Review

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Positive allosteric modulators of metabotropic glutamate 2 receptors in schizophrenia treatment

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The past two decades have witnessed a rise in the 'NMDA receptor hypofunction' hypothesis for schizophrenia, a devastating disorder that affects around 1% of the population worldwide. A variety of presynaptic, postsynaptic, and regulatory proteins involved in glutamatergic signaling have thus been proposed as potential therapeutic targets. This review focuses on positive allosteric modulation of metabotropic glutamate 2 receptors ($mGlu_2Rs$) and discusses how recent preclinical epigenetic data may provide a molecular explanation for the discrepant results of clinical studies, further stimulating the field to exploit the promise of $mGlu_2R$ as a target for schizophrenia treatment.

Schizophrenia: limitations with currently available drugs

Schizophrenia is a chronic debilitating mental disorder that affects approximately 1% of the general population. Symptoms vary from patient to patient but are generally categorized into positive (e.g., hallucinations, delusions, and disorganized speech and behavior), negative (e.g., social withdrawal, lack of motivation, flat affect), and cognitive (e.g., impairments in memory, attention, and executive function). These symptoms are typically associated with social and/or occupational dysfunction. Although the natural course is heterogeneous, the illness typically strikes in late adolescence or early adulthood and usually continues throughout life [1,2]. The prognosis of patients is also variable, but is often poor, with high rates of unemployment, homelessness, violence, and suicide [3,4]. Given the combination of onset in early adulthood and the chronic course, schizophrenia presents a staggering drag on the economy and society. It has been estimated that the cost of schizophrenia in the USA in 2002 exceeded $62 billion [5] and the World Health Organization ranks this disorder among the top 10 causes of disability in developed countries [6].

The classical dopamine (DA) hypothesis (see Glossary) has dominated the theories of schizophrenia since the mid-20th century after the observation that first-generation, or 'typical', antipsychotic drugs, such as chlorpromazine and haloperidol, are high-affinity antagonists of dopamine D_2 receptors. Hyperactivity in the mesolimbic DA pathway was originally proposed to underlie positive symptoms of schizophrenia [7]. While effective against positive symptoms, typical antipsychotic drugs demonstrate limited efficacy against negative symptoms and cognitive impairments which have been shown to contribute to functional impairment and predict poor prognosis [8]. Moreover, these

Glossary

Allosteric site: a binding site on a receptor macromolecule that is non-overlapping and spatially distinct from, but conformationally linked to, the orthosteric binding site.
Dopamine (DA) hypothesis: the most classical schizophrenia hypothesis that attributes psychotic symptoms to hyperactive DA signaling in the brain.
Epigenetics: literally means 'above' or 'on top of' genetics. It refers to external modifications to DNA that turn genes 'on' or 'off' without changes in DNA sequence.
Excitatory postsynaptic potential (EPSP): a temporary depolarization of postsynaptic neuron membrane potential that makes it more likely that this neuron will trigger an action potential.
Glutamate: the main excitatory neurotransmitter in the brain.
G-protein-coupled receptor (GPCR): an integral plasma membrane protein that senses molecules outside the cell and transduces those extracellular signals to intracellular relay proteins, the heterotrimeric GTP-binding proteins (G proteins).
Heteromer: structural assembly composed of two or more different components.
Histone: evolutionarily conserved proteins found in eukaryotic cell nuclei that package DNA into structural units termed nucleosomes. Covalent modifications at the N-terminal tail of histones correlate with open or closed states of chromatin, depending on the type of modification, and thus lead to changes in gene expression.
Histone deacetylase (HDAC): a class of enzymes that remove acetyl groups from histones, allowing the histones to wrap the DNA more tightly and repress gene transcription.
Metabotropic glutamate receptors (mGluRs): GPCRs that bind glutamate and function to modulate, rather than mediate, synaptic transmission. This is to differentiate them from 'ionotropic' glutamate receptors, such as NMDA receptors, which are ion channels mediating excitatory neurotransmission.
***N*-methyl-D-aspartate (NMDA) receptor:** a glutamate receptor and ion channel protein found in nerve cells. It is involved in synaptic transmission and plays an important role in learning and memory.
NMDA hypofunction hypothesis: a glutamate-based hypothesis which postulates that reduced NMDA glutamate receptor activation underlies the development of different schizophrenia symptoms.
Orthosteric site: the binding site on a receptor macromolecule that is recognized by the endogenous agonist for that receptor.

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drugs are associated with several side effects, including hyperprolactinemia and extrapyramidal symptoms (EPS). The second-generation, or 'atypical', antipsychotic drugs were introduced into the clinical practice in the early 1990s in an attempt to improve clinical efficacy and decrease side effects. Second-generation drugs (such as clozapine, olanzapine, risperidone, and quetiapine), which have less potential to induce EPS or hyperprolactinemia, differ pharmacologically from the typical ones in that they have less affinity for D_2 receptors and higher affinity/functional interaction with other monoaminergic receptors, including the serotonin 2A receptor (5-HT_{2A}) [2,9]. Because of the dual dopamine–serotonin mechanism of action of atypical antipsychotic drugs, a serotonin hypothesis for schizophrenia was also proposed [10]. Although highly effective against a wider range of symptoms than typical agents, atypical antipsychotics still have modest efficacy against negative symptoms. In addition, recent clinical evidence does not support the notion that second-generation drugs are superior to first-generation in improving neurocognition [11]. Last but not least, atypical agents are associated with side effects, usually metabolic, and in rare cases severe, such as agranulocytosis seen with clozapine [2]. Despite the availability of many typical and atypical antipsychotic drugs, full functional remission is achieved in fewer than 35% of people with schizophrenia [12]. Moreover, most of the responders are 'partial responders' with whom negative and cognitive symptoms remain problematic, suggesting that impairment and/or dysregulation in DA and serotonin signaling cannot fully account for the underlying pathology. The limitations of the presently available drugs underscore the need for identification of new antipsychotic compounds aimed at new molecular targets. We focus here on positive allosteric modulation of $mGlu_2R$ as a promising therapeutic strategy to treat schizophrenia.

Glutamate and schizophrenia

To account for negative and cognitive symptoms of schizophrenia, the DA hypothesis was expanded to postulate hypoactivity in the mesocortical pathway as well. Despite this expansion, the DA hypothesis still likely oversimplifies the neurocircuitry of schizophrenia and does not explain why the mesocortical DA pathway will be hypoactive while the mesolimbic DA pathway is hyperactive. If anything, this dichotomy suggests that either DA is partially involved in the molecular underpinnings of schizophrenia, or alternatively that multiple defective neurotransmitter systems eventually converge on and disrupt the DA system [7]. In an attempt to account for the shortcomings of the DA hypothesis, the 'NMDA receptor hypofunction' hypothesis was proposed by Olney and Farber in 1995 [13] based on the observation that non-competitive NMDA antagonists such as phencyclidine (PCP) and ketamine induce a psychotomimetic state that closely resembles schizophrenia in healthy human individuals [14,15], and exacerbate preexisting symptoms in schizophrenic patients [16,17]. Although the pharmacology of cocaine and other amphetamine-like psychostimulants is complex [18,19], they share the ability to bind to dopamine transporters and increase synaptic levels of dopamine. Importantly, unlike amphetamine-like psychostimulants, glutamatergic PCP and ketamine dissociative drugs not only induce positive symptoms but also negative symptoms and cognitive dysfunction that better recapitulate the clinical syndrome of schizophrenia. Subsequently, PCP-like drug models have become widely employed in the search for novel treatments. It was proposed that defective NMDA receptors on cortical γ-aminobutyric acid (GABA) interneurons (Figure 1) render these interneurons less effective in inhibiting glutamate projection neurons that project to the ventral tegmental area (VTA). This disinhibition results in excessive glutamate tone, which now overstimulates the DA mesolimbic pathway giving rise to the positive symptoms. An alternative pathway that could be fundamental for cognition might be the functional crosstalk between NMDA and 5-HT_{2A} receptors in pyramidal neurons of prefrontal cortex [20]. Similarly, negative and cognitive symptoms may arise from NMDA receptor hypofunction. In this case hyperactive glutamate projection neurons overactivate GABAergic interneurons, located in the VTA, causing them to release excess GABA and overinhibit the mesocortical DA pathway that now becomes unable to adequately supply DA to the prefrontal cortex [7]. Thus, defective glutamate neurocircuitry might actually drive the excess DA in the mesolimbic pathway as well as the deficiency of DA in the mesocortical pathway. In addition, the resulting glutamate excitotoxicity might drive an ongoing neurodegenerative process responsible for the structural brain changes seen in schizophrenic brains on volumetric MRI [21,22], although the presence of neurodegeneration in schizophrenia remains controversial [23–27]. Interestingly, group II metabotropic glutamate

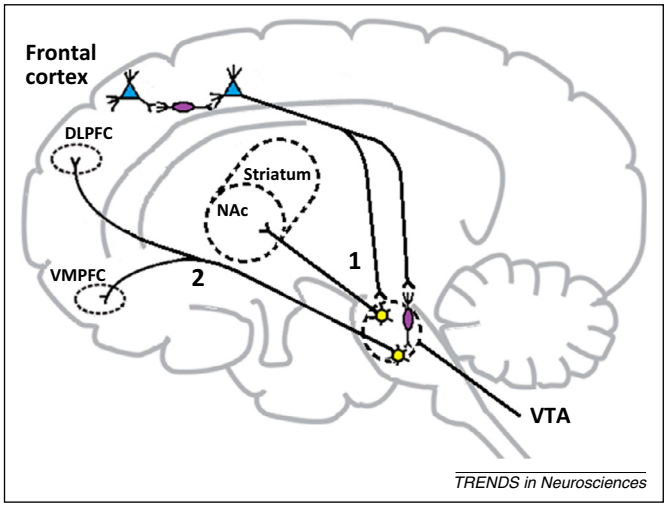

Figure 1. Glutamate neurocircuitry implicated in schizophrenia. Normally, cortical GABA interneurons (in purple) exert an inhibitory tone on glutamate neurons that project to the VTA. When optimal, this inhibitory tone controls the amount of glutamate released in the VTA, and subsequently the activity of the mesolimbic (**1**) and mesocortical (**2**) DA pathways. In schizophrenia, a defective NMDA receptor on the cortical GABA interneurons results in disinhibition of cortical brainstem glutamate projections. Excessive glutamate firing leads to overactivation of the mesolimbic DA pathway (1) and excessive release of DA in the nucleus accumbens. This might be responsible for development of positive symptoms in schizophrenic patients. Similarly, negative and cognitive symptoms may arise from NMDA receptor hypofunction. Hyperactive glutamate tone overactivates GABAergic interneurons in the VTA, overinhibiting the mesocortical DA pathway (2) that now becomes unable to adequately supply DA to the prefrontal cortex resulting in 'hypofrontality'. Abbreviations: DA, dopamine; DLPFC, dorsolateral prefrontal cortex; NAc, nucleus accumbens, VMPFC: ventromedial prefrontal cortex; VTA, ventral tegmental area.

receptors (mGluRs) are located presynaptically on these hyperactive glutamatergic neurons where they function as autoreceptors to help keep glutamate tone in check [7]. Activation of these receptors can thus attenuate schizophrenia symptoms and might prevent potential neurodegeneration. Recent findings suggest that the $mGlu_2Rs$ are also expressed postsynaptically, where they play a key role in the control of the responses induced by atypical antipsychotics [28–31].

Additional evidence for involvement of glutamate in the pathophysiology of schizophrenia comes from postmortem studies which revealed altered ionotropic glutamate receptor binding and gene expression mainly in the prefrontal cortex and the hippocampus [32–35]. Furthermore, functional neuroimaging studies have shown reduced NMDA binding in the hippocampus of medication-free schizophrenic patients compared to healthy controls [36]. More recently, proton magnetic resonance spectroscopy has revealed increased cortical glutamate levels in individuals with poor response to antipsychotic treatment in first-episode schizophrenia compared to those who were responders, supporting the hypothesis that poor responders to antidopaminergic therapy may have a glutamatergic basis for their psychosis [37]. Lastly, many genes involved in glutamatergic neurotransmission were found to be associated with schizophrenia in a recent genome-wide association study (GWAS) involving a consortium of over 200 institutions worldwide in the largest molecular genetic study conducted to date for a neuropsychiatric disorder [38]. Taken together, the above evidence indicates an important role for dysfunctional glutamatergic neurotransmission in the pathophysiology of schizophrenia.

mGluRs and allosteric modulation

mGluRs are class C G-protein-coupled receptors (GPCRs) that are characterized by a conserved heptahelical transmembrane domain (TMD), a large N-terminal extracellular domain (ECD), and a C-terminal intracellular domain. The large ECD, characteristic of family C GPCRs, consists of a Venus flytrap domain (VFD), which contains the orthosteric binding site for glutamate, and a cysteine-rich domain (CRD). Another distinguishing feature of class C GPCRs is constitutive homo- or heterodimerization at the cell surface [39]. It has been demonstrated that class C mGluRs function as homodimers at the plasma membrane in living cells, whereas the class C $GABA_B$ receptor needs to form a heterodimeric complex composed of $GABA_B$-R1 and $GABA_B$-R2 to reach the plasma membrane as a functional receptor complex (see [40] for review). The eight mGluR subtypes identified so far are classified into three groups based on sequence homology, G-protein coupling, and pharmacology [41]. Group I mGluRs ($mGlu_1R$ and $mGlu_5R$) are predominantly coupled to $G_{q/11}$ and activate the phospholipase C enzyme. Group II mGluRs ($mGlu_2R$ and $mGlu_3R$) and group III ($mGlu_4R$, $mGlu_6R$, $mGlu_7R$, and $mGlu_8R$) are coupled to $G_{i/o}$ proteins and typically inhibit adenylyl cyclase activity [42].

In common with the majority of GPCRs, mGluRs have been classically targeted through their 'orthosteric' site (i.e., the binding site recognized by the endogenous ligand). However, because all mGluR orthosteric ligands bind to the VFD, which is highly conserved among all eight mGluRs, subtype selectivity has been difficult to achieve, especially for mGluR members from the same group. Moreover, CNS penetration for many of those orthosteric compounds has been limited by their poor physicochemical properties owing to their glutamate-like structures [43]. The difficulty to identify suitable glutamate analogs with adequate subtype selectivity and pharmacokinetic properties triggered the search for an alternative means to target mGluRs. A promising approach has been the use of allosteric modulators – ligands that bind to sites non-overlapping and spatially distinct from, but conformationally linked to, the orthosteric site (Figure 2) [44]. Mutagenesis studies have revealed that the majority of allosteric modulators identified for mGluRs bind to the TMD, which is relatively less conserved among mGluR subtypes than the VFD [45]. This has been recently confirmed by the crystal structures of the TMDs of both $mGlu_1R$ and $mGlu_5R$ bound by allosteric modulators [46,47].

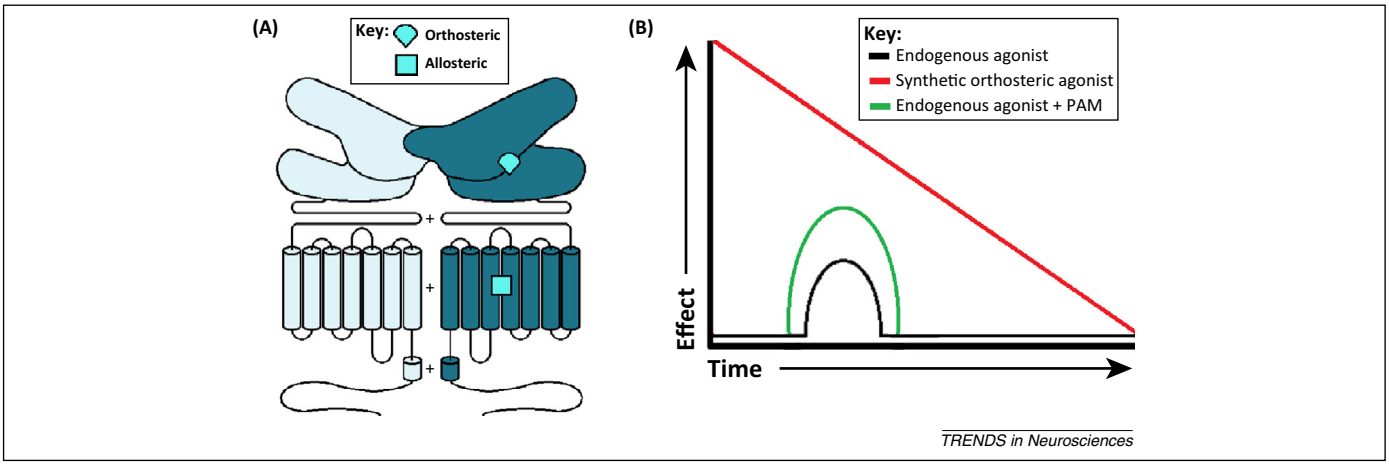

Figure 2. Allosteric versus orthosteric ligands. **(A)** A schematic of the dimeric structure of a class C G-protein-coupled receptor (GPCR). metabotropic glutamate receptors (mGluRs), as well as other receptors in this class, are homodimers that use the Venus flytrap domain (VFD) exclusively to bind orthosteric ligands. The transmembrane domain (TMD) may have one or more potential pockets for allosteric ligands. **(B)** A synthetic orthosteric agonist often produces a bigger effect than the endogenous agonist; however, its effects may decline with time owing to receptor desensitization and/or downregulation. On the other hand, a positive allosteric modulator (PAM) enhances the action of the endogenous agonist on its receptor in a more physiologic temporal pattern, and thus is less likely to cause receptor desensitization and/or downregulation.

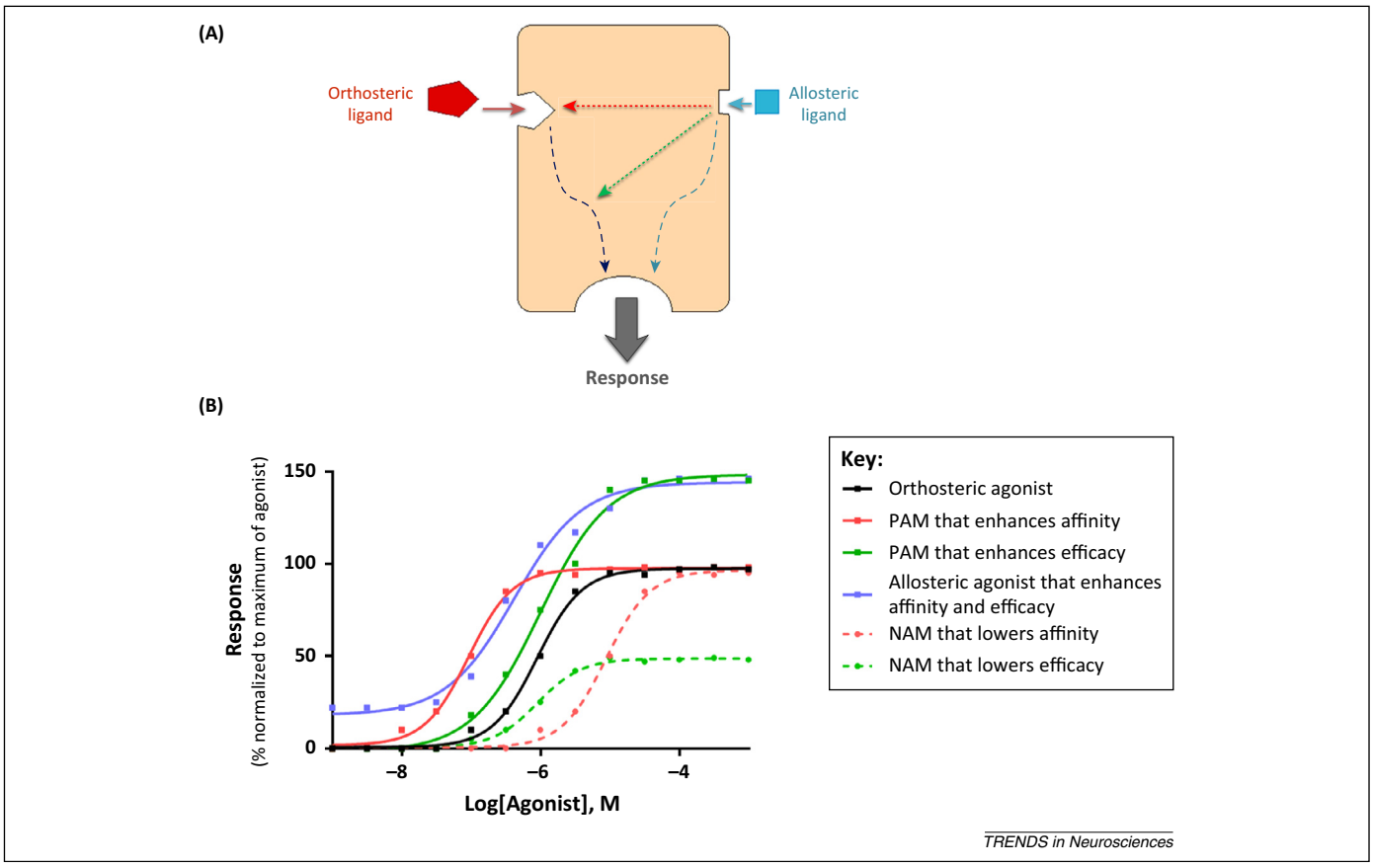

Figure 3. Modes of action of allosteric modulators. **(A)** The allosteric ligand binds to a site topographically distinct from the orthosteric binding site and modulates the affinity (red) and/or efficacy (green) of the orthosteric ligand. Some allosteric ligands are capable of directly eliciting a response on their own (blue). Figure adapted from Conn *et al.* [35]. **(B)** Simple schematic representation of effects mediated by different allosteric ligands on the functional response of an orthosteric agonist. The first (solid red) is a positive allosteric modulator (PAM) that purely enhances orthosteric agonist affinity, as evidenced by an increase in potency (lower EC_{50}) and leftward shift of the concentration–response curve compared to the orthosteric agonist alone (solid black). The second (solid green) is a PAM that purely enhances orthosteric agonist efficacy, as evidenced by an increase in E_{max} and upward shift in its concentration–response curve. The third (blue) demonstrates allosteric agonism that modulates both the affinity and efficacy. The fourth (dashed red) is a negative allosteric modulator (NAM) that lowers the orthosteric agonist affinity, and thus shifts its concentration–response curve to the right. The fifth (dashed green) is a NAM that lowers the orthosteric agonist efficacy and shifts its concentration–response curve downward. Figure adapted from Wootten *et al.* [100].

Allosteric modulators can modify the action of the orthosteric ligand by modulating its affinity and/or efficacy (Figure 3), either in a positive (in case of positive allosteric modulators, PAMs) or a negative direction (in case of negative allosteric modulators, NAMs). This phenomenon is referred to as 'cooperativity' [48]. PAMs potentiate the response to an agonist and cause a leftward (and often an upward) shift in the concentration–response curve for the orthosteric agonist. Depending on the degree of stimulus–response coupling in an assay, a potentiator can behave as a pure PAM – an allosteric ligand that elicits no detectable response in the absence of the orthosteric agonist – or as an ago-PAM that can directly elicit a response like an agonist [49]. NAMs noncompetitively antagonize agonists and cause a rightward (and often downward) shift in the agonist concentration–response curves (Figure 3). Neutral allosteric ligands (previously referred to as silent allosteric modulators, SAMs) occupy the allosteric site without affecting the agonist responses on their own; however, they can block the allosteric effects of both PAMs and NAMs [44].

Advantages of allosteric modulators

Pure allosteric modulators have an advantage over synthetic orthosteric agonists in their ability to preserve the temporal and spatial patterns of receptor signaling because their effects are dependent on the presence of the endogenous ligand. This 'state-dependent' mechanism of action explains, for example, why the ability of $mGlu_2R$ PAMs to inhibit striatal excitatory postsynaptic potentials (EPSPs) shows dependence on the frequency of presynaptic stimulation of corticostriatal afferents. Unlike agonists, $mGlu_2R$ PAMs inhibit EPSPs only upon high-frequency stimulation, indicating that excessive synaptic glutamate release is required for $mGlu_2R$ PAMS to exert their modulatory effect [50]. Because PAMs will not continuously activate the receptor, receptor desensitization and/or downregulation is less likely to occur, as opposed to synthetic orthosteric agonists. In addition, because no effect is expected at saturating concentrations above the concentration determined by cooperativity, an allosteric drug will have a greater potential to fine-tune physiological responses (Figure 2) with less risk of toxicity [51]. This saturability phenomenon is referred to as the 'ceiling level' of the allosteric effect. Lastly, mGluR allosteric modulators generally possess better physicochemical properties and blood–brain barrier (BBB) permeability compared to orthosteric drugs, an important feature considering the therapeutic potential of mGluRs in neuropsychiatric disorders [52].

Challenges with allosteric modulators

Despite the above-mentioned advantages, several characteristics unique to GPCR allosteric modulators may represent a challenge in the translation of data from such agents. For example, the magnitude and direction of the allosteric effect mediated by the same modulator acting on the same receptor can vary depending on the orthosteric ligand that is used to probe receptor function, a phenomenon referred to as 'probe dependence' [51]. Although examples have not yet been discovered for mGluRs [52], the use of glutamate as the orthosteric probe in *in vitro* assays might be more favorable because the results are more likely to be of physiological relevance. Accordingly, any results obtained from assays using non-native agonists as probes should be interpreted cautiously because an allosteric drug may behave differently *in vivo* in the presence of the endogenous agonist [53]. Moreover, potential species differences in the responses to allosteric drugs may exist because the allosteric sites, presumably having undergone less evolutionary pressure to accommodate an endogenous ligand compared to orthosteric sites, are more likely to show sequence divergence between species [48]. Another challenge is that several mGluR allosteric modulator classes are prone to 'molecular switches' by which subtle structural changes within the scaffold can dramatically change the pharmacology of compounds within a class, switching them for example from NAMs to PAMs, PAMs to NAMs, or even change their receptor subtype-selectivity [54–56]. This also raises questions about the metabolism and pharmacology of metabolites [57]. All the above-mentioned complexities, together with the potential implications of mGluR heterodimer formation [58], are issues that need to be addressed before advancing any mGluR allosteric modulator into the clinic.

Potential for biased signaling

Although GPCR activation was first described by a classical two-state model, where the receptor exists in an equilibrium between an active and an inactive state, recent evidence supports an alternative multi-state model where GPCRs can adopt multiple conformational states, with each state possibly activating a discrete subset of cellular behaviors of a broader spectrum than only G-protein coupling or second messenger activation [48,59]. The ability of ligands to stabilize particular unique conformational states activating specific cellular pathways and not others has been termed 'biased signaling', 'functional selectivity', and 'stimulus trafficking' [48,60]. Biased allosteric modulation has been demonstrated for several mGluR allosteric ligands [61–63]. For instance, the gadolinium ion (Gd^{3+}), an allosteric modulator of $mGlu_{1\alpha}R$, potentiates G_s-linked cAMP production but inhibits $G_{q/11}$-linked Ca^{2+} mobilization when administered with glutamate [64]. Biased signaling can also involve non-G-protein-mediated pathways such as the recruitment of β-arrestin. For instance, TRV130, a G-protein-biased agonist of the μ-opioid receptor with minimal β-arrestin recruitment, was shown in mice to produce analgesic effects comparable to morphine, with less respiratory depression and gastrointestinal dysfunction [65]. This strongly suggests that biased allosteric agonism and modulation may help to selectively target signaling pathways crucial for therapeutic efficacy while simultaneously excluding others associated with adverse effects (Figure 4).

$mGlu_2R$ and schizophrenia

Group II mGluRs are widely expressed in the brain, with generally similar distribution patterns in human and rodent brains [66]. $mGlu_2R$ is particularly expressed in

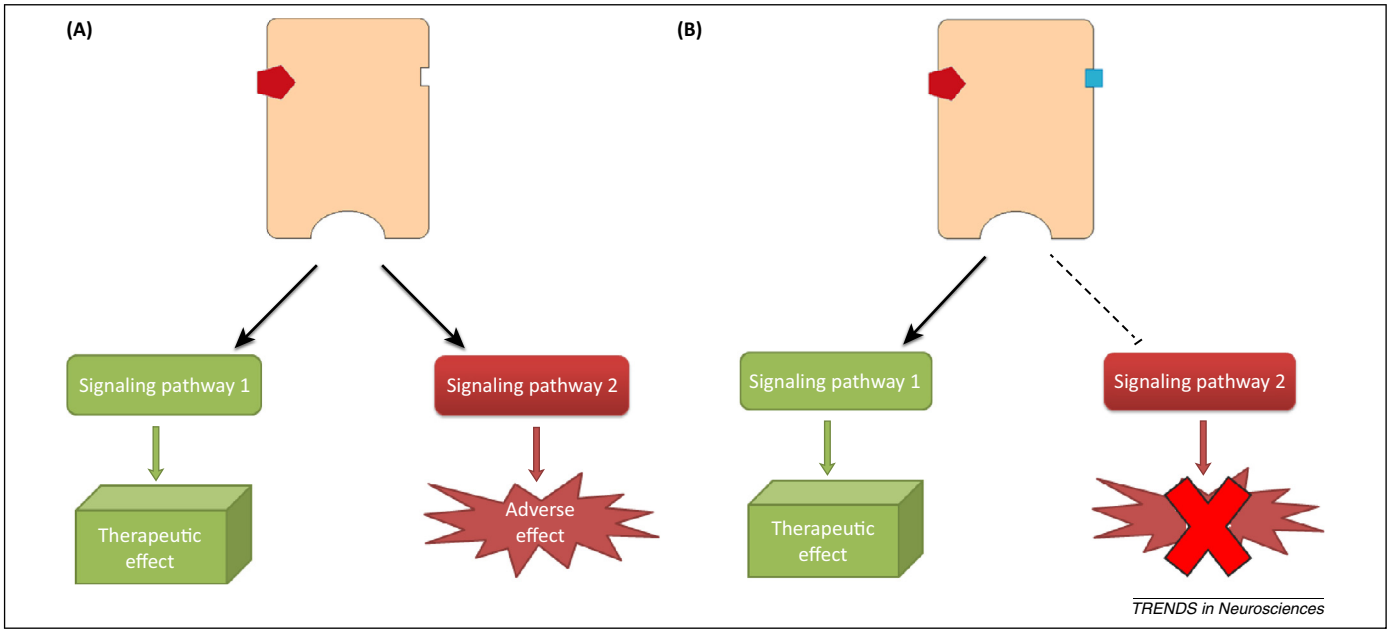

Figure 4. Schematic for biased allosteric modulation. **(A)** An orthosteric agonist turns the receptor 'on', activating signaling pathway 1, associated with therapeutic benefits, and simultaneously pathway 2, associated with adverse effects. **(B)** Co-binding of an allosteric modulator can bias the signaling of the agonist-bound receptor, selectively engaging pathway 1 while inhibiting pathway 2.

regions known to be implicated in schizophrenia such as the prefrontal cortex, hippocampus, striatum, thalamus, and amygdala [67]. Compared to $mGlu_2R$, $mGlu_3R$ shows a considerably more diffuse distribution pattern in the brain, with both showing an overlapping cortical distribution with the 5-HT_{2A} receptor [54]. At the cellular level, although $mGlu_2R$ is also found postsynaptically [30,31], both receptors are located presynaptically, where they function as autoreceptors inhibiting glutamate release and modulating synaptic transmission [68]. In contrast to $mGlu_2R$, $mGlu_3R$ is additionally expressed by astrocytes [69] where it might also be involved in a feedback mechanism to modulate neuronal excitability.

While the $mGlu_3R$ gene (*GRM3*) has been suggested by several independent groups [70–72] as well as by the recent GWAS data [38] to be implicated in schizophrenia, the majority of findings from postmortem human brains have not shown significant changes in $mGlu_3R$ expression in schizophrenia [73–76]. However, when testing either $mGlu_2R$ density or $mGlu_2R$ gene (*GRM2*) mRNA expression in postmortem human brain samples, several reports have suggested both downregulation [73,77,78] and upregulation [74,79] of *GRM2* expression. These results have been obtained in either antipsychotic-free or antipsychotic-treated schizophrenia patients, as revealed by the absence or presence of antipsychotics in postmortem toxicological analysis (see also [66,76,80,81] for studies suggesting absence of changes in $mGlu_2R$ expression in postmortem human brains of schizophrenic subjects). A potential explanation for these discrepancies is that the expression and function of $mGlu_2R$ in schizophrenic subjects might be related to both the profound effects of age on $mGlu_2R$ density in cortical regions [73,76] and of antipsychotic drug treatment on the promoter activity of the *GRM2* gene (for further discussion, see below).

Group II mGluR agonists have been shown to reverse the effects of PCP on locomotion [82,83], working memory, stereotypy, and cortical glutamate efflux [82], and to suppress the head-twitch response induced by 2,5-dimethoxy-4-iodoamphetamine (DOI), a hallucinogenic 5-HT_{2A} agonist, in rats [31]. Owing to the lack of subtype-selective orthosteric ligands, it has been difficult to determine whether the antipsychotic-like effects of $mGlu_{2/3}R$ agonists are mediated via $mGlu_2R$, $mGlu_3R$, or both. Experiments with knockout (KO) mice and experiments using selective $mGlu_2R$ PAMs have both suggested that these effects are predominantly $mGlu_2R$-mediated. Indeed, the inhibitory effects of $mGlu_{2/3}R$ agonists on PCP- and amphetamine-evoked hyperlocomotor activity are absent in $mGlu_2R$-KO but not in $mGlu_3R$-KO mice [84,85].

Recent evidence, however, suggests that $mGlu_3R$ may be a potential therapeutic target for schizophrenia-associated cognitive dysfunction because the $mGlu_3R$ NAMs VU0469942 and VU0477950 reverse the positive effects of the $mGlu_{2/3}R$ orthosteric agonist LY379268 on synaptic plasticity and learning abilities in mice [86].

Crosstalk between mGlu2 and 5-HT_{2A} receptors

Not only does $mGlu_2R$ seem to be the target for many glutamate antipsychotics but it has also been found to crosstalk with 5-HT_{2A} receptor, a key target for atypical antipsychotics [30,73,87–92]. Interestingly, $mGlu_2R$ has been shown to be necessary for pharmacological and behavioral effects induced by hallucinogenic 5-HT_{2A} agonists because these effects were abolished in $mGlu_2R$-KO mice [93]. On the other hand, the locomotor activity induced by the $mGlu_{2/3}R$ antagonist LY341495 is attenuated in 5-HT_{2A}-KO mice [73]. Moreover, chronic administration of the hallucinogenic 5-HT_{2A} agonist 2,5-dimethoxy-4-bromoamphetamine (DOB) in mice attenuates the behavioral effects of the $mGlu_{2/3}R$ agonist LY379268 [94], whereas chronic treatment by LY341495 decreases 5-HT_{2A} binding and the hallucinogenic effects of LSD [95].

Indeed, $mGlu_2R$ and 5-HT_{2A} were found to colocalize in mouse and human cortical pyramidal neurons and form a heterocomplex with unique signaling properties [30,73]. Fribourg and colleagues [30] elucidated the functional coupling between $mGlu_2R$, which signals via a G_i heterotrimeric G protein, and 5-HT_{2A} which signals via G_q protein. Compared to the homomeric responses of each receptor to its endogenous neurotransmitter, heteromerization of mGlu2R with 5-HT_{2A} potentiates glutamate-induced $mGlu_2R$-coupled G_i signaling and attenuates 5-HT-induced 5-HT_{2A}-coupled G_q signaling. Moreover, drugs that stabilize the active or inactive conformation in one receptor cause the opposite conformation of the partner receptor. This inverse conformational coupling of the two receptors as parts of a heteromer unifies the mechanism of action of antipsychotic drugs targeting these receptors (Figure 5). Using the PCP-like drug MK801 as a model of psychosis, it was demonstrated that the $mGlu_2R$-dependent antipsychotic-like behavioral effects of LY379268 were absent in 5-HT_{2A}-KO mice, while the 5-HT_{2A}-dependent antipsychotic-like behavioral effects of clozapine were absent in $mGlu_2R$-KO mice. In schizophrenia, where the relative expression of these two receptors is dysregulated, a combination of an inverse agonist of 5-HT_{2A} and a strong agonist of $mGlu_2R$ may prove beneficial, as suggested by Fribourg and colleagues [30]. Collectively, these data suggest that a common target for psychedelics, and for atypical and glutamate antipsychotics, is the 5-HT_{2A}–$mGlu_2R$ complex, for which designing a bivalent ligand might be a rational therapeutic strategy.

Clinical effects of $mGlu_{2/3}R$ agonists

In 2007 a Phase II proof-of-concept clinical trial compared pomaglumetad methionil (LY2140023 monohydrate; a prodrug of the $mGlu_{2/3}R$ agonist LY404039) to the atypical antipsychotic olanzapine or placebo. The study showed a positive effect for pomaglumetad against positive and negative symptoms of schizophrenia compared to placebo. Although the efficacy was not as significant as for olanzapine, pomaglumetad was safe and well-tolerated and, importantly, was neither associated with extrapyramidal symptoms nor with metabolic abnormalities [96]. Interestingly, a pharmacogenetic analysis identified 23 single nucleotide polymorphisms (SNPs) significantly associated with pomaglumetad response, 16 of which were located in the 5-HT_{2A} receptor (*HTR2A*) gene [97]. However, subsequent clinical trials with pomaglumetad showed either inconclusive results [98] or results that were no different from placebo [99]. Importantly, results from a recent *post hoc* analysis suggest that the therapeutic effects of

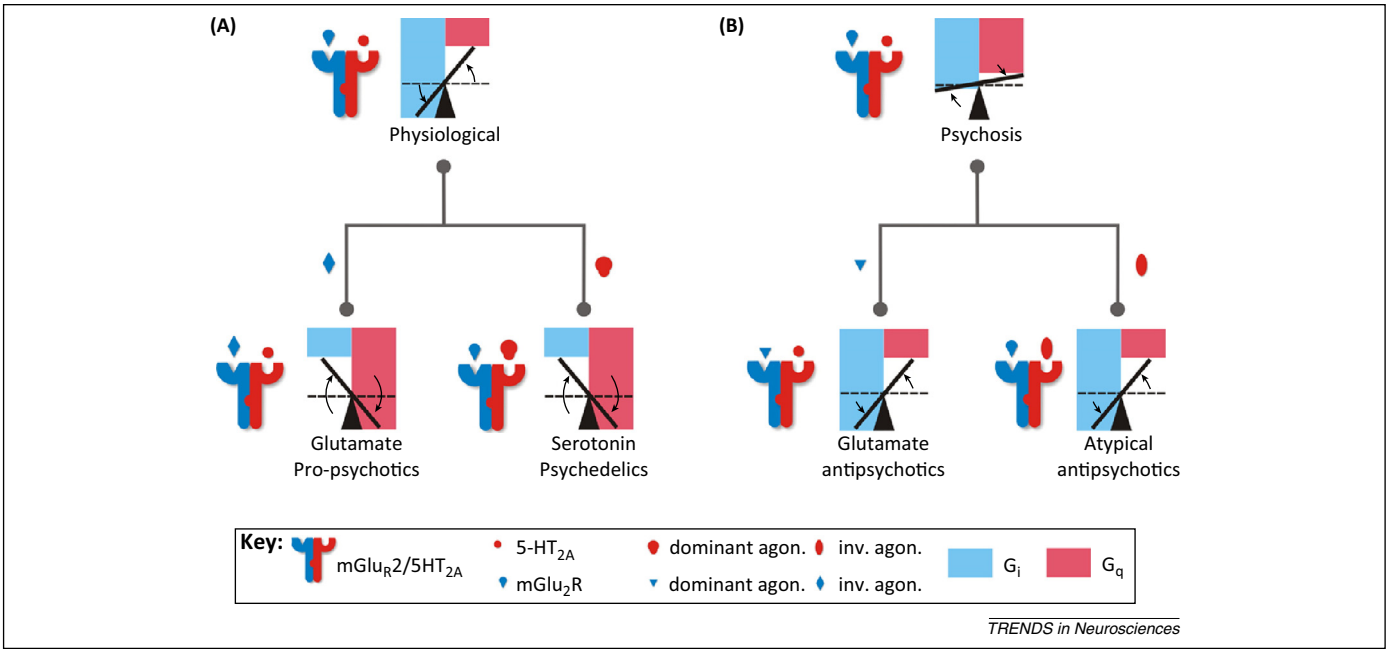

Figure 5. G_i–G_q balance model of the mechanism of action of antipsychotic and psychedelic drugs through the mGlu$_2$R/5-HT$_{2A}$ complex. **(A)** Heteromerization of mGlu$_2$R and 5-HT$_{2A}$ establishes an optimal Gi–Gq signaling balance in response to the endogenous neurotransmitters glutamate (Glu) and serotonin (5-HT): an increase in G_i and a decrease in G_q activation compared to the homomeric receptor signaling (dashed lines). Psychedelics (mGlu$_2$R inverse agonists and 5-HT$_{2A}$ agonists) invert the signaling balance through the complex, tipping the balance from being in favor of G_i signaling (normal complex) to becoming in favor of G_q signaling (propsychotic). **(B)** Disruption of the optimal balance in psychotic states can be compensated for by antipsychotics (mGlu$_2$R agonists and 5-HT$_{2A}$ inverse agonists) that tip the balance in favor of G_i signaling, thus restoring physiological signaling. Figure reprinted from Fribourg *et al.* [21]. Abbreviations: Agon, agonist; inv., inverse.

pomaglumetad depend on previous exposure to either typical or atypical antipsychotic drugs [100]. These findings and their basic mechanism based on previous preclinical findings [101] are discussed in Box 1.

Promise of mGluR positive allosteric modulators

LY487379, reported by researchers at Eli Lilly in 2003, was the first-identified mGlu$_2$R-selective PAM [102]. LY487379 showed comparable efficacy to mGlu$_{2/3}$R agonists in preclinical psychosis models [103,104]. However, the poor bioavailability, modest potency, and short duration of action limited the further *in vivo* characterization of compounds within the LY487379 class. Soon after, the biphenyl-indanone class was reported [105,106]. Biphenyl-indanone A (BINA) became the prototype mGlu$_2$R PAM for *in vivo* studies after it was shown to be a potent mGlu$_2$R-selective PAM with robust long-lasting *in vivo* activity [107]. BINA blocks the PCP-induced hyperlocomotor activity [107,108] as well as the DOB-induced head twitch response [88]. Pharmacologic magnetic resonance imaging (phMRI) revealed that BINA blocks PCP-induced blood oxygenation level-dependent (BOLD) activation in the rat brain. This allowed defining the mechanism of action of BINA at the brain circuitry level where its effect was apparent in the prefrontal cortex, caudate putamen, nucleus accumbens, and mediodorsal thalamus, structures linked to schizophrenia [108]. After the introduction of LY487379 and BINA, a variety of structurally distinct classes of mGlu$_2$R PAMs were discovered, many of which proved to have *in vivo* activity as well [109]. Early mutagenesis studies identified three amino acid residues in transmembrane (TM) segments IV and V of mGlu$_2$R that are crucial for the activity of LY487379 and several other mGlu$_2$R PAMs [110,111]. A more recent extensive study identified additional residues in TMs III, V, and VI that play an important role in the activity of mGlu$_2$R PAMs [112]. Interestingly, three residues in TM IV of mGlu$_2$R have been found to be essential for the heteromerization of 5-HT$_{2A}$–mGlu$_2$R [31], indicating that the TMD region of mGlu$_2$R is implicated in different types of allosteric interactions. However, the implications of mGlu$_2$R involvement in heteromeric receptor complexes for PAM pharmacology remain unknown. Although glutamate-induced signaling through mGlu$_2$R can be potentiated by a PAM, mGlu$_2$R in its heteromeric state may not necessarily respond to a PAM in the same manner. Moreover, it is not known yet whether mGlu$_2$R PAMs can crosstalk and alter the signaling of 5-HT$_{2A}$ through the 5-HT$_{2A}$–mGlu$_2$ receptor heteromer.

So far, two mGlu$_2$R PAMs have reached clinical trials. The first compound, ADX71149 from Addex and Janssen, showed the first successful clinical proof-of-concept in a Phase IIa study. Data reported in late 2012 demonstrated safety and tolerability, and identified patients with residual negative symptoms as the population most likely to benefit from adjunctive treatment with ADX71149 [113]. The second compound, AZD8529 from Astrazeneca, failed to separate from placebo in a Phase IIa study, unlike the active control risperidone [114]. However, it is worth mentioning that this study tested a single dose of AZD8529 as a monotherapy, and further investigation is therefore warranted.

One more ongoing effort by Janssen is to identify potential positron emission tomography (PET) tracers for imaging mGlu$_2$R [115]. Preliminary evaluation of [^{11}C]JNJ-42491293 in rat brain demonstrated specific and reversible binding to an mGlu$_2$R allosteric site [116]. Subsequent evaluation in 20 healthy male subjects confirmed radioactivity uptake consistent with the reported distribution of

Box 1. Can epigenetics explain discrepant clinical data?

Interestingly, recent preclinical and postmortem human brain findings may provide an epigenetic explanation of the apparently discordant results with both $mGlu_{2/3}R$ agonists and $mGlu_2R$ PAMs. It was demonstrated that chronic atypical antipsychotic therapy induces a highly selective downregulation of $mGlu_2R$ (*Grm2*) gene expression in mouse frontal cortex (Figure I). This effect was mediated via a signaling mechanism that involved 5-HT_{2A} receptor-dependent modulation of the promoter activity of the histone deacetylase 2 (*Hdac2*) gene. Thus, the presence of serotonin resulted in inhibition of *Hdac2* promoter activity, an effect that was reversed *in vitro* and in mouse frontal cortex by clozapine. Importantly, chronic treatment with atypical antipsychotics induced 5-HT_{2A} receptor-dependent upregulation of HDAC2 and increased binding of HDAC2 to the *Grm2* promoter. These epigenetic changes occurred in association with repressive histone modifications at the promoter region of the *Grm2*/*GRM2* gene in mouse and human frontal cortex [101]. Because chronic antipsychotic drug treatment induced a 5-HT_{2A} receptor-dependent decrease in $mGlu_2R$ transcription and its electrophysiological properties, together these findings suggest that previous medication with atypical antipsychotic drugs may prevent the therapeutic responses to $mGlu_2R$ ligands in schizophrenia patients. This is supported by results from a recent *post hoc* analysis disclosed by Eli Lilly investigators where the subgroup of patients who were at an earlier stage of the course of the disease (<3 years of illness) responded to pomaglumetad, as opposed to patients at a later stage in the disease (>10 years of illness). More interestingly, in patients with previous exposure to predominantly D_2-blocking drugs, the efficacy of pomaglumetad was comparable to that of risperidone, whereas it did not differ from placebo in patients previously treated predominantly with 5-HT_{2A} antagonists [100]. Together, these findings also suggest that inhibition of HDAC2 may reverse the repressive epigenetic changes induced by chronic atypical antipsychotic drugs and hence improve the antipsychotic properties of $mGlu_{2/3}R$ agonists and $mGlu_2R$ PAMs.

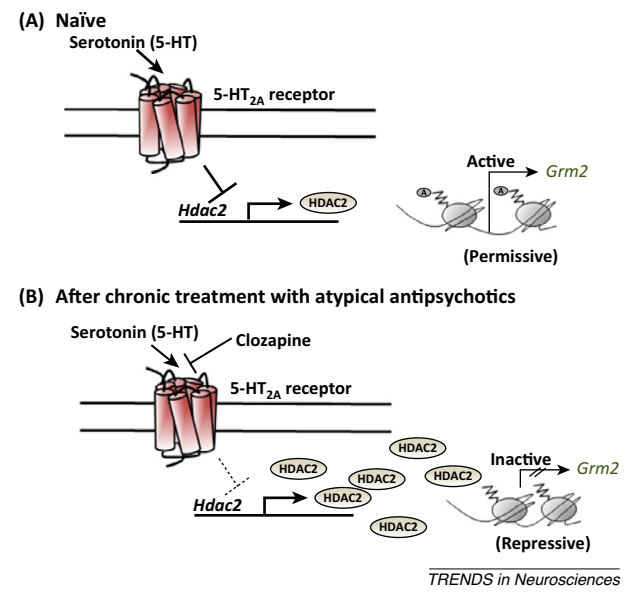

Figure I. Chronic atypical antipsychotic therapy downregulates $mGlu_2R$. Schematic model of the effect of chronic atypical antipsychotic treatment to the epigenetic status of the $mGlu_2R$ (*Grm2*) gene. (**A**) Activation of the 5-HT_{2A} receptor by the endogenous neurotransmitter serotonin represses the promoter activity of the *Hdac2* gene in mouse and in human frontal cortex. (**B**) Atypical antipsychotic drugs, such as clozapine and risperidone, reverse the 5-HT_{2A} receptor-dependent repression of *Hdac2*, an effect that is associated with increased *Hdac2* promoter activity and repressive histone modifications at the *Grm2* promoter. This epigenetic effect of chronic atypical antipsychotic treatment may limit the therapeutic effects of $mGlu_{2/3}R$ agonists such as pomaglumetad.

$mGlu_2R$ in human brain [117]. These encouraging results would likely allow quantifying $mGlu_2R$ in human brain as well as permit assessment of occupancy by drug candidates targeting this allosteric site.

$mGlu_5R$ is another receptor within the mGluR superfamily for which positive allosteric modulation is a potential treatment strategy for schizophrenia. The efficacy of $mGlu_5R$ PAMs in animal models of schizophrenia and cognitive dysfunction was thought to be mediated by $mGlu_5R$-induced potentiation of NMDA receptors [118,119]. However, a recent study has challenged this hypothesis by showing that VU0409551, a novel $mGlu_5R$ PAM with robust antipsychotic and cognition-enhancing effects in animal models, exhibits biased allosteric modulation by selectively potentiating $mGlu_5R$ coupling to G_q-mediated signaling but not $mGlu_5R$ modulation of NMDA receptors [61]. This raises an interesting question regarding the signaling mechanisms underlying the antipsychotic effects of $mGlu_2R$ PAMs, and whether they could exert some of their beneficial effects through NMDA-independent signaling as well. It would also be interesting to compare $mGlu_2R$ versus $mGlu_5R$ PAMs, particularly to elucidate which classes of drugs are superior for treatment of cognitive dysfunction associated with schizophrenia.

Recent findings suggest that ligands that interact with putative receptor heteromers, such as μ-opioid–$mGlu_5R$ and μ–δ opioid receptor heteromers, modulate unique phenotypes in rodent models [120,121]. Because the heteromer between $mGlu_2R$ and 5-HT_{2A} has been suggested to function as the primary molecular target responsible for the therapeutic effects of both atypical and glutamate antipsychotic drugs (Figure 5), further medicinal chemistry work with bivalent compounds that specifically target the 5-HT_{2A}–$mGlu_2R$ heteromer to induce an antipsychotic-like pattern of G-protein signaling in frontal cortex pyramidal neurons may provide a route for the development of new and more-effective agents for the treatment of schizophrenia.

Concluding remarks

This review has summarized recent findings suggesting that $mGlu_2R$ activation may provide a novel therapeutic strategy for the treatment of schizophrenia and help to avoid the adverse effects associated with currently available antipsychotic drugs. As pharmacologic tools, PAMs might be superior to orthosteric agonists because they have a unique ability to modulate glutamate release in a

Box 2. Outstanding questions

- How do $mGlu_2R$ PAMs modulate $mGlu_2R$ in its heteromeric states?
- Do $mGlu_2R$ PAMs have the ability to crosstalk to 5-HT_{2A} receptors?
- Can an $mGlu_2R$ PAM be a part of a bivalent ligand targeting the 5-HT_{2A}–$mGlu_2$ receptor complex implicated in schizophrenia? If yes, can a bivalent PET tracer be used diagnostically for quantifying such receptor complex?
- Would an HDAC2 inhibitor improve the therapeutic efficacy of $mGlu_2R$ antipsychotics in patients with a history of chronic atypical antipsychotic therapy?
- What is the right patient population that is likely to benefit from $mGlu_2R$ allosteric modulation?

'state-dependent' manner that helps to fine-tune physiological responses. An important challenge in the field is related to the potential adverse signaling effects induced by mGluR PAMs in the absence of orthosteric agonists [122]. This is particularly important considering the recent findings that mGluRs with VFDs deleted, when reconstituted into nanodiscs, are able to couple to and activate heterotrimeric G proteins in the presence of PAMs [123]. Whether the expression of alternatively spliced variants that generate truncated isoforms of mGluRs [124] might lead to unfavorable PAM-dependent signaling events in the absence of endogenous agonists needs to be explored. Despite the advances in our knowledge of medicinal chemistry and the basic mechanism of action of mGluRs, many questions are yet to be addressed with both biophysical approaches and whole-animal experimental systems (Box 2). Only time and additional research will provide us with answers.

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