations may lead to subtle though critical differences in the degree of inhibition in different target regions. The competitive compounds currently in development will hopefully provide the proof whether GlyT1 inhibition represents an efficacious antipsychotic mechanism at safe doses.

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### **mGlu5 receptor potentiators**

The mGlu5 receptor mRNA and protein is distributed widely throughout the limbic forebrain (e.g., cortex, hippocampus, dorsal and ventral striatum) with a relatively sparse distribution in much of the brainstem (Abe et al., 1992; Romano et al., 1995). One of the interesting relationships of the postsynaptic mGlu5 receptor is coupling to NMDA receptors through the Homer family of scaffolding proteins. Most of the immunoreactivity with antibodies recognizing mGlu5 is found on membranes of dendritic spines and shafts. One function of certain Homer proteins may be to bring the mGlu5 receptor into close physical proximity with the NMDA receptor at synaptic sites. A second cellular compartment implicated in the pathophysiology of schizophrenia that mGlu5 receptors may share with NMDA receptors are glial cells. The understanding that much of the signal in 2-deoxyglucose experiments is derived from the functional coupling of astrocytes and nerve terminals highlights the potential importance of these glial glutamate receptors in modulating local circuit activity. A third shared cellular compartment between mGlu5 receptors and NMDA receptors may be interneurons in a variety of cortical and subcortical sites. However, while NMDA receptors with various heteromeric combinations of subunits are present in almost all neurons in the brain, mGlu5 receptors may be restricted to certain subclasses of GABAergic interneurons as appears the case in the primate prefrontal cortex (Muly et al., 2003) and hippocampal formation (Kerner et al., 1997). A numerically minor cellular compartment where mGlu5 receptors are also found are in axons of presumed glutamatergic neurons where they may enhance glutamate release in the prefrontal cortex and neocortex (Marek and Zhang, 2008; Muly et al., 2003; Musante et al., 2008; Romano et al., 1995; Thomas et al., 2000).

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Homer1 proteins may play a regulatory role in targeting mGlu5 receptors to axonal or dendritic compartments (Ango et al., 2000).

Amongst the effects seen in mice with a deletion of the mGlu5 receptor, sensorimotor gating as reflected by prepulse inhibition was disrupted in using several mice knockout strains with different backgrounds (Brody et al., 2004b). Neither clozapine nor haloperidol reversed the prepulse inhibition deficit in the mGlu5 receptor -/- strain at doses which were active in reversing either amphetamine- or ketamine-induced deficits in wild type mice (Brody et al., 2004a). The effects of mGlu5 receptor PAMs in animals and mGlu5 receptor negative allosteric modulators in man described below, however, suggest caution in over-interpreting data with transgenic mice lines.

For both the group I (mGlu1 and mGlu5) and group II (mGlu2 and mGlu3) mGlu receptors, the development of selective agonists within a group with appropriate drug-like properties has been very difficult. As a result, the focus shifted developing selective compounds modulating a single mGlu receptor has focused on developing positive or negative allosteric agonists/modulators. A number of series of mGlu5 receptor PAMs have been developed which enhance  $Ca^{2+}$  mobilization in response to glutamate in cells transfected with mGlu5 receptors. Several different allosteric binding sites have been identified for these mGlu5 receptor PAMs; one site appears to bind both PAMs and NAMs. A further complication is that different mGlu5 PAMs appear to differentially recruit different signaling pathways in cortical astrocytes (Zhang et al., 2005); this effect may relate to the two different allosteric binding sites identified. The mGlu5 receptor PAM N-{4-chloro-2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide (CPPHA) has been shown to enhance NMDA receptor activity via

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potentiation of mGlu5 receptor function in hippocampal slices (O'Brien et al., 2004).

Both in vitro and in vivo studies supporting utility in treating cognitive dysfunction may be found in a recent report that the mGlu5 receptor PAMs facilitated both hippocampal long-term potentiation and long-term depression in addition to enhancing performance in the Morris water maze (Ayala et al., 2009). Additional evidence for a pro-cognitive effect of mGlu5 receptor PAMs was found in attenuation of performance impairments induced by MK-801 on a operant set-shifting task when a mGlu5 receptor PAM was administered following the channel blocking NMDA receptor antagonist (Darrah et al., 2008). The Addex mGlu5 receptor PAM ADX47273 was also reported to attenuate natural or scopolamine-induced forgetting in the novel object recognition task (Epping-Jordan et al., 2005).

Further evidence consistent with antipsychotic action for mGlu5 receptor PAMs has been collected for different chemical platforms originating from Merck and Addex scientists, respectively, using classical antipsychotic screening tests (Table 1). For example, CDPPB and/or ADX47273 have been found to suppress amphetamine-induced hyperactivity or amphetamine-induced deficits in prepulse inhibition in rats (Epping-Jordan et al., 2005; Kinney et al., 2005; Liu et al., 2008). Several different mGlu5 receptor PAMs have also been reported to attenuate locomotor hyperactivity induced by channel blocking NMDA receptor antagonists (Chan et al., 2008; Homayoun and Moghaddam, 2008; Liu et al., 2008); while mGlu5 receptor NAMs have been reported to enhance locomotor hyperactivity induced by channel blocking NMDA receptor antagonists. The mGlu5 receptor PAM ADX47273 also exerted an antipsychotic-like



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effect in a classical screen by reducing conditioned avoidance responding in the rat (Liu et al., 2008).

While mGlu5 receptor PAMs have not yet reached phase 2 proof-of-concept testing in patients, a putative anxiolytic drug, fenobam, was investigated in phase 2 trials that was subsequently determined to be a selective mGlu5 receptor NAM (Porter et al., 2005). While there was modest clinical evidence for an anxiolytic effect of fenobam in patients, it was discontinued after this medication appeared to induce derealization and depersonalization in patients with anxiety disorders. While these observations may portend positive effects for mGlu5 receptor PAMs in the clinic for schizophrenia, it still remains unknown if these adverse events were due to the pharmacology of the parent molecule.

Many questions remain for this target. What symptom domains of schizophrenia will mGlu5 PAMs effectively treat? Which cellular locus of action(s) will be responsible for these effects? If mGlu5 receptor PAMs are effective in treating schizophrenia, will these effects be related to coupling of mGlu5 receptors with NMDA receptors through scaffolding proteins or will they be independent of this physiological coupling?

## **Conclusions**

Over the last 25 years, hypotheses reflecting glutamate hypofunction and then later NMDA receptor hypofunction in schizophrenia have been reinterpreted by the growing recognition that this pathophysiological principle acts within the context of dysfunctional local circuits and miswired long-loop pathways between the prefrontal cortex, hippocampus, striatum, thalamus, amygdala, and the ventral tegmental area. A

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number of the leading genetic susceptibility candidates are intimately involved in glutamatergic neurotransmission. Furthermore, there has been a growing understanding in the neurodevelopmental biology for NMDA and mGlu receptors. In this context, it is not especially surprising that targets which profoundly alter the synaptic availability of glutamate and/or directly modulate NMDA receptor function demonstrate strong localization within cortical and subcortical circuits implicated in schizophrenia. It is expected that better understanding the nature by which ensembles of neurons lead to oscillatory behavior both within a given region and between cortical and subcortical regions will help us optimize glutamatergic treatments for schizophrenia. It is also hoped that such translational work between rodents, non-human primates and humans will also lead to additional breakthroughs to treat this devastating illness.

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## Legends for Figures

Fig.1. Distributed dysfunctional cortical-subcortical circuits in schizophrenic patients. Schematic of hypothetical neural systems underlying the three major symptom domains of schizophrenia: positive symptoms or psychosis, cognitive dysfunction, and negative affect. AH = anterior hippocampus; ACC = anterior cingulate cortex; mPFC = medial prefrontal cortex; DLPFC = dorsolateral prefrontal cortex; V. Pall = ventral pallidum; V Str. = ventral striatum; SN/VTA = substantia nigra / ventral tegmental area. (Adapted from (Ghose and Tamminga, 2007) )

Fig. 2. Glutamatergic neurotransmission with and primary localization of mGlu and ionotropic glutamate receptors with respect to presynaptic glutamatergic nerve terminals, post-synaptic cells, presynaptic GABAergic nerve terminals and glia. A primary function of mGlu2 receptors is as an autoreceptor for glutamatergic nerve terminals. The mGlu3 receptor is present as a heteroreceptor on a number of GABAergic interneurons throughout the brain. This receptor is one of two primary mGlu receptors, along with mGlu5 receptors, that modulate the function of glia. The mGlu3 receptor is also present as a postsynaptic receptor as well. The mGlu5 receptor has been intensely studied with respect to functional positive coupling with NMDA receptors. A portion of these receptors also appear located on glutamatergic axons/nerve endings in addition to the glial localization noted above. This figure was originally published by Schoepp (2001).

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## Tables

Table 1. Effects of atypical antipsychotic drugs and drugs modulating targets modulating the glutamatergic system on positive and negative symptoms of schizophrenia and preclinical behavioral paradigms relevant to these symptom domains in addition to cognitive dysfunction.

Behavioral measure	D2/5HT2A antagonists	mGlu2/3 agonists	mGlu2 PAMs	GlyT1 Inhib.	mGlu5 PAMs
POSITIVE SYMPTOMS (clinic)	YES	YES	?	? <sup>1</sup>	?
NMDA-R antagonist -induced hyperlocomotion	Yes <sup>2</sup>	Yes <sup>3</sup>	Yes <sup>4</sup>	Yes <sup>5</sup>	Yes <sup>6</sup>
Amphetamine-induced hyperlocomotion	Yes <sup>2</sup>	Yes <sup>7</sup>	Yes <sup>8</sup>	Yes <sup>9</sup> /No <sup>10</sup>	Yes <sup>11</sup>
Serotonergic hallucinogen- induced head shakes/twitches	Yes <sup>12</sup>	Yes <sup>13</sup>	Yes <sup>14</sup>	n.r.	n.r.
Conditioned avoidance response	Yes <sup>2</sup>	Yes <sup>15</sup>	n.r.	n.r.	Yes <sup>16</sup>
NEGATIVE SYMPTOMS (clinic)	YES	YES	?	? <sup>1</sup>	?
PCP-induced social interaction deficits	Yes <sup>17</sup>	n.r.	n.r.	n.r.	n.r.
Improve COGNITION* (preclinical)	no	yes/no	yes	yes	yes

n.r., not reported

\* see text for discussion of improvement from baseline cognitive performance or provocation-induced impairment of cognition/executive function

<sup>1</sup> Results of double-blind, placebo-controlled studies not reported yet for a number of GlyT1 inhibitors tested (phase 1/2 studies) including GSK1018921, ORG 25935 (SCH900435), RG1678, R231857

<sup>2</sup> Geyer and Ellenbroek for review, 2003

<sup>3</sup> Moghaddam and Adams, 1998; Cartmell et al., 1999; Rorick-Kehn et al., 2007

<sup>4</sup> Johnson et al., 2005; Galici et al., 2005

<sup>5</sup> Boulay et al., 2008; Depoortere et al., 2005; Harsing et al., 2003; Singer et al., 2009

<sup>6</sup> Chan et al., 2008; Homayoun and Moghaddam, 2008; Liu et al., 2008

<sup>7</sup> Gallici et al., 2005; Rorick-Kehn et al., 2007

<sup>8</sup> Gallici et al., 2005

<sup>9</sup> see Depoortere et al. (2005) for positive results in rats treated with PCP as neonates

<sup>10</sup> see Singer et al (2009) for GlyT1 inhibitor potentiation

<sup>11</sup> Kinney et al., 2005; Liu et al., 2008

<sup>12</sup> Sanchez and Arnt, 2000

<sup>13</sup> Gewirtz and Marek, 2000; Klodzinska et al., 2002; Benneyworth et al., 2007

<sup>14</sup> Benneyworth et al., 2007

<sup>15</sup> Takamori et al., 2003; Rorick-Kehn et al., 2007

<sup>16</sup> Liu et al., 2008

<sup>17</sup> Nilsson et al., 2006



