Published in final edited form as:

Trends Pharmacol Sci. 2019 April ; 40(4): 240–252. doi:10.1016/j.tips.2019.02.006.

Neuropharmacological Insight from Allosteric Modulation of mGlu Receptors

Branden J. Stansley^{1,2} and P. Jeffrey Conn^{1,2,*}

¹Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA

²Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

Abstract

The metabotropic glutamate (mGlu) receptors are a family of G protein-coupled receptors that regulate cell physiology throughout the nervous system. The potential of mGlu receptors as therapeutic targets has been bolstered by current research that has provided insight into the diverse modes of mGlu activation and signaling. Particularly, the allosteric modulation of mGlu receptors represents a major area of focus in studies of basic pharmacology as well as drug development, largely due to the high subtype specificity achievable by targeting allosteric sites on mGlu receptors. These provide sophisticated regulation of neuronal excitability and synaptic transmission to influence behavioral output. Here, we review how these allosteric mechanisms have been leveraged preclinically to demonstrate the therapeutic potential of allosteric modulators for neurological and neuropsychiatric disorders such as autism, cognitive impairment, Parkinson's disease, stress, and schizophrenia.

Keywords

mGlu; allosteric modulators; CNS; stimulus bias; drug discovery; neuropsychiatric disorders

Targeting mGlu Receptors

The metabotropic glutamate (mGlu) receptors are a family of cell surface G protein-coupled receptors (GPCRs) consisting of seven transmembrane domains that are activated endogenously by glutamate, the primary excitatory transmitter in the central nervous system (CNS) [1]. As class GPCRs, mGlu receptors form constitutive homo- or heterodimers mediated by the N-terminal extracellular domain [2]. This N-terminal domain is also known

* Correspondence: jeffrey.conn@vanderbilt.edu, Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, 1205 Light Hall, Nashville, TN 37232-0697, (615) 875-9770.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of interest statement

P.J.C. receives research support from Lundbeck Pharmaceuticals and are inventors on multiple patents for allosteric modulators for several classes' metabotropic glutamate receptors.

as the ‘venus flytrap domain’ (VFD) and contains the orthosteric glutamate binding site. Eight mGlu receptor subtypes have been identified and are expressed throughout the CNS, both presynaptically and postsynaptically in neurons and also in glial cells. The mGlu receptors can be subdivided into three groups based on G-protein coupling, sequence homology and ligand selectivity. Group I mGlu receptors (mGlu₁ and mGlu₅) couple to G_{q/11} and related signaling pathways, whereas group II (mGlu₂ and mGlu₃) and group III (mGlu₄, 6, 7, 8) mGlu receptors couple to G_{i/o} signaling (Table 1). Figure 1, Key Figure lays out the location of the mGlu receptors within the prefrontal cortex (PFC) and hippocampus that will be discussed in this review.

It is now clear that group I mGlu receptors can signal through pathways that are independent of G_{q/11}, and group II and III mGlu receptors are capable of signaling through other non-canonical pathways that lead to potentiation of G_{q/11} signaling. One such example of this is the potentiation of phosphoinositide hydrolysis in brain tissue produced by activation of mGlu₅ receptors through co-activation of mGlu₃ receptors. Moreover, the effects of mGlu₅ on calcium signaling are similarly enhanced by pharmacological activation of mGlu₃ in cortical pyramidal neurons [3]. This exemplifies a complexity of pharmacologically targeting these receptors, which has led to many recent novel discoveries, as discussed in subsequent sections. Uncovering the physiological roles of these unique downstream pathways is essential to determining the temporal and site-specific dynamics regulating secondary signaling by mGlu receptors, and thus serves as crucial information in guiding current and future drug discovery efforts.

Allosteric Modulation of mGlu Receptors

Allosteric modulators act to alter the conformational state of the receptor through binding a topographically distinct non-orthosteric site of the receptor, typically found within the heptahelical domain of mGlu receptors [2]; hence the term allosteric, a Greek word meaning ‘other site’ [4]. Pure allosteric modulators do not activate the receptor directly but potentiate or decrease the response to the endogenous **orthosteric ligand** (see Glossary) (i.e. glutamate for mGlu receptors). Allosteric modulators that potentiate the functional response of the orthosteric agonist are termed positive allosteric modulators (PAMs), whereas those that decrease the functional response to the orthosteric agonist are termed negative allosteric modulators (NAMs). A compound that binds to an orthosteric site without effect on receptor response is called neutral allosteric ligand (NAL) [5]. In functional assays such as those that measure calcium mobilization, the effect of a PAM is often to induce a leftward shift of the agonist concentration-response curve, while a NAM will decrease the maximal effect of the response. The first allosteric modulator of mGlu receptors identified was the mGlu₁ NAM, CPCCOEt [6]; a compound that was shown to inhibit mGlu₁ receptor signaling without affecting glutamate binding [7]. This was followed shortly by the discovery of the first selective mGlu₅ NAMs, SIB-1757 and SIB-1893 [8]. Since the discovery of these prototypical compounds, many compounds for mGlu receptor subtypes within each of the three groups have been developed [9]. Many of these compounds are highly potent, selective and CNS penetrant and they have provided novel drug candidates that have advanced into clinical development for treatment of neurological and neuropsychiatric disorders.

From a theoretical perspective, the **ternary-complex-mass-action model** provides a framework to define the allosteric relationship between two ligands simultaneously binding to separate sites on the same receptor. Considering the affinity of both ligands as well as the ‘**cooperativity factor**’, this framework provides a theoretical direction and magnitude of functional response to the orthosteric ligand. As knowledge of GPCR allosteric pharmacology has advanced, so in turn has the operational modeling of allostereism. Current models allow for the description of allosteric modulation of both affinity and efficacy, as well as **allosteric agonism** (for detailed review see [10, 11]). Indeed, some allosteric modulators exert efficacy in the absence of the orthosteric ligand (**ago-PAMs**), and importantly, these models also include parameters to account for intrinsic efficacy of orthosteric and allosteric ligands. These models offer value in terms of providing a mechanism to hone drug discovery efforts.

Targeting the allosteric sites offer several advantages in development of pharmacotherapies. For example, the amino acid sequences making up the orthosteric site across mGlu receptor subtypes is highly conserved. In contrast, allosteric sites are less conserved and thus provide greater opportunities for the development of compounds to differentiate between subtypes. Moreover, subtype selectivity can be achieved through differential cooperativity [12]. The cooperativity between allosteric and orthosteric sites is not correlated with affinity of allosteric modulators for the binding site, and therefore allows certain allosteric modulators to bind multiple mGlu receptor subtypes with a similar affinities, but exert selective effects through differential cooperativity between subtypes.

Another unique characteristic of allosteric modulators is the ability of these compounds to regulate receptor responses only in brain areas where the endogenous agonist exerts its physiological effect. Allosteric modulators allow for ‘tuning’ of active synapses versus non-physiological activation of synapses that display low glutamatergic tone. Certainly, the aberrant activation of receptors in absence of the endogenous orthosteric ligand, as observed with orthosteric agonism, can lead to overactivation and disruptive circuit modulation. For example, mGlu₅ agonists have been found to cause epileptic seizure activity [13], an effect that is mitigated by administration of pure-mGlu₅ PAMs (PAMs displaying no agonist activity) [14]. This spatial and temporal restriction of allosteric modulators imbued by their functional requirement for the presence of the endogenous agonist provides a large therapeutic advantage by achieving physiologically appropriate modulation of synaptic transmission and signaling.

Functional selectivity

The concept of ‘pluridimensional efficacy’ for mGlu receptors is the phenomenon that individual ligands can display distinct efficacy towards a host of physiological parameters [15], leading to signaling pathways activating G-proteins, β -arrestins, receptor endocytosis, phosphorylation, etc. With multiple pathways available for activation by an agonist, the possibility of a specific signaling pathway being activated becomes an important characteristic when considering the ability for mGlu allosteric modulators to selectively enhance coupling of mGlu receptors to specific signaling pathways.

The conformation of mGlu receptors is not static, but exists in a spectrum of conformational states that activate a range of signaling pathways. Allosteric modulation that allows for biasing mGlu receptors towards conformations that selectively activate a particular signaling pathway over another is referred to as ‘functional selectivity’, or ‘stimulus bias’ [16]. Allosteric modulators have the ability to alter the conformational state of the receptor upon agonist (glutamate) binding, leading to selective activation, or bias, of specific signaling pathways by the endogenous agonist (Figure 2). This is exemplified by mGlu₅ allosteric modulators that have demonstrated stimulus bias, and has important therapeutic implications (see Box 1).

In addition to biased mGlu receptor PAMs having the ability to preferentially activate certain signaling pathways over others, mGlu receptor NAMs can also confer biased signaling and are capable of exhibiting varied efficacy on signaling pathways within a given system or tissue. This is exemplified by experiments designed to test mGlu₇ NAM effects on signaling pathways using several different G_{i/o} coupled assay readouts, including both cell systems and hippocampal tissue preparations. Interestingly, these studies found that the mGlu₇ NAM MMPiP demonstrated strong effects at inhibiting calcium mobilization in Chinese Hamster Ovary (CHO) cells expressing recombinant mGlu₇, but lacked any effects at modulating group III mGlu receptor agonist-induced depression of synaptic transmission at the Schaffer Collateral-CA1 (SC-CA1) synapse (for reference, see Figure 1) [17]. These differences in functional efficacy between model systems are referred to as “**context-dependent pharmacology**”, and highlight the importance of testing allosteric modulators for mGlu receptors in several model systems as well as disease relevant states, as the functional efficacy of a given compound may vary depending on context. Taken together, understanding of the signal bias displayed by specific PAMs and NAMs may be a crucial component to reducing adverse effect liability of allosteric modulators, but also improving desired pharmacological responses in a given system within the CNS.

Heterodimerization

The conformational dynamics of mGlu receptors are crucial in determining ligand recognition, effector transduction and downstream signaling. As class GPCRs, mGlu receptors are characterized by the necessity of dimerization for proper function. Furthermore, the stoichiometry of these dimers is an important aspect in determining how the receptors will transduce signal in response to allosteric modulation. Many *in vitro* cellular model systems utilize homodimeric receptor complexes; however, studies have begun to uncover the impact of heterodimeric receptors in regard to these signaling dynamics. For example, experiments using photoswitchable tethered ligands revealed that group II mGlu receptors, mGlu₂ and mGlu₃, form heterodimers *in vitro* and display an asymmetric activation cooperativity relative to mGlu₂ homodimers [18]. The differential pharmacological response between mGlu₂ homodimers and mGlu_{2/3} heterodimers suggests that these receptors may offer unique targets for impaired neural circuits in which these heterodimers are expressed, although *in vivo* identification of mGlu_{2/3} heterodimers has yet to be reported.

Additionally, evidence for mGlu₂ and mGlu₄ receptor heterodimers have been described *in vitro* using cell culture systems [19], and more recently *in vivo* in rodent CNS tissue [20]. Using biochemical co-immunoprecipitation assays, Yin et al. [20] found that mGlu_{2/4} heterodimers exist at the corticostriatal synapse, and these heterodimers display a unique functional response to certain mGlu₄ PAMs as measured by electrophysiology. As this corticostriatal synapse has been implicated in Parkinson's disease pathophysiology, allosteric modulators that engage mGlu₄ homo-versus mGlu_{2/4} hetero-dimers may allow for more precise modulation of these circuits. Indeed, Niswender et al. discovered a selective mGlu₄ PAM VU0418506 that did not show mGlu_{2/4} activity, but did demonstrate antiparkinsonian like effects in rodents [21]. These seminal studies of *in vivo* heterodimerization were built upon recently by the empirical identification of the mGlu_{2/4} heterodimer pharmacological fingerprint [22] by using fluorescence resonance energy transfer (FRET)-based assays. These fingerprints were then used to identify mGlu_{2/4} heterodimeric complexes in the lateral perforant path terminals of the dentate gyrus within the hippocampus, a key brain area involved in cognitive functions such as spatial learning and memory [23]. These studies highlight the importance of mGlu receptor heterodimers within CNS circuits that govern key physiological processes, and also provide the exciting possibility for selectively engaging brain regions in which these heterodimers are selectively expressed for the treatment of neurological disorders.

Therapeutic Potential of mGlu Receptor Allosteric Modulators

Schizophrenia

Selective mGlu₅ PAMs have received a great deal of attention as potential therapeutic agents for treatment of schizophrenia (see Box 1). In addition, extensive efforts have been focused on development of mGlu_{2/3} agonists and selective mGlu₂ PAMs as a novel approach to treatment of schizophrenia. Studies focused on targeting mGlu₂ and mGlu₅ for treatment of this disorder have been the subject of multiple previous reviews [24, 25] While clinical studies with mGlu_{2/3} agonists and mGlu₂ PAMs have been disappointing [26, 27], these approaches continue to provide viable possibilities as potential treatment strategies.

More recently, mGlu₁ has emerged as a potential target for treatment of schizophrenia. Multiple studies reveal that single nucleotide polymorphisms (SNPs) within the gene encoding for mGlu₁, *GRM1*, are associated with schizophrenia [28] and mGlu₁ mRNA is significantly altered in postmortem tissue samples from schizophrenia patients [29]. Interestingly, these mutations result in reduced mGlu₁ signaling *in vitro* and selective mGlu₁ PAMs potentiate responses to activation in these mutant mGlu₁ receptors [30]. Finally, mice harboring genetic deletions of mGlu₁ display a behavioral phenotype that mirrors the symptomology of schizophrenia, such as deficits in prepulse inhibition (PPI), a model of sensory motor gating that is disrupted in schizophrenia patients [31].

Consistent with these mouse and human genetic studies, exciting new studies reveal that mGlu₁ PAMs have antipsychotic-like effects in preclinical models of NMDAR-hypofunction [32]. Extensive *ex vivo* and *in vivo* **fast-scan cyclic voltammetry** studies suggest that mGlu₁ PAMs exert their antipsychotic-like effects by reducing striatal dopamine release [32]. However, unlike dopamine receptor antagonists that are currently used for treatment of

schizophrenia, mGlu₁ PAMs selectively inhibit dopamine signaling in the striatum and are not likely to impair cognitive function. Furthermore, mGlu₁ PAMs do not impair motivation in an operant conditioning paradigm [33], suggesting that these compounds could have significant advantages relative to existing antipsychotics.

Separately, mGlu₁ NAMs have also been demonstrated to reverse methamphetamine induced hyperlocomotion [34]. However, c-Fos expression studies point to the nucleus accumbens and medial prefrontal cortex regions being involved in mGlu₁ NAM effects, whereas the striatal region was not affected. Together these reports suggest similar preclinical efficacies by mGlu₁ PAMs and NAMs, but more research is necessary to discern possible desperate mechanisms of action. While these studies mentioned focus primarily on the positive symptoms related to schizophrenia, mGlu₁ receptors are positioned in critical brain areas such as the hippocampus [35] and prefrontal cortex [36] to suggest that they can be modulated to enhance cognitive function and possibly treat cognitive and negative domains of this disorder as well. These other domains are likely to be thoroughly investigated as mGlu₁ is still in a preclinical stage of validation as a target for schizophrenia.

Genetic studies have also provided a strong link between mutations in *GRM3*, the gene encoding for mGlu₃, with schizophrenia and deficits in cognitive function [37]. Based on these genetic studies and recent advances with novel selective allosteric modulators of mGlu₃, a role for this receptor as a target for cognitive impairments associated with schizophrenia is also beginning to emerge. While the physiological roles of mGlu₃ that could contribute to regulation of cognitive function have not been fully elucidated, preclinical studies are beginning to accumulate that are consistent with a key role of mGlu₃ in regulating multiple aspects of cognitive function. For instance, mGlu₃ knockout (KO) mice show deficits in working memory [38], and recent discovery of the first highly selective mGlu₃ NAMs have provided the first pharmacological tools that allow exploration of the specific physiological roles of mGlu₃ [39, 40].

New reports suggest that mGlu₃ may play a key role in regulating hippocampal synaptic plasticity and cognitive function. For example, mGlu_{2/3} agonists enhance **long-term potentiation (LTP)** at the hippocampal SC-CA1 synapse. This effect was shown to be dependent on mGlu₃ through experiments using mGlu₂ and mGlu₃ KO mice [41], and pharmacologically using highly selective mGlu₂ and mGlu₃-selective allosteric modulators [42]. Consistent with its ability to enhance hippocampal LTP, selective activation of mGlu₃ enhances cognition in hippocampal dependent temporal associative tasks [42]. Furthermore, these hippocampal studies corroborate a functional partnership between mGlu₃ with mGlu₅, specifically in their shared ability to facilitate G_{q/11} signaling-dependent pathways and downstream synaptic plasticity responses throughout the brain [3]. mGlu₃ activation was also capable of reversing deficits in LTP and working memory in an NMDAR hypofunction model of schizophrenia pathology in mice [42]. Although selective mGlu₃ PAMs have not been reported, discovery efforts could be directed based upon these signaling mechanisms observed by selective orthosteric mGlu₃ activation, which may increase the chances of enhancing cognition for schizophrenia. While these studies primarily focus on the hippocampus, mGlu₃ in other brain areas pathologically involved in schizophrenia have also been investigated.

Several novel studies have revealed that mGlu₃ also plays key roles in regulating information processing and synaptic plasticity in the PFC (for reference, see Figure 1) that could be critical for aspects of FC-dependent cognitive function that are impaired in schizophrenia patients. Recent research found that mGlu₃ receptors located postsynaptically in the dorsolateral PFC of non-human primates contribute to working memory, such that pharmacologically activating mGlu₃ enhances delayed cell firing during a working memory task [43]. Furthermore, synaptic plasticity studies using *ex vivo* PFC slices demonstrate that mGlu₃ activation causes **long-term depression (LTD)** of electrically evoked field potentials [44]. This mGlu₃-mediated LTD was found to be postsynaptically mediated via a pathway involving AKT signaling and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) internalization [45]. Interestingly, mGlu₅ activation was required for mGlu₃-mediated LTD to occur in the PFC [45], highlighting that this mGlu₃ and mGlu₅ interaction contributes to multiple forms of plasticity throughout the brain. Taken together, these results point to mGlu₃ PAMs as having extremely high potential therapeutic utility for disorders involving cognitive impairment such as schizophrenia.

Autism and related disorders

Autism spectrum disorders (ASD) and autism-related disorders are clinically diagnosed by observable deficits in social communication and interaction, repetitive patterns of behavior or activity which occur early in development and are not due to overarching intellectual disability. While the etiology can vary between patients diagnosed with ASD, genetic evidence strongly implicates the glutamatergic system as being involved in pathologies related to ASD and autism-related disorders [46]. There is a high incidence of ASD diagnosis within developmental disorders such as Fragile X syndrome [47] or Rett syndrome [48], which are genetically defined conditions.

Extensive preclinical studies suggested that mGlu₅-selective NAMs may be efficacious for treatment of Fragile X syndrome [49], and these compelling results motivated efforts to advance mGlu₅ NAMs into clinical development for treatment of this disorder. Unfortunately, initial clinical studies have been disappointing and mGlu₅ NAMs failed to meet their primary endpoints in reducing symptoms in Fragile X patients [50]. However, a recent report suggests that β -arrestin-2 mediates mGlu₅-stimulated protein synthesis in the hippocampus, a response that is thought to be key to pathological increases in mGlu₅ signaling in Fragile X syndrome [51]. Furthermore, genetic reduction of β -arrestin-2 reverses impaired synaptic plasticity and cognitive function in a mouse model of Fragile X [51]. These effects were independent of mGlu₅-mediated G_{q/11} signaling [51], raising the possibility that mGlu₅ NAMs that confer biased inhibition of mGlu₅ coupling to β -arrestin-2 signaling may be more favorable than non-biased mGlu₅ NAMs that inhibit G_{q/11} signaling, and could potentially lead to unwanted side-effects such as cognitive disruption.

Rett syndrome is a neurodevelopmental disorder caused by mutations in the methyl-CpG-binding protein 2 (MeCP2) gene. Patients bearing these mutations present with symptoms including but not limited to apneas, cognitive impairment, and social deficits [52]. New research has found that mGlu₇ protein expression is reduced in postmortem brain samples of patients diagnosed with Rett syndrome that is paralleled by similar deficits in mouse models

of *Mecp2* genetic reduction. Furthermore, mGlu₇ activation not only restored proper synaptic plasticity within the hippocampus of Rett model mice, but also reversed deficits in cognition, sociability, as well as respiration [53]. These advances highlight the benefits of potentially using allosteric modulation of mGlu receptors to treat these genetic forms of autism disorders that currently lack any approved efficacious medicines.

Parkinson's disease

For over a decade, mGlu₄ receptors have been investigated as potential therapeutic targets for the motor symptoms associated with Parkinson's disease [54] (for review see [55]). Parkinson's disease is currently treated primarily with L-dopa, but over time the chronic treatment of L-dopa leads to uncontrollable movement in the patient referred to as 'dyskinesia'. Recently, clinical trials have been initiated to test mGlu₄ PAMs that have shown efficacy in reducing motor symptoms associated with Parkinson's disease, as well as L-dopa induced dyskinesia (LID) in non-human primates [56]. The introduction of mGlu₄ PAMs to the clinic could fill a critical unmet need for Parkinson's patients.

Stress-related disorders

Stress and anxiety involve both 'bottom-up' and 'top-down' control, primarily by limbic regions of the brain. Many stressors result in a dysregulation of glutamatergic transmission [57], and thus the mGlu receptors are in prime locations within these regions to modulate stress response and resilience to stressors. Within the hippocampus, mGlu₅ function is critical for long-term plasticity such as LTP and LTD [58]. The hippocampus is also a critical node in the formation of fear memories. Pavlovian fear conditioning and extinction provides a preclinical paradigm that is analogous to the current standard treatment for traumatic stress, which is exposure therapy [59]. Selective mGlu₅ NAMs injected into the hippocampus impair fear extinction by blocking hippocampal metaplasticity mechanisms that lead to enhanced LTP [60]. Correspondingly, some preclinical work has demonstrated efficacy of mGlu₅ PAM enhancement of fear extinction [61]. In addition, mGlu₁ activation was shown to enhance hippocampal LTP via small conductance calcium-activated potassium (SK) channel inhibition, leading to disinhibition of NMDARs [62]. This points to the possibility of mGlu₅ and mGlu₁ PAMs as being potential adjunctive pharmacotherapies to enhance exposure therapy.

The effects of stress on hippocampal plasticity also involve mGlu₃ in astrocytes within the hippocampal area CA1 (see Figure 1). Specifically, they have been shown to be activated during stress and act to reduce LTP by coupling to β -adrenergic receptors co-localized on astrocytes, leading to adenosine autocoid signaling that inhibits presynaptic glutamate transmission. This decrease in LTP correlated with an impairment in memory consolidation when mGlu₃ was activated [63]. The hippocampus also gates information flow to the PFC, an area highly involved in reciprocal signaling to areas such as the amygdala during stress [64]. While the mGlu receptors expressed in the PFC and hippocampus are somewhat overlapping, several recent studies reveal an interesting dichotomy in mGlu receptor mediated plasticity effects driven by these receptors between the two regions (see Figure 1). For example, mGlu₃ activation in the PFC leads to an LTD through a postsynaptic neuronal mechanism. This mGlu₃ mediated LTD at PFC synapses has been demonstrated to be

dependent on mGlu₅ signaling [3]. Interestingly, experiments focused on mGlu₃ function after stress found that stress impaired cognitive function in mice, which correlated with a deficit in mGlu₃-LTD. These stress-induced impairments were reversed by mGlu₅ PAM VU0409551, further demonstrating the utility of targeting receptors that maintain functional partnerships, such as mGlu₃ and mGlu₅, in order to treat stress related psychiatric disorders [45]. These data also suggest that mGlu₃ NAMs would be beneficial prophylactically prior to a stress event. This is supported by studies by Joffe et al. that show that administration of a selective mGlu₃ NAM that exhibits a desirable pharmacokinetic profile and CNS penetration, prior to acute stress, prevents impairments in mGlu₃ mediated LTD at amygdala-cortical synapses, as well as operant behavior [65].

Taken together, these studies reveal that selective mGlu receptor modulation within specific brain areas can have different effects on synaptic physiology. Moreover, mGlu₁ modulation of inhibitory transmission [36], and mGlu₂ acting as an autoreceptor at excitatory terminals in the PFC [43] may provide fine modulation of synaptic transmission by exploiting site specific differences (see Figure 1).

Concluding remarks

The investigation of mGlu receptor allosteric modulators has led to many fascinating insights into neuropharmacology, and surely more discoveries are on the horizon. There are many outstanding questions that the field is primed to address that will propel research on allosteric modulators forward (see Outstanding Questions). For example, several mGlu receptor agonists have shown efficacy in preclinical models of schizophrenia, cognitive impairment, stress (mGlu group II agonist), and autism-related disorders (mGlu group III agonist). What mGlu subtype(s) specifically contribute to the efficacy in these models? In future studies, it will be interesting to elucidate the specific subtypes mediating the efficacy behind these agonist compounds, through the use of subtype selective PAMs. Additionally, allosteric-related factors such as signal bias and heterodimer engagement will be keys to the translation of these PAMs into the clinical setting. A pertinent question then becomes, what signal biases and/or heterodimer complexes are most beneficial to restoring normal physiology within the circuits underlying specific disease states, and can we develop compounds displaying these signal biases and/or heterodimer selectivity, respectively?

As the pharmacology and physiology of mGlu allosteric modulators advance, basic science findings within CNS regions can be applied to new potential indications and model systems to test therapeutic utility. Examples of this include mGlu₁ modulation of basal ganglia circuitry, which has been investigated for positive symptoms of schizophrenia, but could also influence cognition and certain forms of executive functioning. Additionally, mGlu₃ has been demonstrated to enhance hippocampal and PFC-dependent forms of cognition, but may also influence affective behavior that is dependent upon striatal or amygdala function, as mGlu₃ has been shown to be expressed and modulate cellular signaling in these areas. Therefore, could mGlu₁ or mGlu₃ allosteric modulators have therapeutic potential for multiple schizophrenia symptom domains (positive, negative and cognitive)?

Recent reports have demonstrated that signal bias can have crucial implications for therapeutic efficacy, as evidenced by mGlu₅ PAMs biased away from NMDAR modulation for schizophrenia or mGlu₅ NAMs biased toward inhibition of β -arrestin-2 signaling in models of Fragile X. How do we translate these preclinical findings to drug candidates that maintain proper biased signaling throughout different systems and species to avoid 'context-dependent pharmacology' that could compromise efficacy? Drug discovery programs that account for these pharmacological phenomena throughout model systems will likely increase the competitiveness of future drug candidates.

We discussed several relevant findings and how they relate to the potential utility of selective allosteric modulators of mGlu receptors for disorders of the CNS. However, there are also many indications that would be predicted to be extremely amenable to mGlu allosteric modulation that were not covered within the scope of this review. For example, a recent study has provided evidence for mGlu₅ in sleep/wake architecture, suggesting that mGlu₅ allosteric modulators may have potential to remedy certain sleep disorders [66]. Certainly, investigation of mGlu allosteric modulators for substance use disorders [67], epilepsy [68], major depressive disorder [69], and Alzheimer's disease [70] are contexts in which there is exciting work being accomplished within the field of allosteric modulators and mGlu receptors. As the drug discovery process is a circular method, continued research efforts will likely lead to even more insights into the neuropharmacology surrounding mGlu allosteric modulators and their usefulness as therapeutics in CNS disorders.

Acknowledgements

PJC receives funding from the National Institutes of Health (R01MH062646, R37NS031373). BJS is the recipient of a postdoctoral fellowship from the National Institute of Health (F32MH111124). Special thank you to members of the Conn Laboratory for their insightful input.

GLOSSARY

Ago-PAM

positive allosteric modulator that displays agonist activity

Allosteric agonism

binding of ligand to allosteric site that causes direct activation of receptor, without co-activation of orthosteric site

Context-dependent pharmacology

the concept of a single ligand exhibiting different pharmacological characteristics in a given cell, synapse or tissue.

Cooperativity factor

the effect that the binding of a molecule to the receptor has on the binding affinity of another molecule to another site on that receptor

Fast-scan cyclic voltammetry

an electrochemical technique with high sampling rate, often used to monitor extracellular concentration of electroactive molecules

LTD

the persistent weakening of synaptic efficacy in response to previous patterned activity

LTP

the persistent strengthening of synaptic efficacy in response to previous patterned activity

Orthosteric ligand

molecule that binds to active site on the receptor

Ternary-complex-mass-action model

pharmacological model used to describe the behavior of transmembrane mGlu receptors, accounting for second messenger signaling mechanisms

REFERENCES:

1. Niswender CM and Conn PJ (2010) Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol* 50, 295–322. [PubMed: 20055706]
2. Wu H et al. (2014) Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator. *Science* 344 (6179), 58–64. [PubMed: 24603153]
3. Di Menna L et al. (2018) Functional partnership between mGlu3 and mGlu5 metabotropic glutamate receptors in the central nervous system. *Neuropharmacology* 128, 301–313. [PubMed: 29079293]
4. Monod J et al. (1965) On the Nature of Allosteric Transitions: Plausible Model. *J Mol Biol* 12, 88–118. [PubMed: 14343300]
5. Conn PJ et al. (2009) Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat Rev Drug Discov* 8 (1), 41–54. [PubMed: 19116626]
6. Annoura H,FA, Uesugi M, Tatsuoka T, Horikawa Y. (1996) A novel class of antagonists for metabotropic glutamate receptors, 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylates. *Bioorg Med Chem Lett* 6, 763–766.
7. Litschig S et al. (1999) CPCCOEt, a noncompetitive metabotropic glutamate receptor 1 antagonist, inhibits receptor signaling without affecting glutamate binding. *Mol Pharmacol* 55 (3), 453–61. [PubMed: 10051528]
8. Varney MA et al. (1999) SIB-1757 and SIB-1893: selective, noncompetitive antagonists of metabotropic glutamate receptor type 5. *J Pharmacol Exp Ther* 290 (1), 170–81. [PubMed: 10381773]
9. Maksymetz J et al. (2017) Targeting metabotropic glutamate receptors for novel treatments of schizophrenia. *Mol Brain* 10 (1), 15. [PubMed: 28446243]
10. Gregory KJ et al. (2010) Overview of receptor allosterism. *Curr Protoc Pharmacol Chapter 1, Unit 1* 21. [PubMed: 21935895]
11. Changeux JP and Christopoulos A (2016) Allosteric Modulation as a Unifying Mechanism for Receptor Function and Regulation. *Cell* 166 (5), 1084–1102. [PubMed: 27565340]
12. Christopoulos A (2002) Allosteric binding sites on cell-surface receptors: novel targets for drug discovery. *Nat Rev Drug Discov* 1 (3), 198–210. [PubMed: 12120504]
13. Tizzano JP et al. (1995) Receptor subtypes linked to metabotropic glutamate receptor agonist-mediated limbic seizures in mice. *Ann N Y Acad Sci* 765, 230–5; discussion 248. [PubMed: 7486609]
14. Rook JM et al. (2015) Biased mGlu5-Positive Allosteric Modulators Provide In Vivo Efficacy without Potentiating mGlu5 Modulation of NMDAR Currents. *Neuron* 86 (4), 1029–1040. [PubMed: 25937172]
15. Galandrin S and Bouvier M (2006) Distinct signaling profiles of beta1 and beta2 adrenergic receptor ligands toward adenylyl cyclase and mitogen-activated protein kinase reveals the pluridimensionality of efficacy. *Mol Pharmacol* 70 (5), 1575–84. [PubMed: 16901982]

16. Kenakin T and Christopoulos A (2013) Signalling bias in new drug discovery: detection, quantification and therapeutic impact. *Nat Rev Drug Discov* 12 (3), 205–16. [PubMed: 23411724]
17. Niswender CM et al. (2010) Context-dependent pharmacology exhibited by negative allosteric modulators of metabotropic glutamate receptor 7. *Mol Pharmacol* 77 (3), 459–68. [PubMed: 20026717]
18. Levitz J et al. (2016) Mechanism of Assembly and Cooperativity of Homomeric and Heteromeric Metabotropic Glutamate Receptors. *Neuron* 92 (1), 143–159. [PubMed: 27641494]
19. Doumazane E et al. (2011) A new approach to analyze cell surface protein complexes reveals specific heterodimeric metabotropic glutamate receptors. *FASEB J* 25 (1), 66–77. [PubMed: 20826542]
20. Yin S et al. (2014) Selective actions of novel allosteric modulators reveal functional heteromers of metabotropic glutamate receptors in the CNS. *J Neurosci* 34 (1), 79–94. [PubMed: 24381270]
21. Niswender M et al. (2016) Development and Antiparkinsonian Activity of VU0418506, a Selective Positive Allosteric Modulator of Metabotropic Glutamate Receptor 4 Homomers without Activity at mGlu2/4 Heteromers. *ACS Chem Neurosci* 7 (9), 1201–11. [PubMed: 27441572]
22. Moreno Delgado D et al. (2017) Pharmacological evidence for a metabotropic glutamate receptor heterodimer in neuronal cells. *Elife* 6.
23. Madronal N et al. (2016) Rapid erasure of hippocampal memory following inhibition of dentate gyrus granule cells. *Nat Commun* 7, 10923. [PubMed: 26988806]
24. Conn PJ and Jones CK (2009) Promise of mGluR2/3 activators in psychiatry. *Neuropsychopharmacology* 34 (1), 248–9. [PubMed: 19079073]
25. Conn PJ et al. (2009) Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. *Trends Pharmacol Sci* 30 (1), 25–31. [PubMed: 19058862]
26. Li ML et al. (2015) Perspectives on the mGluR2/3 agonists as a therapeutic target for schizophrenia: Still promising or a dead end? *Prog Neuropsychopharmacol Biol Psychiatry* 60, 66–76. [PubMed: 25724760]
27. Litman RE et al. (2016) AZD8529, a positive allosteric modulator at the mGluR2 receptor, does not improve symptoms in schizophrenia: A proof of principle study. *Schizophr Res* 172 (1–3), 152–7. [PubMed: 26922656]
28. Ayoub MA et al. (2012) Deleterious GRM1 mutations in schizophrenia. *PLoS One* 7 (3), e32849. [PubMed: 22448230]
29. Volk DW et al. (2010) Alterations in metabotropic glutamate receptor 1alpha and regulator of G protein signaling 4 in the prefrontal cortex in schizophrenia. *Am J Psychiatry* 167 (12), 1489–98. [PubMed: 20889653]
30. Cho HP et al. (2014) Chemical modulation of mutant mGlu1 receptors derived from deleterious GRM1 mutations found in schizophrenics. *ACS Chem Biol* 9 (10), 2334–46. [PubMed: 25137254]
31. Brody SA et al. (2003) Disruption of prepulse inhibition in mice lacking mGluR1. *Eur J Neurosci* 18 (12), 3361–6. [PubMed: 14686909]
32. Yohn SE et al. (2018) Activation of the mGlu1 metabotropic glutamate receptor has antipsychotic-like effects and is required for efficacy of M4 muscarinic receptor allosteric modulators. *Mol Psychiatry*
33. Yohn SE et al. (2015) The role of dopamine D1 receptor transmission in effort-related choice behavior: Effects of D1 agonists. *Pharmacol Biochem Behav* 135, 217–26. [PubMed: 26022661]
34. Satow A et al. (2009) Unique antipsychotic activities of the selective metabotropic glutamate receptor 1 allosteric antagonist 2-cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1H-1,2,3-triazol-4-yl]-2,3-dihydro-1H-isoindol-1-one. *J Pharmacol Exp Ther* 330 (1), 179–90. [PubMed: 19359526]
35. Foster WJ et al. (2018) Hippocampal mGluR1-dependent long-term potentiation requires NAADP-mediated acidic store Ca(2+) signaling. *Sci Signal* 11 (558).
36. Sun H and Neugebauer V (2011) mGluR1, but not mGluR5, activates feed-forward inhibition in the medial prefrontal cortex to impair decision making. *J Neurophysiol* 106 (2), 960–73. [PubMed: 21613584]
37. Saini SM et al. (2017) Meta-analysis supports GWAS-implicated link between GRM3 and schizophrenia risk. *Transl Psychiatry* 7 (8), e1196. [PubMed: 28786982]

38. Lainioli M et al. (2014) mGluR3 knockout mice show a working memory defect and an enhanced response to MK-801 in the T- and Y-maze cognitive tests. *Behav Brain Res* 266, 94–103. [PubMed: 24631392]
39. Engers JL et al. (2017) Design and Synthesis of N-Aryl Phenoxyethoxy Pyridinones as Highly Selective and CNS Penetrant mGlu3 NAMs. *ACS Med Chem Lett* 8 (9), 925–930. [PubMed: 28947938]
40. Sheffler DJ et al. (2010) Development of the First Selective mGlu3 NAM from an mGlu5 PAM Hit. In *Probe Reports from the NIH Molecular Libraries Program*
41. Rosenberg N et al. (2016) Activation of Group II Metabotropic Glutamate Receptors Promotes LTP Induction at Schaffer Collateral-CA1 Pyramidal Cell Synapses by Priming NMDA Receptors. *J Neurosci* 36 (45), 11521–11531. [PubMed: 27911756]
42. Stansley B et al. (2018) T39. NEURAL MECHANISMS OF METABOTROPIC GLUTAMATE RECEPTOR 3 MEDIATED ENHANCEMENT OF SYNAPTIC PLASTICITY AND COGNITION. *Schizophrenia Bulletin* 44 (suppl_1), S127–S128.
43. Jin L. et al. (2018) mGluR2 versus mGluR3 Metabotropic Glutamate Receptors in Primate Dorsolateral Prefrontal cortex: Postsynaptic mGluR3 Strengthen Working Memory Networks. *Cereb Cortex* 28 (3), 974–987. [PubMed: 28108498]
44. Walker G et al. (2015) Metabotropic glutamate receptor 3 activation is required for long-term depression in medial prefrontal cortex and fear extinction. *Proc Natl Acad Sci U S A* 112 (4), 1196–201. [PubMed: 25583490]
45. Joffe ME et al. (2019) Mechanisms underlying prefrontal cortex mGlu3/mGlu5-dependent plasticity and reversal learning deficits following acute stress. *Neuropharmacology* 144, 19–28. [PubMed: 30326237]
46. Rojas DC (2014) The role of glutamate and its receptors in autism and the use of glutamate receptor antagonists in treatment. *J Neural Transm (Vienna)* 121 (8), 891–905. [PubMed: 24752754]
47. Abbeduto L et al. (2014) The fragile X syndrome-autism comorbidity: what do we really know? *Front Genet* 5, 355. [PubMed: 25360144]
48. Percy AK (2011) Rett syndrome: exploring the autism link. *Arch Neurol* 68 (8), 985–9. [PubMed: 21825235]
49. Hagerman RJ et al. (2014) Translating molecular advances in fragile X syndrome into therapy: a review. *J Clin Psychiatry* 75 (4), e294–307. [PubMed: 24813413]
50. Berry-Kravis E et al. (2016) Mavoglurant in fragile X syndrome: Results of two randomized, double-blind, placebo-controlled trials. *Sci Transl Med* 8 (321), 321ra5.
51. Stoppel LJ et al. (2017) beta-Arrestin2 Couples Metabotropic Glutamate Receptor 5 to Neuronal Protein Synthesis and Is a Potential Target to Treat Fragile X. *Cell Rep* 18 (12), 2807–2814. [PubMed: 28329674]
52. Zoghbi HY (2016) Rett Syndrome and the Ongoing Legacy of Close Clinical Observation. *Cell* 167 (2), 293–297. [PubMed: 27716498]
53. Gogliotti RG et al. (2017) mGlu7 potentiation rescues cognitive, social, and respiratory phenotypes in a mouse model of Rett syndrome. *Sci Transl Med* 9 (403).
54. Hopkins CR et al. (2009) mGluR4-positive allosteric modulation as potential treatment for Parkinson's disease. *Future Med Chem* 1 (3), 501–13. [PubMed: 20161443]
55. Charvin D (2018) mGlu4 allosteric modulation for treating Parkinson's disease. *Neuropharmacology* 135, 308–315. [PubMed: 29578036]
56. Charvin D et al. (2018) An mGlu4-Positive Allosteric Modulator Alleviates Parkinsonism in Primates. *Mov Disord* 33 (10), 1619–1631. [PubMed: 30216534]
57. Popoli M et al. (2011) The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat Rev Neurosci* 13 (1), 22–37. [PubMed: 22127301]
58. Ayala JE et al. (2009) mGluR5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. *Neuropsychopharmacology* 34 (9), 2057–71. [PubMed: 19295507]
59. VanElzakker MB et al. (2014) From Pavlov to PTSD: the extinction of conditioned fear in rodents, humans, and anxiety disorders. *Neurobiol Learn Mem* 113, 3–18. [PubMed: 24321650]

60. Stansley BJ et al. (2017) Contextual Fear Extinction Induces Hippocampal Metaplasticity Mediated by Metabotropic Glutamate Receptor 5. *Cereb Cortex*, 1–14. [PubMed: 28365777]
61. Sethna F and Wang H (2014) Pharmacological enhancement of mGluR5 facilitates contextual fear memory extinction. *Learn Mem* 21 (12), 647–50. [PubMed: 25403451]
62. Tigaret CM et al. (2018) Convergent Metabotropic Signaling Pathways Inhibit SK Channels to Promote Synaptic Plasticity in the Hippocampus. *J Neurosci* 38 (43), 9252–9262. [PubMed: 30242046]
63. Walker AG et al. (2017) Co-Activation of Metabotropic Glutamate Receptor 3 and Beta-Adrenergic Receptors Modulates Cyclic-AMP and Long-Term Potentiation, and Disrupts Memory Reconsolidation. *Neuropsychopharmacology* 42 (13), 2553–2566. [PubMed: 28664928]
64. Stujenske JM and Likhtik E (2017) Fear from the bottom up. *Nat Neurosci* 20 (6), 765–767. [PubMed: 28542155]
65. Joffe ME et al. (2017) Metabotropic glutamate receptor subtype 3 gates acute stress-induced dysregulation of amygdalo-cortical function. *Mol Psychiatry*
66. Holst SC et al. (2017) Cerebral mGluR5 availability contributes to elevated sleep need and behavioral adjustment after sleep deprivation. *Elife* 6.
67. Joffe ME et al. (2018) Metabotropic Glutamate Receptors in Alcohol Use Disorder: Physiology, Plasticity, and Promising Pharmacotherapies. *ACS Chem Neurosci* 9 (9), 2188–2204. [PubMed: 29792024]
68. Umpierre D et al. (2019) Conditional Knock-out of mGluR5 from Astrocytes during Epilepsy Development Impairs High-Frequency Glutamate Uptake. *J Neurosci* 39 (4), 727–742. [PubMed: 30504280]
69. Joffe ME and Conn PJ (2019) Antidepressant potential of metabotropic glutamate receptor mGlu2 and mGlu3 negative allosteric modulators. *Neuropsychopharmacology* 44 (1), 214–236. [PubMed: 30202048]
70. Haas LT et al. (2017) Silent Allosteric Modulation of mGluR5 Maintains Glutamate Signaling while Rescuing Alzheimer's Mouse Phenotypes. *Cell Rep* 20 (1), 76–88. [PubMed: 28683325]
71. Kahn RS et al. (2015) Schizophrenia. *Nat Rev Dis Primers* 1, 15067. [PubMed: 27189524]
72. Balu DT (2016) The NMDA Receptor and Schizophrenia: From Pathophysiology to Treatment. *Adv Pharmacol* 76, 351–82. [PubMed: 27288082]
73. Balu DT et al. (2016) An mGlu5-Positive Allosteric Modulator Rescues the Neuroplasticity Deficits in a Genetic Model of NMDA Receptor Hypofunction in Schizophrenia. *Neuropsychopharmacology* 41 (8), 2052–61. [PubMed: 26741285]
74. Parmentier-Batteur S et al. (2014) Mechanism based neurotoxicity of mGlu5 positive allosteric modulators--development challenges for a promising novel antipsychotic target. *Neuropharmacology* 82, 161–73. [PubMed: 23291536]
75. Xu J et al. (2014) Hippocampal metaplasticity is required for the formation of temporal associative memories. *J Neurosci* 34 (50), 16762–73. [PubMed: 25505329]
76. Chevalleyre V and Castillo PE (2003) Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron* 38 (3), 461–72. [PubMed: 12741992]
77. Suzuki G et al. (2010) Effect of CFMTI, an allosteric metabotropic glutamate receptor 1 antagonist with antipsychotic activity, on Fos expression in regions of the brain related to schizophrenia. *Neuroscience* 168 (3), 787–96. [PubMed: 20399255]
78. Youssef EA et al. (2018) Effect of the mGluR5-NAM Basimglurant on Behavior in Adolescents and Adults with Fragile X Syndrome in a Randomized, Double-Blind, Placebo-Controlled Trial: FragXis Phase 2 Results. *Neuropsychopharmacology* 43 (3), 503–512. [PubMed: 28816242]
79. Gogliotti RG et al. (2016) mGlu5 positive allosteric modulation normalizes synaptic plasticity defects and motor phenotypes in a mouse model of Rett syndrome. *Hum Mol Genet* 25 (10), 1990–2004. [PubMed: 26936821]
80. Tao J et al. (2016) Negative Allosteric Modulation of mGluR5 Partially Corrects Pathophysiology in a Mouse Model of Rett Syndrome. *J Neurosci* 36 (47), 11946–11958. [PubMed: 27881780]
81. Petrov D et al. (2014) Mavoglurant as a treatment for Parkinson's disease. *Expert Opin Investig Drugs* 23 (8), 1165–79.

82. Griebel G et al. (2016) The mGluR2 positive allosteric modulator, SAR218645, improves memory and attention deficits in translational models of cognitive symptoms associated with schizophrenia. *Sci Rep* 6, 35320. [PubMed: 27734956]
83. Engers JL et al. (2015) Discovery of a Selective and CNS Penetrant Negative Allosteric Modulator of Metabotropic Glutamate Receptor Subtype 3 with Antidepressant and Anxiolytic Activity in Rodents. *J Med Chem* 58 (18), 7485–500. [PubMed: 26335039]
84. Panarese JD et al. (2019) The discovery of VU0652957 (VU2957, Valiglurax): SAR and DMPK challenges en route to an mGlu4 PAM development candidate. *Bioorg Med Chem Lett* 29 (2), 342–346. [PubMed: 30503632]
85. Wieronska JM et al. (2013) The reversal of cognitive, but not negative or positive symptoms of schizophrenia, by the mGlu(2)/(3) receptor agonist, LY379268, is 5-HT(1)A dependent. *Behav Brain Res* 256, 298–304. [PubMed: 23948211]
86. Kalinichev M et al. (2013) ADX71743, a potent and selective negative allosteric modulator of metabotropic glutamate receptor 7: in vitro and in vivo characterization. *J Pharmacol Exp Ther* 344 (3), 624–36. [PubMed: 23257312]
87. Duvoisin RM et al. (2010) Acute pharmacological modulation of mGluR8 reduces measures of anxiety. *Behav Brain Res* 212 (2), 168–73. [PubMed: 20385173]

Highlights

- mGlu allosteric modulators are highly selective, and have the ability to induce multiple modes of transduction, aiding in drug discovery efforts by providing the ability to target specific signaling cascades of particular mGlu receptor subtypes.
- mGlu₁ PAMs may provide a novel target for the positive symptom domains of schizophrenia, without compromising motivated behavior.
- mGlu₃ activity gates synaptic plasticity in brain areas involved in cognition and represents a potential target for disorders of cognitive impairment.
- mGlu₇ potentiation is efficacious in preclinical models of the autism-related disorder, Rett syndrome.
- Stress response of the CNS is amenable to modulation by several mGlu receptor subtypes.

Outstanding questions

- Given that several non-subtype selective mGlu orthosteric agonists have demonstrated efficacy in preclinical models of disease pathologies, can allosteric modulators be used to tease apart the contribution of these mGlu subtype(s) to determine which receptors specifically contribute to the efficacy in these models?
- What signal biases are most beneficial to restoring normal physiology within the critical brain areas afflicted by specific disease states, and can we develop compounds displaying these signal biases?
- Can mGlu receptor heterodimer complexes be identified within unique brain circuits that could allow for systemically administered mGlu allosteric modulators to selectively restore physiological function, while reducing off-target effects within the brain?
- Could mGlu1 or mGlu3 allosteric modulators have therapeutic potential for multiple schizophrenia symptom domains (positive, negative and cognitive)?
- What are the best methods to translate these preclinical findings on mGlu allosteric modulators into drug candidates that maintain proper biased signaling throughout different model systems and species to avoid ‘context-dependent pharmacology’ that could compromise efficacy?

BOX 1:**Stimulus bias of mGlu5 PAMs**

There have been several examples of stimulus bias of mGlu receptor allosteric modulators. For instance, mGlu5 PAMs have been a long sought-after target for all three symptom domains of schizophrenia (positive, negative, and cognitive) [71]. Interestingly, the initial impetus of mGlu5 PAM drug discovery for schizophrenia was based on the hypothesis that N-Methyl-D-aspartate receptor (NMDAR)-hypofunction may contribute to the pathophysiology underlying some aspects of schizophrenia. Multiple studies suggest that reduced function of NMDARs on inhibitory interneurons can lead to disinhibition and overactivation of glutamatergic circuits in key forebrain regions and that this contributes to positive, negative, and cognitive symptoms in schizophrenia patients [72]. Therefore, the structural and functional link between NMDARs with mGlu5 receptors in the postsynaptic cell provided rationale for activating mGlu5 receptors to restore NMDAR-mediated currents back to physiological levels. While mGlu5 PAMs proved extraordinarily efficacious in reversing deficits in animal models such as transient pharmacological NMDAR-antagonism [14] and genetic knockdown of NMDAR subunits [73], some of these specific mGlu5 PAMs were accompanied by adverse effects such as seizures and neuronal cytotoxicity [74]. These effects halted many discovery programs focused on mGlu5 PAMs. However, recent research on mGlu5-induced stimulus bias has demonstrated that some mGlu5 allosteric modulators can confer biased signaling to mGlu5 and selectively potentiate coupling to $G_{q/11}$ without potentiating coupling to NMDAR potentiation. For example, experiments demonstrated that the mGlu5 PAM, VU0409551, did not potentiate mGlu5 modulation of NMDAR receptor currents in hippocampal neurons (see Figure 1), but still displayed antipsychotic-like effects, as well as enhanced hippocampal-dependent cognitive function in assays of spatial learning and social behavior in rodents. Importantly, VU0409551 was devoid of severe adverse effect liability that is often observed with mGlu5 PAMs that potentiate coupling to NMDAR signaling [14]. Therefore, optimization of biased mGlu5 PAMs that do not potentiate coupling to NMDAR currents may provide therapeutic efficacy without inducing the adverse effects and CNS toxicity observed with non-biased mGlu5 PAMs.

The downstream signaling cascades that enable the efficacy of mGlu5 PAMs that do not directly modulate NMDAR currents, has yet to be fully elucidated. However, previous studies have described a signaling pathway involving mGlu5 coupling to $G_{q/11}$ and subsequent release of an endocannabinoid leading to decreased inhibitory tone onto glutamatergic neurons, thus altering excitatory synaptic plasticity [75, 76]. Determining if certain mGlu5 PAMs selectively bias transduction toward this mechanism versus NMDAR current modulation, and the extent to which differentiating PAMs based on these properties would provide added therapeutic value has yet to be tested.

Figure 1, Key Figure. Synaptic mGlu receptor locations within the prefrontal cortex and hippocampus.

mGlu receptors mediate disparate effects at synapses of different brain regions. (A) Prefrontal cortex glutamatergic synapse. Postsynaptic mGlu₃ and mGlu₅ receptor activation synergizes to enhance signaling cascades. Presynaptic mGlu₂ receptors act to reduce synaptic transmission. mGlu₁ receptors located on interneurons mediate feed forward inhibition of pyramidal cells. (B) Hippocampal SC-CA1 synapse. mGlu₃ and mGlu₅ receptors interact postsynaptically to enhance signaling cascades. mGlu₁ receptors activation results in SK channel inhibition, promoting excitability and NMDAR disinhibition. mGlu₇ receptors located on inhibitory interneurons reduce GABA transmission. Astrocytic mGlu₃ receptors lead to enhanced cAMP production and retrograde signaling onto presynaptic terminals to reduce excitatory transmission.

Figure 2. mGlu receptor allosteric modulation induced stimulus bias.

Schematic depicting the differential effects of mGlu receptor PAMs on downstream signaling cascades. (A) Activation of mGlu receptors by the endogenous ligand (glutamate) causes activation of downstream signaling cascades. (B) Non-biased PAM causes potentiation of all downstream signaling cascades equally. (C) Biased PAM causes selective potentiation of signaling cascades.

mGlu receptor subtypes, signaling, localization and potential indications

Table 1.

Metabotropic Glutamate Receptors (mGlu)			Potential therapeutic indications	Mode of action *	
	Subtype	Signaling		Positive allosteric modulators	Negative allosteric modulators
Group I	mGlu ₁	G _{q/11} coupled	Post-synaptic	VU0469650	CFMTI
	mGlu ₅		Post-synaptic/glia	VU0409551, CDPPB	CTEP, Basimglurant, Mavoglurant
Group II	mGlu ₂	G _{i/o} coupled	Pre- and post-synaptic	SAR218645	VU6001966
	mGlu ₃		Pre- and post-synaptic/glia	N/A	VU0650786
Group III	mGlu ₄		Pre-synaptic	VU0652957, (Valiglurax), PTX002331	N/A
	mGlu ₆		Post-synaptic (retina)	N/A	N/A
	mGlu ₇		Pre-synaptic	N/A	ADX71743
	mGlu ₈		re-synaptic	AZ12216052	N/A

Abbreviations used- PTSD: Post-traumatic Stress Disorder, ASD: Autism Spectrum Disorder, PD-LID: Parkinson’s Disease-L-dopa induced dyskinesia, PD: Parkinson’s disease

* Listed PAM/NAM compounds are derived from corresponding references per indication within the row