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Targeting glutamate synapses in schizophrenia

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Abstract

Although early clinical observations implicated dopamine dysfunction in the neuropathology of schizophrenia, accumulating evidence suggests that multiple neurotransmitter pathways are dysregulated. The psychotomimetic actions of NMDA receptor antagonists point to an imbalance of glutamatergic signaling. Encouragingly, numerous preclinical and clinical studies have elucidated several potential targets for increasing NMDA receptor function and equilibrating glutamatergic tone, including the metabotropic glutamate receptors 2, 3 and 5, the muscarinic acetylcholine receptors M₁ and M₄, and the glycine transporter GlyT1. Highly specific allosteric and orthosteric ligands have been developed that modify the activity of these novel target proteins, and in this review we summarize both the glutamatergic mechanisms and the novel compounds that are increasing promise for a multifaceted pharmacological approach to treat schizophrenia.

Dysfunction of multiple neurotransmitter pathways in schizophrenia

Schizophrenia is a potentially debilitating psychiatric disorder comprised of multiple overlapping symptom clusters, notably positive symptoms, negative symptoms, and cognitive disturbances. Positive symptoms include hallucinations and delusions, negative symptoms include depression and social withdrawal, and cognitive disturbances include disordered thought processing and disruptions in working memory (see Glossary). Although traditional antipsychotic drugs have demonstrated utility in treating the positive symptoms of schizophrenia, current treatments are limited in their ability to alleviate the negative and cognitive symptom clusters. Furthermore, the biological underpinnings of schizophrenia still remain to be elucidated.

The etiology of schizophrenia is hypothesized to be the result of both abnormalities in local activity within several brain regions and dysfunctional interactions between cortical and subcortical circuits [1]. Histological and structural imaging studies of schizophrenic brains have identified neuroanatomical alterations, such as the loss of glutamatergic pyramidal cell spines and axons, loss of GABAergic interneurons, and decreased mesocortical dopaminergic innervation, in the prefrontal cortex (PFC), and many studies have attributed cognitive impairments to cell loss within thalamic subregions and a subsequent decrease in excitatory thalamic afferents to the PFC [2–4]. Aberrant glutamatergic, GABAergic and dopaminergic signaling, owing to a loss of neuronal connections or neurons themselves, in brain regions such as the hippocampus, striatum, and amygdala, as well as auditory and visual cortex, have also been reported [5–9], and a recent meta-analysis of diffusion tensor

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imaging studies concludes that white matter tracts connecting cortico-thalamic and cortico-limbic structures are reduced in schizophrenic patients, highlighting the disconnection of these networks [10]. Thus, it is increasingly clear that there is not a single locus of dysfunction within the schizophrenic brain, and symptoms likely arise from impaired interactions between multiple brain regions [1, 11].

Pharmacological exploration of schizophrenia began with the serendipitous observation that antagonists of the dopamine receptor D₂, such as chlorpromazine, induced marked improvements in positive symptoms. Indeed, subsequent traditional antipsychotics have relied on antagonism of the D₂ receptor. Conversely, indirect dopamine agonists, such as amphetamine, have psychotomimetic effects in healthy individuals. In addition, it has been shown that excessive dopamine is released in the striatum of the schizophrenic patient, while dopamine in the PFC is depleted, lending support to a dopaminergic hypothesis of schizophrenia [12]. This dopamine hypothesis, however, cannot explain all behaviors associated with the three symptom clusters, nor do dopamine receptor antagonists demonstrate utility in treating all symptoms. The observations that phencyclidine (PCP) and ketamine, both NMDA receptor antagonists, induce psychotic behaviors reminiscent of schizophrenia contributed to the hypothesis that disruption of glutamatergic signaling may also play a significant role in the disease (for review see [15]) [13, 14]. The NMDA receptor is activated by the binding of co-agonists glutamate and glycine, in concert with membrane depolarization, to open the receptor channel and allow an influx of calcium, and, along with AMPA glutamate receptors, mediates fast synaptic transmission in the central nervous system. From these observations have developed several variants of the NMDA hypofunction hypothesis which postulates that changes in brain circuits induced by reduced NMDA receptor function are similar to those underlying the primary pathophysiology that occurs in schizophrenia patients [16-18]. Expanding on this central tenet, several studies suggest that decreased NMDA receptor function in subcortical regions leads to disinhibition of glutamatergic and dopaminergic signaling in the cortex, resulting in sensory, cognitive and behavioral deficits [11, 18, 19].

Because NMDA receptor hypofunction is hypothesized to be important for all three symptom clusters of schizophrenia, it is also predicted that interventions that potentiate NMDA receptor function have the potential to alleviate these symptoms. However, treatments acting directly on the glutamate binding site of NMDA receptors are considered to be impractical due to this receptor's widespread distribution in the CNS and the potential toxicity induced by glutamatergic overactivation of NMDA receptor cation channels [20]. Therefore, increased attention has been placed on identifying pharmacological targets that indirectly increase NMDA receptor function or mitigate the consequences of NMDA receptor hypofunction without inducing glutamatergic overexcitation. In this review, we will discuss the therapeutic potential of several such targets: the metabotropic glutamate receptors (mGlu receptors) 2, 3 and 5, muscarinic acetylcholine receptors M₁ and M₄, and the glycine transporter GlyT1 (Figure 1).

mGlu receptors as modulators of NMDA activity

Recent evidence has raised the possibility that several mGlu receptor subtypes could provide novel drug targets for the treatment of schizophrenia. While ionotropic glutamate receptors, such as NMDA and AMPA receptors, are responsible for fast excitatory synaptic responses, the mGlu receptors, which are G protein-coupled receptors (GPCRs), play a role in modulation of synaptic transmission [21, 22] (Box 1). Due to the highly conserved nature of the glutamate binding site, selective orthosteric ligands for mGlu receptors have been difficult to develop. In addition, persistent activation of mGlu receptors, like many GPCRs, with the orthosteric agonist glutamate often results in desensitization of receptor response

and down-regulation of receptor expression. For these reasons, increasing interest has been placed on identifying compounds that interact with mGlu receptors at allosteric, rather than orthosteric, sites to modulate receptor function. Positive modulation can lead to potentiation of receptor function (positive allosteric modulators, or PAMs) or direct activation of the receptor (allosteric agonists). Pure PAMs are inactive until the endogenous orthosteric ligand is present, resulting in the potential to maintain the temporal and spatial conformity of endogenous physiological signaling (Figure 2). Negative modulation of receptor function (negative allosteric modulators, or NAMs) leads to antagonism of orthosteric agonist activity. Because allosteric binding sites are topographically distinct from the orthosteric site and display far less sequence conservation, allosteric modulators offer greater potential for receptor subtype selectivity [23].

Interestingly, mGlu₅ has been shown to both physically and functionally interact with NMDA receptors within GABAergic interneurons of the forebrain. Numerous electrophysiological studies have demonstrated that activation of mGlu₅ potentiates the activity of NMDA receptors in the striatum, hippocampus and subthalamic nucleus [24]. In addition, mGlu₅ knock-out (KO) mice display deficits in NMDA receptor-dependent learning as well as pre-pulse inhibition (PPI), an animal model of the sensory-motor gating impairment that is a hallmark of schizophrenia [25, 26]. In contrast, inhibition of mGlu₅ activity by the selective allosteric antagonist MPEP potentiates the psychotomimetic effects of PCP, further suggesting a critical interplay between these two receptors in maintaining glutamatergic tone [27]. Thus, activation of mGlu₅ offers a novel mechanism to increase NMDA receptor function that may not induce the toxicity associated with NMDA agonists.

Development of mGlu₅ agonists has also been met with challenges. It has been shown that activation of mGlu₅ with the Group I orthosteric agonist DHPG induces both long-term potentiation (LTP) and long-term depression (LTD), as well as epileptiform activity in hippocampal slices and seizures in animals [28, 29]. Significant strides, however, have been made in the development of mGlu₅ PAMs. The first mGlu₅ PAM, DFB, demonstrated the ability to increase mGlu₅'s response to agonist - in effect, shifting the concentration response curve for glutamate to the left (Figure 2) - without inducing a response in the absence of agonist [30]. Building on this proof-of-concept compound was the discovery of CPPHA. An mGlu₅ PAM that is structurally distinct from DFB, CPPHA possesses sufficient aqueous solubility to be used in native preparations and, importantly, demonstrated that application of an mGlu₅ PAM concurrently with agonist potentiates NMDA receptor function in hippocampal slices [31]. The properties of CPPHA, however, were not sufficient for utilization in rodent behavioral models. A third generation of mGlu₅ PAM is represented in CDPPB, a potent and highly selective potentiator that acts at the site of MPEP binding. As the first mGlu₅ PAM to possess sufficient pharmacokinetic properties for *in vivo* applications, CDPPB demonstrated robust efficacy in reversing amphetamine-induced hyperlocomotion and disruption of PPI, two animal models widely used to predict efficacy in treating the positive symptoms of schizophrenia [32]. CDPPB also has efficacy in increasing hippocampal plasticity [33] as well as blocking the effects of NMDA receptor antagonists on neuronal firing in the prefrontal cortex [34]. CDPPB blocks impairments in set-shifting tasks induced by the NMDA receptor antagonist MK-801 [35, 36], and an optimized analog of CDPPB, VU-29, potentiates both LTD and LTP in hippocampal slices [28]. Recently, the structurally distinct mGlu₅ PAMs VU0360172, CPPZ, and ADX47273 have been shown to reverse amphetamine-induced hyperlocomotion, contributing further diversity to the growing collection of mGlu₅ PAMs that have *in vivo* efficacy [37-40]. While these preclinical findings are encouraging, human clinical trials will be critical for establishing the viability of mGlu₅ PAMs as therapeutics in the treatment of schizophrenia.

Group II mGlu receptors offer additional opportunities for therapeutic intervention

Agonists for the Group II mGlu receptors (mGlu₂ and mGlu₃) may also have potential as novel antipsychotics. The Group II mGlu receptors are primarily expressed in cortical and limbic areas of the forebrain and function as inhibitory receptors both pre- and postsynaptically. As discussed, it is hypothesized that hypofunction of NMDA receptors in subcortical regions leads to decreased activation of inhibitory GABAergic interneurons, thereby disinhibiting glutamatergic output in the PFC [11]. Evidence from animal models treated with PCP or ketamine suggests that Group II agonists may decrease this excessive glutamate release by acting at presynaptic autoreceptors on glutamatergic neurons [41]. The highly selective mGlu_{2/3} agonists LY379268 and LY354740 block the hyperlocomotion induced by PCP [42], and LY354740 attenuates MK801-induced abnormal neuronal firing in the PFC [43], as well as amphetamine-induced hyperlocomotion and striatal dopamine release [44]. In some preclinical studies, LY354740 reduces PCP-induced cognitive deficits in working memory tasks [19]; however, in other animal models, LY354740 induced deficits in spatial learning and working memory tasks, suggesting that its influence on cognitive function warrants further study [45, 46]. The antipsychotic efficacy of LY354740 in schizophrenic patients has yet to be examined, but interestingly, this agonist has been shown to improve working memory performance in healthy adults treated with ketamine [47]. In addition, a structurally distinct Group II agonist, LY404039, has advanced into clinical trials for treatment of schizophrenia. In one study, LY404039, administered as the oral pro-drug LY2140023, was shown to significantly improve both positive and negative symptoms in schizophrenic patients when compared to placebo [48]; however, additional clinical trials of LY2140023 have yielded inconclusive results [49] and follow-up studies are ongoing.

The respective contributions of mGlu₂ and mGlu₃ in mediating these antipsychotic effects remain the subject of intense investigation. Several conflicting studies have identified alterations in both the protein and mRNA levels of mGlu₂ and mGlu₃ in brains of schizophrenic subjects, but have yet to bring clarity to their respective contributions to the disease [41, 50, 51]. Perhaps the most compelling evidence implicating an individual mGlu subtype in the antipsychotic efficacy of mGlu_{2/3} agonists comes from studies of mGlu₂ and mGlu₃ KO mice. The antipsychotic-like effects of LY404039 and LY379268 are absent in mGlu₂ KO mice but not mGlu₃ KO mice, suggesting that inhibition of mGlu₂ may be driving the antipsychotic effects observed clinically [52, 53]. In addition, mGlu₂ has been shown to physically and functionally associate with the serotonin receptor 5HT_{2A} [54, 55], and recent analyses of these clinical trials have linked clinical response to LY2140023 to single nucleotide polymorphisms in the 5HT_{2A} receptor gene [56]. Such evidence for the involvement of mGlu₂ has led to focused efforts to develop mGlu₂-specific PAMs, which may avoid the tolerance observed for mGlu_{2/3} agonists in reversing PCP- and amphetamine-induced hyperlocomotion [57]. Among a wide array of structurally diverse mGlu₂ PAMs, the two prototypical mGlu₂ PAMs LY487379 and BINA have been shown to enhance the receptor's response to orthosteric agonists, including both glutamate and Group II agonists [58, 59]. Specifically, BINA modulates excitatory neurotransmission in the medial PFC, attenuating 5HT_{2A}-induced increases in spontaneous excitatory postsynaptic firing in the PFC and suppressing the resulting head twitch associated with 5HT_{2A} activation [60] as well as reversing PCP-induced hyperlocomotion [61]. These results suggest that inducing activation of Group II mGlu receptors, specifically mGlu₂, may be a viable strategy for the treatment of schizophrenia, but additional clinical data is needed to validate this hypothesis.

Targeting glutamate signaling via non-glutamatergic receptors

In addition to aberrant monoaminergic and glutamatergic signaling, changes in cholinergic activity have also been implicated in the etiology of schizophrenia. Postmortem biochemical analyses of schizophrenic subjects have revealed a reduction in muscarinic cholinergic receptor density in both the PFC and the hippocampus and early studies of acetylcholinesterase inhibitors demonstrated antipsychotic activity, albeit with significant limiting side effects (see [62] for review). Several atypical antipsychotics have activity at the muscarinic cholinergic receptors, as well as dopaminergic and serotonergic receptors, further indicating that muscarinic receptor activity may contribute to antipsychotic effects.

Of the five muscarinic acetylcholine receptors (Box 1), M_1 and M_4 have been strongly implicated as potential targets for ameliorating the cognitive and behavioral deficits of schizophrenia. Studies of M_1 KO mice have revealed reductions in hippocampal LTP and impaired working memory, as well as increased striatal dopamine levels and hyperlocomotion, mimicking several hallmarks of schizophrenia [62, 63]. M_4 KO mice display increased deficits in PPI following PCP administration as well as elevated basal and amphetamine-induced levels of dopamine [63]. Taken together, these studies suggest that cholinergic activity via M_1 and M_4 may intersect with both glutamatergic and dopaminergic pathways in the pathogenesis of schizophrenia. At the molecular level, the M_1 receptor has been shown to colocalize with NMDA receptors in hippocampal pyramidal cells, and activation of M_1 potentiates NMDA-mediated currents in the hippocampus [64]. In addition, the clinical effects of xanomeline have demonstrated the potential antipsychotic effects of M_1 and M_4 activation. Xanomeline is an M_1/M_4 -preferring agonist that has been demonstrated to cause dramatic improvements in psychotic symptoms as well as significant improvements in cognitive symptoms in patients with Alzheimer's disease [65]. In a double-blind, placebo-controlled trial, xanomeline demonstrated robust efficacy in ameliorating positive symptoms such as hallucinations and vocal outbursts in schizophrenic patients, as well as modest gains in cognitive function [66]. Due to its lack of true selectivity in the periphery, resulting in excessive salivation, lachrymation and GI disturbances in some patients, xanomeline's clinical utility is limited [67, 68]. In addition, the respective contributions of M_1 and M_4 to the clinical efficacy of xanomeline remain to be confirmed.

Similar to orthosteric agonists for the mGlu receptors, orthosteric agonists for the muscarinic receptors have been difficult to develop due to the high conservation of the acetylcholine (ACh) binding site. Thus, significant efforts have focused on developing highly specific allosteric ligands for M_1 and M_4 [69]. Building on the allosteric potential demonstrated by the early M_1 agonists brucine and MCNA343, the selective agonists AC-42 and N-desmethylozapine (NDMC) have demonstrated robust activation of M_1 , high specificity for M_1 over other muscarinic receptors, and potentiation of NMDA receptor activity in hippocampal slices [70]. Of these, the finding that NDMC is an M_1 agonist is especially interesting because this compound is the major circulating metabolite of clozapine, an atypical antipsychotic with a unique efficacy profile. Interestingly, the circulating levels of NDMC are more closely related to measures of efficacy in patients than are the levels of the parent compound [71, 72]; however, the independent efficacy of NDMC is hypothesized to occur at prohibitively high doses [73].

Systemically active selective M_1 agonists, such as TBPB, 77-LH-28-1 and AC-260584, offered renewed promise for developing M_1 agents for treatment of schizophrenia. In cell-based assays, electrophysiological preparations, and behavioral models, TBPB, 77-LH-28-1, and AC-260584 have antipsychotic and cognitive-enhancing properties with reduced side effects compared with less-specific acetylcholinesterase inhibitors [74-76]. Further improving the pharmacokinetic profile of M_1 modulators are VU0184670 and VU0357017,

which possess high selectivity for M₁ versus a large panel of ancillary targets, excellent brain penetration, and robust efficacy in reversing cognitive deficits induced by the muscarinic antagonist scopolamine [77]. Interestingly, recent evidence suggests that some M₁ agonists previously characterized as allosteric may, in fact, be bitopic, acting at both allosteric and orthosteric sites [78, 79].

In addition to M₁ agonists, significant progress has also been made in the development of M₁ allosteric potentiators. Historically, M₁ PAMs have been shown to occupy a single allosteric binding site despite great diversity in their chemical structures [78]; the prototypical M₁ PAMs, however, have shown poor selectivity and failed to be useful for *in vivo* studies. Representing another unique structure, the M₁ PAM benzyl quinalone carboxylic acid (BQCA) has demonstrated robust selectivity and efficacy in potentiating M₁ responses to ACh [80, 81]. BQCA has been shown to increase spontaneous excitatory postsynaptic potentials in pyramidal neurons of the medial PFC as well as increase PFC neuron firing *in vivo* [81]. In behavioral paradigms, BQCA reverses scopolamine-induced deficits in memory task performance and improves discrimination reversal learning in a rodent model of Alzheimer's disease, suggesting the therapeutic potential of M₁ activation in both schizophrenia and Alzheimer's disease [80, 81].

While M₁ is thought to mediate, in part, the effects of xanomeline in schizophrenia patients, it is thought that M₄ is also involved. Recent evidence suggests that xanomeline's M₄-mediated antipsychotic activity can be attributed to a subset of M₄ receptors co-expressed with D₁ receptors on GABAergic interneurons of the striatum [82]. Interestingly, the drug development efforts of multiple groups have not yielded allosteric agonists for M₄, but significant progress has been made in the development of M₄ PAMs. An early M₄ PAM, thiochrome, demonstrated the unique ability to bind to multiple muscarinic receptor subtypes while potentiating the activity of only M₄, suggesting a selective influence on cooperativity as opposed to a selective mode of binding [83]. However, a significant breakthrough in the specific targeting of M₄ came with the discoveries of VU0010010 and LY2033298, both novel pure PAMs of M₄ [84, 85]. VU0010010 increases both the affinity of the receptor for ACh as well as the efficiency of M₄ coupling to downstream effectors. VU0010010 also potentiates the responses of hippocampal neurons to the muscarinic agonist carbachol [85], but does not possess the requisite solubility and pharmacokinetic properties for *in vivo* characterization. The optimized VU0010010 analogs VU0152099 and VU0152100 maintain M₄ specificity and potency and, importantly, reverse amphetamine-induced hyperlocomotion in rats, confirming the antipsychotic potential of specific M₄ activation [86]. In addition, LY2033298 has been shown to potentiate the antipsychotic activity of the muscarinic receptor agonist oxotremorine in animal models [84]. Taken together, the results of M₁- and M₄-specific allosteric modulators suggest that the clinical efficacy of xanomeline results from synergistic activation of both receptor subtypes. For M₁, it is likely that modulation of NMDA receptor function may contribute to any antipsychotic and cognition-enhancing effects. By contrast, M₄ is unlikely to modulate NMDA receptor function directly but may regulate midbrain circuits that are ultimately important for the roles of both glutamatergic and dopaminergic systems in schizophrenia. Recently reported novel PAMs that are highly selective for M₁ and M₄ will certainly aid in the dissection of these contributions [87-89].

Enhancing NMDA receptor function via inhibition of the glycine transporter, GlyT1

Although much focus has been placed on the activation of NMDA receptors by glutamate, NMDA receptor activation requires coactivation by glutamate and its important co-agonist glycine. In addition to modulating NMDA receptor function by targeting specific GPCRs,

the glycine transporter GlyT1 has also shown promise as a therapeutic target in schizophrenia. Clinical studies have demonstrated that co-administration of glycine or glycine site agonists, such as D-serine, with some atypical antipsychotics improves symptoms in schizophrenic patients, suggesting that increasing the local concentration of glycine by inhibiting its uptake into neurons via GlyT1 may be of significant utility in a multifaceted approach to treatment [90].

As an alternative to orally administering high doses of glycine, significant efforts have recently been undertaken to develop specific GlyT1 inhibitors. GlyT1 is largely expressed on glia but is also found on the presynaptic terminals of glutamatergic neurons of the forebrain, and blockade of this transporter is hypothesized to elevate the glycine concentration around NMDA receptors, thereby facilitating their function in a clinically meaningful mechanism (for reviews, see [91, 92]). The naturally occurring GlyT1 substrate and antagonist sarcosine has been used as a template for design of novel inhibitors, most notable N-(3-4'-fluorophenyl)-3-[4'phenylphenoxy]propylsarcosine or NFPS, that have been shown to reduce PPI and enhance hippocampal LTP in animal models [93]. However, sarcosine-based GlyT1 inhibitors also induce motor side effects, such as compulsive walking and respiratory distress, which are hypothesized to result from increased activity at the GlyA receptor in the caudal brainstem [94-96]. Thus, efforts to develop specific GlyT1 inhibitors without sarcosine moieties have been undertaken by several groups (for review, see [97]), although many published structures have not been tested in preclinical models. Lindsley and colleagues reported a [4-phenyl-1-(propylsulfonyl)piperidin-4-yl]methyl benzamide, termed Merck (S)-13h, that demonstrated high selectivity for GlyT1 versus GlyT2, selectively elevated glycine in the PFC, and enhanced PPI with a level of efficacy comparable to clozapine in animal models [98]. The novel GlyT1 inhibitors SSR103800 and SSR504743 demonstrated reversal of amphetamine or MK-801-induced deficits in latent inhibition paradigms, a collection of animal models hypothesized to represent the positive, negative and cognitive symptoms of schizophrenia [99]. These and similar inhibitors do not ameliorate amphetamine-induced hyperactivity, suggesting that their pharmacological activity does not duplicate the effects of dopamine receptor antagonists and that they may be useful in combination with typical and atypical antipsychotics [100]. In a human clinical trial, the optimized GlyT1 inhibitor RG1678 demonstrated significant improvement in negative symptoms in schizophrenic patients without significant motor side effects ([101]; see also <http://www.roche.com/med-cor-2010-12-06b>). It has been recently reported that, regardless of inhibitor chemotype, GlyT1 inhibitors with short mean residence times at the transporter reduced PPI without inducing motor side effects, suggesting that highly efficacious blockade of GlyT1 may be clinically deleterious [96]. Although GlyT1 inhibitors have demonstrated milder side effect profiles than their typical and atypical antipsychotic counterparts in preclinical studies [91, 96, 100, 101], the potential consequences of glycine transport inhibition require further exploration.

A multifaceted approach to treatment of schizophrenia

In conclusion, ongoing research suggests that the multidimensional symptoms of schizophrenia likely arise from dysregulation in multiple signaling pathways and that successful treatment will require a multi-target strategy. Although targeting the monoaminergic systems has increased our understanding of this disorder and elucidated treatments that ameliorate patients' positive symptoms, the development of novel therapeutics to address schizophrenia's glutamatergic imbalance and the resulting positive, negative, and cognitive symptoms is urgently needed. Here, we have outlined multiple potential targets that directly or indirectly influence NMDA receptor function and offer potential therapeutic strategies to complement currently available antipsychotics. Significant preclinical and clinical evidence suggests that selective activation of the metabotropic

glutamate receptors mGlu₅ and mGlu₂ and the muscarinic receptors M₁ and M₄, as well as blockade of the glycine transporter GlyT1, may address the myriad consequences of NMDA receptor hypofunction and exert antipsychotic effects in schizophrenic patients (Table 1). However, few preclinical studies have compared these mechanisms in animal models to ascertain their relative benefit [102]. These studies demonstrate the potential power of allosteric modulation of GPCRs in treating complex CNS disorders, and our growing understanding of the interplay between glutamate, acetylcholine and the monoamines in the schizophrenic brain is advancing the promise of these approaches in the clinic.

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Glossary

Positive symptoms of schizophrenia

include hallucinations, delusions, paranoia, disordered thinking and agitated movement

Negative symptoms of schizophrenia

include lack of emotional expression, lack of pleasure in daily life, inability to sustain activities, and self-neglect

NMDA

N-methyl-D-aspartate, an amino acid derivative that acts as a specific agonist at the NMDA receptor (to which it lends its name) and replicates the action of the endogenous agonist glutamate. The NMDA receptor is a ligand-gated cation channel that requires coactivation by glutamate and glycine as well as membrane depolarization

AMPA

2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid, a compound that acts as a specific agonist at the AMPA receptor (to which it lends its name) and mimics receptor activation by glutamate. The AMPA receptor is a ligand-gated cation channel that requires activation by glutamate

Interneuron

in the CNS, a multipolar neuron that is typically inhibitory, serves as a connection within the local circuitry of a brain region, and does not send projections to other areas

Pyramidal neuron

a neuron characterized by the pyramidal shape of its cell body and pronounced apical dendrite, multiple basilar dendrites, and a single axon; the pyramidal neuron is the primary excitatory neuron of the cortex and subcortical structures such as the hippocampus and amygdala.

Allosteric

describing a site on an enzyme or receptor that is separate and distinct from the site typically bound by the endogenous substrate or agonist

Orthosteric

describing the site on an enzyme or receptor that is typically bound by the endogenous substrate or agonist

Positive allosteric modulator (PAM)

a ligand that, when bound to an allosteric site, potentiates the response of the receptor to an orthosteric agonist, i.e. shifts the agonist concentration response curve to the left

Negative allosteric modulator (NAM)

a ligand that, when bound to an allosteric site, antagonizes the response of the receptor to an orthosteric agonist, i.e. shifts the agonist concentration response curve to the right

Pre-pulse inhibition (PPI)

a behavioral task in which a weak stimulus presented prior to a strong stimulus inhibits the startle response to the strong stimulus for example a weak acoustic tone played immediately prior to a loud acoustic tone limits the startle induced by the loud tone. Administration of PCP or amphetamine to animals prevents the ability of the weaker stimulus to inhibit the response to the stronger, an observation also present in schizophrenics and thought to be reflective of sensory deficits

Set-shifting task

a behavioral task in which goal-directed focus on one object must be abandoned to accommodate focus on another object. This task measures cognitive flexibility in response to changing environmental demands

Box 1. GPCR-coupled glutamate and acetylcholine receptors of the CNS

Eight receptor subtypes of mGlu receptors have been cloned, which are delineated based upon G protein-coupling specificity, pharmacology, and sequence homology. Group I mGlu receptors (mGlu1 and mGlu5) are typically located postsynaptically and are traditionally coupled to $G_{\alpha q}$, leading to the stimulation of phospholipase $C\beta$ and subsequent release of intracellular calcium. Group II mGlu receptors (mGlu2 and mGlu3) are coupled to $G_{\alpha i/o}$ and the downstream inhibition of adenylyl cyclase and are expressed both pre- and post-synaptically. Group III mGlu receptors (mGlu4, mGlu6, mGlu7, and mGlu8) are also coupled to $G_{\alpha i/o}$ and are predominantly localized presynaptically[22]. Due to the structural and functional diversity and heterogeneous distribution of the mGlu subtypes, the opportunity to develop therapeutic agents that selectively interact with specific mGlu subtypes under specific conditions is promising[23].

The muscarinic cholinergic receptors are a family of GPCRs found widely throughout the CNS, with roles in learning and memory, arousal, and motor control. The muscarinic receptors include five subtypes, M_1 - M_5 . M_1 , M_3 , and M_5 receptors are coupled to the $G_{\alpha q}$ signaling pathway, exerting downstream effects via activation of phospholipase $C\beta$ and subsequent calcium release, while M_2 and M_4 receptors couple to $G_{\alpha i/o}$, thus inhibiting adenylyl cyclase and downstream kinase activation [103].

Figure 1.

Model of NMDA receptor hypofunction in schizophrenia and potential interventions at glutamate synapses. The diagram illustrates the consequences of diminished NMDA receptor function on subcortical GABAergic interneurons. Reduced glutamatergic excitation of these GABAergic neurons (a) leads to disinhibition of thalamocortical glutamatergic neurons (b) projecting to pyramidal cells of the cortex (c). This disinhibition results in excessive excitation of cortical neurons. The synaptic architecture, however, offers multiple targets for intervention, including mGlu₅ and M₁/M₄ receptors located on the subcortical GABAergic interneurons, mGlu_{2/3} autoreceptors located perisynaptically on glutamatergic projections, and GlyT1 glycine transporters on both neurons and glia.

Figure 2.

Schematic representation of concentration response curves of a positive allosteric modulator (PAM) compared to an orthosteric agonist. (a) Increasing concentrations of a PAM or agonist are plotted against a functional response. Note, in the absence of agonist, the PAM exhibits no activity. (b) A constant concentration of PAM is added in the presence of an increasing concentration of orthosteric agonist. Here, the PAM shifts the agonist CRC to the left, signifying a gain in agonist potency.

Table 1

Pharmacology of novel selective ligands for metabotropic glutamate receptors, muscarinic receptors and glycine transporters.

	Activity	Compound	Effect	Refs.
mGlu ₅	mGlu ₅ PAM	DFB	MPEP site ligand; Shifts glutamate concentration response curve in cell-based assays	[30]
		CPPHA	Potentiates NMDA receptor function in hippocampal slices	[31]
		CDPPB	MPEP site ligand; Antipsychotic efficacy in animal models	[32]
		VU-29	Potentiates LTP and LTD in hippocampal slices	[104]
		VU0360172	Antipsychotic efficacy in animal models	[38]
		ADX47273		[105]
mGlu _{2/3}	mGlu _{2/3} agonist	LY379268	Antipsychotic efficacy in animal models	[42]
		LY354740	Antipsychotic efficacy in animal models and human clinical trials	[19, 42]
		LY404039 (LY2140023)		[106, 107]
	mGluR ₂ PAM	LY487379	Antipsychotic efficacy in animal models	[108]
		BINA		[58]
M ₁ /M ₄	M ₁ /M ₄ agonist	xanomeline	Robust efficacy in reducing both positive and cognitive symptoms in humans	[66]
	M ₁ agonist	AC-42	First allosteric agonist of M ₁	[109]
		NMDC	Potentiates NMDA receptor function in hippocampal slices	[70]
		TBPB	Antipsychotic efficacy in animal models; Reduced side effects	[75]
		77-LH-28-1		[76]
		AC-260584		[110]
		VU0184670	Improved pharmacokinetic profile; antipsychotic efficacy in animal models	[77]
		VU0357017		[77]
	M ₁ PAM	VU0090157	Pure PAM in cell-based assays	[111]
		VU0029767		[111]
		BQCA	Antipsychotic efficacy in animal models	[81]
	M ₄ PAM	thiochrome	PAM with selective effect on M ₄ cooperativity	[83]
		VU0010010	Increases ACh affinity and cooperativity; potentiates M ₄ receptor function in hippocampal slices	[85]
		LY2033298	Potentiates M ₄ receptor function in hippocampal slices	[84]
		VU0152099	Optimized VU0010010 analog; antipsychotic efficacy in animal models	[86]
		VU0152100		[86]
GlyT1	Inhibitor	sarcosine	Endogenous antagonist; antipsychotic efficacy in animal models with limiting motor side effects	[93]
		NFPS/ALX-5407	Antipsychotic efficacy in animal models	[93]
		Merck (S)-13h		[98]
		SSR103800		[99, 100]
		SSR504743		[99, 112]
		RG1678	Antipsychotic efficacy in both animal models and human clinical trial	[101]