Allosteric Modulators of Metabotropic Glutamate Receptors as Novel Therapeutics for Neuropsychiatric Disease

Deborah J. Luessen and P. Jeffrey Conn

Abstract—Metabotropic glutamate (mGlu) receptors, a family of G-protein-coupled receptors, have been identified as novel therapeutic targets based on extensive research supporting their diverse contributions to cell signaling and physiology throughout the nervous system and important roles in regulating complex behaviors, such as cognition, reward,
I. Introduction

Glutamate is the primary excitatory neurotransmitter within the central nervous system (CNS). Glutamate modulates cell excitability and synaptic transmission through actions on glutamate receptors, including ionotropic glutamate and metabotropic glutamate (mGlu) receptors. Ionotropic glutamate receptors, which include amino-3-hydroxy-5-methyl-isoxazolopropionic acid, N-methyl-d-aspartate (NMDA), and kainate receptors, are ligand-gated ion channels that mediate fast excitatory synaptic transmission (Trayanos, 2010). mGlu receptors are members of the G-protein-coupled receptor superfamily and can be classified into three distinct groups based on their sequence homology, G-protein coupling, and ligand selectivity (Conn and Pin, 1997). The mGlu receptor subtypes are differentially expressed pre- and postsynaptically throughout the CNS and are located on both neurons and glial cells.

A. Metabotropic Glutamate Receptors: Structure and Signal Transduction

1. Structural Components of Metabotropic Glutamate Receptors.

mGlu receptors feature a large extracellular N-terminal domain, coined the Venus flytrap domain (VFD), which contains the orthosteric glutamate binding site and is critical for homo- and heterodimerization of these receptors (Yin and Niswender, 2014). Extensive evidence shows that VFDs form dimers, which can exist in three main states: open-open, open-closed, and closed-closed. Antagonist binding stabilizes the open-open (inactive) conformation, whereas ligand binding induces open-closed and closed-closed conformations. Distinct residues that are associated with closure of the VFD strongly contribute to functional switching of ligands from antagonists to agonists (Bessis et al., 2002; Jingami et al., 2003; Niswender and Conn, 2010), highlighting the important role of these domains and their respective orientations for receptor activation. Importantly, a number of conserved residues interact directly with glutamate as well as divalent cations, such as calcium or magnesium, which have the ability to activate the receptor (Kubo et al., 1998; Kunishima et al., 2000; Francesconi and Duvoisin, 2004). Ligand binding results in conformational changes, originating from the VFD via cysteine-rich domains (CRDs) to the heptahelical domain (HD)–C-terminal tail. Studies using mutagenesis and crystallization have shown that the CRD, which consists of nine cysteine residues, are critical to ligand-induced signal transduction, in part via a disulfide bridge formed between a cysteine in lobe 2 of the VFD and the ninth CRD cysteine (Rondard et al., 2006; Muto et al., 2007). Additionally, the second intracellular loop of mGlu receptors regulate selectivity of G protein coupling (Pin et al., 1994; Gomeza et al., 1996) and acts as an important regulatory site for kinases, like G-protein-coupled receptor kinase 2 (Dhami et al., 2005). Importantly, allosteric modulators of mGlu receptors that bidirectionally alter glutamate activity largely bind within the HD (Niswender and Conn, 2010). The C-terminus region of mGlu receptors is important for...
modulating G protein coupling and undergo alternative splicing, modulatory protein-protein interactions, and regulation by phosphorylation (Niswender and Conn, 2010; Enz, 2012). In addition to interacting with G-proteins, the C-terminal domains of mGlu receptor subtypes directly interact with many proteins, including enzymes, ion channels, receptors, scaffolds, and cytoskeletal proteins (Enz, 2012).

2. Metabotropic Glutamate Receptor Signal Transduction. As detailed in Table 1, group I mGlu receptor subtypes (mGlu1-5) are widely expressed in CNS neurons (MakSYMetz et al., 2017). mGlu1 and mGlu5 are primarily expressed postsynaptically but are also located at presynaptic terminals of GABA and glutamate neurons (Higley, 2014). Group I mGlu receptor subtypes can also signal through alternative pathways including the G protein Gq/11 alpha subunit (Gαq/11) and activate phospholipase C beta (β), which in turn results in the hydrolysis of phosphoinositides and generation of inositol 1,4,5-trisphosphate and diacyl-glycerol. This signaling pathway promotes calcium mobilization and downstream activation of protein kinase C. Additionally, group I mGlu receptor subtypes can also signal through alternative pathways including the G protein Gi/o alpha subunit (Gαi/o) and activate phospholipase D, protein kinase cascades like kinase 1, cyclin-dependent protein kinase 5, mitogen-activated protein kinase (MAPK/extracellular receptor kinase, Jun kinase, and the mammalian target of rapamycin/p70 S6 kinase (p70-S6) kinase pathways (Page et al., 2006; Li et al., 2007). These signaling cascades, such as mammalian target of rapamycin/p70 S6 kinase and MAPK/extracellular receptor kinase, are critical to synaptic plasticity mediated by group I mGlu receptors. Additionally, group I mGlu receptors represent promising therapeutic targets, based on their ability to directly couple to NMDA receptors via intracellular signaling pathways and scaffolding proteins, such as SRC Homology 3 Domain (SH3), Homer, and multiple ankyrin repeat domains protein, and guanylate kinase-associated protein–postsynaptic density-95 (Aniksztejn et al., 1991; Harvey and Collingridge, 1993; Yu et al., 1997) and their subsequent capacity to activate NMDA receptors in acute brain slices (Fitzjohn et al., 1996).

The group II mGlu receptors, mGlu2 and mGlu3, are expressed presynaptically (Niswender and Conn, 2010) on axonal preterminal regions where they can be activated by excessive synaptic or astrocytic glutamate release (Nicoletti et al., 2011; Maksymetz et al., 2017). mGlu3 is located postsynaptically as well as on astrocytes where it promotes neuroprotective effects (Nicoletti et al., 2011) and facilitates astrocytic-neuronal communication (Winder and Conn, 1996; Winder et al., 1996). Group II mGlu receptor

| Group  | Receptor | CNS Expression          | Synaptic Localization | G protein Coupling         | Primary Signaling Pathways | Interacting Partners                                      |
|--------|----------|-------------------------|-----------------------|---------------------------|----------------------------|-----------------------------------------------------------|
| I      | mGlu1    | Widespread in neurons    | Presynaptic and       | Primarily Gαq/11            | PLCβ - IP3 + DAG hydrolysis| Activates NMDA receptors                                  |
|        |          |                         | postynaptic           | noncanonical Gαq/11         | phospholipase D             | receptors activates Ca2⁺ channels (e.g.,                               |
| mGlu4  |          | Widespread in neurons    | Predominantly         | Primarily Gαq/0             | Inhibition of adenylyl cyclase | Ca₂⁺ channels (e.g., N-type)                                    |
| II     | mGlu2    | Widespread in neurons    | Postsynaptic and      |                         | MAPK/ERK                   | Activation of K⁺ channels                                    |
|        |          |                         | postynaptic           | Primarily Gαq/0             | mTOR/p70 S6 kinase          | inhibition of voltage-gated Ca²⁺ channels                    |
|        | mGlu3    | Widespread in neurons,   | Presynaptic           |                         | IP3 kinase                 |                                                                           |
|        |          | astrocytes               | high in cerebellum    | Primarily Gαq/0             | Inhibition of adenylyl cyclase |                                                                           |
| Group III | mGlu4    | Widespread in neurons,   | Presynaptic           |                         | MAPK/ERK                   | Activation of K⁺ channels                                    |
|         |          | high in retinal cells    |                        | Primarily Gαq/0             | mTOR/p70 S6 kinase          | inhibition of Ca²⁺ channels (P/Q subtype)                      |
| mGlu5  | Retina, select | Widespread in neurons,    |                        | Primarily Gαq/0             | IP3 kinase                 |                                                                           |
|        | neuron populations,   | high in cerebellum    |                        | Primarily Gαq/1             | stimulation of cGMP         |                                                                           |
| mGlu7  | Microglia  | Widespread in neurons    | Active zone of         |                         | phosphodiesterase           |                                                                           |
|        |          |                         | presynaptic terminals | Primarily Gαq/0             | (mGlu6)                     |                                                                           |
| mGlu8  | Lower and more restricted expression than mGlu5/7 | Predominantly         |                        | Primarily Gαq/0             |                                                                           |
subtypes predominantly couple to G_{ia/o} proteins, which classically inhibit adenyl cyclases and downstream production of 3',5'-cAMP. Group II mGlu receptors also directly regulate ion channels, including potassium (K^+) and calcium (Ca^{2+}) channels, and other downstream signaling components via G protein beta/gamma (G_{b/c}) subunits (Niswender and Conn, 2010). Additionally, group II mGlu receptors can engage in a multitude of signal transduction pathways, including activation of phosphatidyl inositol 3-kinase and MAPK pathways (Muguruza et al., 2016), demonstrating the great complexity by which these receptors regulate neuronal signaling and synaptic plasticity.

The group III mGlu receptors consist of mGlu4 (Tanabe et al., 1992), mGlu6 (Nakajima et al., 1993), mGlu7 (Saugstad et al., 1994), and mGlu8 (Niswender and Conn, 2010). mGlu4 receptors are largely expressed in the retina, however, these receptors have also been shown to be expressed in the CNS, including cortical areas, superior colliculus, the accessory olfactory bulb, and axons of the corpus calleous (Vardi et al., 2011; Palazzo et al., 2020). Alternatively, other group III mGlu receptors are largely expressed within the CNS (Nakajima et al., 1993). Group III mGlu receptors canonically signal via the G_{z/o} subunits of the heterotrimeric G protein complex, resulting in inhibition of adenyl cyclase and cAMP production (Niswender and Conn, 2010). Activation of group III mGlu receptors can also regulate a variety of ion channels and inhibition of vesicular fusion via G_{b/c} subunits (Cartmell and Schoepp, 2000). In addition to canonical G_{z/o}-mediated downstream signaling, group III mGlu receptors engage in a multitude of other signal transduction cascades. For instance, mGlu4 has been shown to selectively inhibit P/Q-type (P/Q)-type Ca^{2+} channels through a phospholipase C-dependent mechanism resulting in subsequent Ca^{2+} release from intracellular stores and diacyl-glycerol-mediated activation of protein kinase C (Perroy et al., 2000). Furthermore, activation of mGlu4 results in MAPK pathway signaling via G_{b/c} subunits (Iacovelli et al., 2004). Evidence suggests that the mGlu4 receptor signals through G_{z/o} to a cyclic guanosine monophosphate-prefering phosphodiesterase (Shiels and Falk, 1990; Thoreson and Miller, 1994; Nawy, 1999).

**B. Metabotropic Glutamate Receptor Regulation of Neurotransmission and Synaptic Plasticity**

mGlu receptors are distributed widely throughout the CNS in a vast array of major brain regions and are localized at discrete synaptic and extrasynaptic sites in both neurons and glia. Based on their robust CNS expression and diverse signal transduction pathways, activation of mGlu receptors elicits a multitude of outcomes on synaptic transmission and contributes to many forms of synaptic plasticity (Crupi et al., 2019). These activity-dependent modifications of synaptic transmission are critical to learning and memory, for example and thus, represent important mechanisms underlying many neuropsychiatric disorders, such as schizophrenia and substance use disorders (SUDs). Here, we will briefly summarize select functional roles of mGlu receptor subtypes in neurotransmission and synaptic plasticity. In-depth discussion of the vast array of physiological roles of mGlu receptors has been presented in numerous previous reviews (Benarroch, 2008; Niswender and Conn, 2010; Mukherjee and Manahan-Vaughan, 2013; Maksymetz et al., 2017). mGlu receptors expressed presynaptically have the ability to increase or decrease neurotransmitter release at excitatory (glutamate), inhibitory (GABA), and neuromodulatory (i.e., monoamines, acetylcholine, peptides) synapses (Niswender and Conn, 2010). In most cases, mGlu receptor-mediated regulation of neurotransmitter release is mediated by mGlu receptors that are localized presynaptically; however, this can also occur via postsynaptic mGlu receptors and release of retrograde messengers, such as endocannabinoids (Yohn et al., 2020). mGlu receptor-mediated neuromodulation, in turn, has a wide array of downstream effects on neuronal activity and firing. For example, mGlu receptor-mediated inhibition of GABA transmission in the cerebellum results in a local reduction in inhibition, enhancing the efficacy of the more active fibers and accentuating the contrast between inputs with differential firing rates (Mitchell and Silver, 2000).

**1. Group I: Metabotropic Glutamate1/5**

Group I mGlu receptors, which include mGlu1 and mGlu5, are heterogeneously expressed throughout the brain, with high levels in regions critical to cognition, reinforcement learning, and motivation, such as the nucleus accumbens (NAC), hippocampus, medial prefrontal cortex (mPFC), and thalamus (Cleva and Olive, 2012). Further studies have revealed that the vast majority of mGlu1 and mGlu5 receptors are located postsynaptically on dendritic spines (Olive, 2009) and on axon terminals in brain regions, such as the hippocampus (Romano et al., 1995) and cerebral cortex (Muly et al., 2003; Paquet and Smith, 2003). Additionally, group I mGlu receptors in globus pallidus of nonhuman primates are found in the main body of symmetric synaptic junctions established by striatal GABA terminals as well as perisynaptic to asymmetric glutamatergic synapses (Hanson and Smith, 1999). Their activation leads to cell depolarization and increases in neuronal excitability (Niswender and Conn, 2010). Group I mGlu receptor-mediated modulation of neuronal excitability is driven by regulation of numerous ion channels, which enable fine-tuning of neuronal excitability (Conn and Pin, 1997; Anwyl, 1999; Coutinho and Knöpfel, 2002; Valenti et al., 2002).

Activation of group I mGlu receptor subtypes leads to alterations in excitability and spontaneous synaptic
transmission in the mPFC and cornu ammonis (CA1) region of the hippocampus, among other brain regions (Zho et al., 2002; Yin and Niswender, 2014; Turner et al., 2018; Maksymetz et al., 2021). For example, activation of group I mGlur receptors by selective orthosteric agonists, such as (S)-3,5-dihydroxyphenylglycine (DHPG), results in direct excitatory effects on CA1 pyramidal cells, including increased cell firing and depolarization (Charpak et al., 1990; Desai and Conn, 1991; Pedarzani and Storm, 1993; Davies et al., 1995; Gereau and Conn, 1995; Mannaioni et al., 1999). These downstream effects are mediated by inhibition of K+ currents and activation of both Ca2+-dependent and independent conductance (Crépel et al., 1994; Guérineau et al., 1995). Additionally, recent studies have directly demonstrated for the first time that activation of mGlur1 increases inhibitory transmission in the mPFC by excitation of somatostatin-expressing interneurons (SST-INs) (Maksymetz et al., 2021). Extensive evidence also supports the role of mGlur1 and mGlur5 receptor subtypes in numerous forms of long-term synaptic plasticity, including long-term depression (LTD) and long-term potentiation (LTP) of transmission. At excitatory synapses in hippocampal CA1, activation of mGlur1 and subsequent Ca2+ mobilization has been shown to induce LTP, a modality of synaptic plasticity thought to underlie learning and memory, at numerous glutamatergic synapses (Bashir et al., 1993; Frenguelli et al., 1993; Petrozzino and Connor, 1994; Balschun et al., 1999; Chevaleyre and Castillo, 2003; Gladding et al., 2009). However, there are mixed reports regarding the role of mGlur5 in N-methyl-D-aspartate receptor (NMDAR)-dependent LTP (Fitzjohn et al., 1996, 1998; Lu et al., 1997; Francesconi and Duvoisin, 2004; Bortolotto et al., 2005; Neyman and Manahan-Vaughan, 2008). Several studies have also revealed that mGlur1 and mGlur5 subtypes regulate LTP in hippocampal SST-INs (McBain et al., 1994; Perez et al., 2001; Le Duigou and Kullmann, 2011; Pelkey et al., 2017). There is evidence for the role of group I mGlur receptors in regulation of LTP extrahippocampal brain regions. For instance, it has been reported that application of DHPG facilitated LTP of the evoked excitatory postsynaptic currents (EPSCs) in SST-INs of the prefrontal cortex (PFC) (Crowley and Joffe, 2021). Furthermore, a wealth of studies has shown that activation of group I mGlur receptors induces a LTD of synaptic transmission in rat hippocampal CA1 (Palmer et al., 1997; Chevaleyre and Castillo, 2003; Tan et al., 2003; Gladding et al., 2009) and dentate gyrus (O’Mara et al., 1995; Camodeca et al., 1999) among other brain regions (Kano and Kato, 1987; Kato, 1993; Conquet et al., 1994; Wang et al., 2015).

The development of mouse models with selective deletion of group I mGlur receptors have corroborated these findings. For instance, electrophysiological recordings from mGlur1−/− mice show that these animals display impaired hippocampal LTP, which correlates with impairments in context-specific learning and impaired LTD in the cerebellum (Aiba et al., 1994a,b; Gil-Sanz et al., 2008). A wealth of studies using constitutive knockout models further confirmed the role of mGlur5 in hippocampal LTP (Bashir et al., 1993; Lu et al., 1997). Using genetic deletion or pharmacological inhibition, it has been demonstrated that mGlur5 reduces LTP at Shaffer collateral (SC)-CA1 synapses of the hippocampus in freely moving rats and ex vivo slice preparations (Lu et al., 1997; Francesconi et al., 2004; Shalin et al., 2006). In addition, LTP induction can be primed by DHPG (Cohen et al., 1998; Raymond et al., 2000), and multiple mGlur5 positive allosteric modulators (PAMs) can induce LTP at SC-CA1 synapses (Ayala et al., 2009; Noetzel et al., 2013; Rook et al., 2015). More recently, studies using conditional knockout of Glutamate Metabotropic Receptor 5 gene (GRM5) in hippocampal CA1 pyramidal cells showed that loss of mGlur5 in this cellular population impaired LTD of inhibitory synapses compared with wild-type control mice (Xu et al., 2014), suggesting a specific role of mGlur5 in hippocampal CA1 pyramidal cells in metaplasticity by regulating inhibition. These findings are of particular importance as hippocampal LTP is known to be altered in models that recapitulate the physiologic and behavioral phenotypes associated with neuropsychiatric diseases, including schizophrenia. For example, subchronic phencyclidine (PCP) treatment in mice increases the threshold for LTP of CA1 excitatory synapses, and this effect is directly related to enhanced inhibitory input to CA1 pyramidal cells through increased activity of GABAergic neurons (Nomura et al., 2016). Therefore, the contributions of group I mGlur receptors in regulating LTP at excitatory synapses is critical for our understanding of the pathophysiology of neuropsychiatric diseases and development of novel treatments.

2. Group II: Metabotropic Glutamate2/3. The group II mGlur receptor subtypes, mGlur2 and mGlur3, are expressed throughout the CNS, notably in brain regions central to motivation, learning, and memory (Moussawi and Kalivas, 2010; Muguruza et al., 2016). In some instances, they modulate synaptic transmission and alter neuroplasticity by acting at preterminal regions away from the active zone of NMDAR or GABAergic synapses (Nicoletti et al., 2011). mGlur2 receptors located on the presynaptic membrane can be activated by substantial synaptic or astrocytic glutamate release (Muguruza et al., 2016; Maksymetz et al., 2017). Additionally, further evidence supports the existence of mGlur2 and mGlur3 expressed postsynaptically (Muguruza et al., 2016).

At many synapses, mGlur2/3 receptor activation decreases spontaneous excitatory transmission (Marek et al., 2000;
Kiritsoti and Neugebauer, 2015; Bocchio et al., 2019). Studies have shown that group II mGlu receptor agonist LY 354740 decreases frequency, but not amplitude, of miniature EPSCs in the presence of tetrodotoxin, which suggests that the site of action is primarily presynaptic on the glutamatergic terminals (Han et al., 2006; Kiritsoti and Neugebauer, 2015). In the infralimbic mPFC, electrophysiology experiments in rat brain slices showed that group II mGlu receptor subtypes decrease the output of layer V pyramidal cells as the result of an inhibitory action on glutamatergic synapses (Kiritsoti and Neugebauer, 2015; Thompson and Neugebauer, 2017). More specifically, LY379268, a selective group II mGlu receptor agonist, regulates activity of pyramidal cells by modulating glutamate-driven feedforward inhibitory transmission (inhibitory postsynaptic currents) in addition to direct EPSCs. In addition to regulation of neurotransmitter release and short-term plasticity, group II mGlu receptors play important roles in long-term synaptic plasticity, which underlies many cognitive and behavioral processes disrupted in neuropsychiatric disease. Activation of either mGlu2 or mGlu3 receptor subtypes induces a robust, postsynaptic LTD of evoked synaptic responses in the PFC. Interestingly, mGlu2 and mGlu3 receptors induce LTD by divergent presynaptic and postsynaptic mechanisms, respectively (Walker et al., 2015; Joffé et al., 2019a,b). At mossy fiber pyramidal cell synapses, prolonged low-frequency stimulation results in a presynaptic form of LTD that is absent in mGlu2-deficient mice and blocked by a nonselective group II antagonist. At this synapse, the activation of mGlu2 induces LTD selectively when it is coupled to a synaptically-driven increase in presynaptic Ca2+. Additionally, mossy fiber LTD is reversible by low-frequency stimulation via the activation of group II mGlu receptors (Chen et al., 2001). Finally, exciting recent reports have demonstrated that activation of mGlu3 induces metaplastic changes, biasing stimulation ofafferents to induce LTP through an mGlu3 receptor-dependent, endocannabinoid-mediated mechanism of action (Dogra et al., 2021). In this same study, targeted genetic deletion of mGlu3 from hippocampal pyramidal cells prevented the LTP-inducing effects of mGlu3 activation, revealing a novel avenue by which mGlu3 regulates long-term hippocampal synaptic plasticity.

3. Group III: Metabotropic Glutamate1/6/7/8

Group III mGlu receptors are differentially expressed in the CNS and peripheral nervous system. mGlu4 and mGlu8 subtypes are expressed in the brain but in a restricted manner (Pilc et al., 2008; Julio-Piiper et al., 2011). Although mGlu4 is primarily found in the cerebellum (Kinoshita et al., 1996b; Shigemoto et al., 1997), expression has also been reported in the cerebral cortex, striatum, olfactory bulb, pontine nuclei, lateral septum, hippocampus, thalamic nuclei, and dorsal horn (Fotuhi et al., 1994; Azkue et al., 2001). Within the CNS, mGlu6 is found presynaptically in the hippocampus, cerebellum, olfactory bulb, and cortical areas (Ferraguti and Shigemoto, 2006). Although mGlu7 is expressed widely throughout the brain, mGlu8 only exhibits limited expression in the retina (Crupi et al., 2019). mGlu7 expression has been reported in the amygdala, hypothalamus, hippocampus, thalamus, and locus coeruleus (Ngomba et al., 2011). Similar to group II mGlu receptors, group III mGlu receptor subtypes are most commonly located in or near presynaptic active zones of GABAergic and glutamatergic neuronal cells (Shigemoto et al., 1997; Ferraguti and Shigemoto, 2006). Activation of group III mGlu receptors inhibits the release of neurotransmitters, such as glutamate, GABA, and dopamine, via modulation of a variety of ion channels and Gβγ subunit-dependent inhibition of vesicular fusion (Cartmell and Schoepp, 2000).

Until recent advances in group III mGlu receptor subtype-selective pharmacological compounds, most of the research pertaining to group III mGlu receptors used the broad-spectrum agonist, L-2-amino-4-phosphonobutyric acid (L-AP4). A multitude of studies have shown that L-AP4 reduces excitatory transmission in numerous brain regions, including the hippocampus, amygdala, striatum, globus pallidus, thalamus, hypothalamus, cerebellum, NAc, and substantia nigra (Mercier and Lodge, 2014). Application of L-AP4 typically increases paired-pulse ratio (Harris and Cotman, 1983; Manzoni et al., 1997; Lorez et al., 2003), and its application has also been shown to reduce the frequency, but not the amplitude, of miniature excitatory postsynaptic events (Harris and Cotman, 1983; Gereau and Conn, 1995; Manzoni et al., 1997; Schoppa and Westbrook, 1997; Schrader and Tasker, 1997), both of which indicate the role of group III mGlu receptors as presynaptic autoreceptors in the CNS. Group III mGlu receptors are also involved in the regulation of GABA and monoamine neurotransmission (Cartmell and Schoepp, 2000; Schoepp, 2001). In addition to its ability to regulate glutamate release, L-AP4 has been found to reduce inhibitory transmission in many brain regions, including the midbrain, globus pallidus, striatum, thalamus, and hippocampus (Mercier and Lodge, 2014). Importantly, hippocampal electrophysiological studies bolstered evidence for the localization of group III mGlu receptors on both glutamate and GABA terminals, such that L-AP4 has reduced both excitatory and inhibitory transmission onto hippocampal interneurons and pyramidal cells (Semyanov and Kullmann, 2000; Kogo et al., 2004; Klar et al., 2015). The critical role of group III mGlu receptors on interneurons highlights their utility in regulating the balance of excitation and inhibition of these cells, thus regulating overall network excitability (Ferraguti and Shigemoto, 2006; Klar et al., 2015).
A multitude of studies have leveraged selective pharmacological tools and transgenic knockout lines to determine the roles of group III mGlu receptors in long-term synaptic plasticity. For instance, application of α-Cyclopropyl-4-phosphonophenylglycine (CPPG), the group III mGlu receptor antagonist, prevents LTD, but not LTP, in the CA1 region of mice (Altinbilek and Manahan-Vaughan, 2007). However, the development of selective pharmacological and genetic tools has provided additional insight to the role of Group III mGlu receptor subtypes in long-term synaptic plasticity. A recent study shows that inhibition of group III mGlu receptors elicits an NMDA receptor-dependent LTD in SC-CA2 synapses (Dasgupta et al., 2020). Further, disinhibition produced by activation of mGlu7 induces LTP at SC-CA1 synapses in the hippocampus (Klar et al., 2015). Interestingly, one study using mGlu4 knockout mice found enhanced LTP in hippocampal CA1 region but not in the PFC compared with wild-type controls (Iscru et al., 2013). Other studies have shown that mGlu receptor-dependent LTD can be induced after activation of mGlu7 (Bellone et al., 2008). Together, these demonstrate critical roles of group III mGlu receptor subtypes in regulating various forms of long-term synaptic plasticity in brain regions and circuits dysregulated in neuropsychiatric disease.

II. Targeting Metabotropic Glutamate Receptors for the Treatment of Neuropsychiatric Disease

A. Metabotropic Glutamate Receptor Allosteric Modulators

The vast diversity and distribution of mGlu receptors provides an unparalleled opportunity for selective targeting of individual mGlu receptor subtypes as novel treatment strategies for neuropsychiatric and neurologic disorders. However, longstanding efforts to develop ligands that target mGlu receptors have largely focused on agonists and antagonists that interact with the orthosteric glutamate binding sites of these receptors to mimic or block the endogenous actions of glutamate. Although this strategy has proven to be somewhat fruitful, the high conservation of orthosteric binding sites across receptor subtypes has served as a critical barrier to the development of subtype-selective orthosteric ligands. To address this issue, recent efforts have been focused on developing allosteric modulators for mGlu receptor subtypes. Allosteric modulators act by altering the receptor conformational state by binding a topographically distinct nonorthosteric site, typically found within the HD of mGlu receptors (Wu et al., 2014). Thus, allosteric modulators potentiate or attenuate the response to the endogenous orthosteric ligand (i.e., glutamate for mGlu receptors) without activating the receptor directly. Allosteric modulators can be categorized based on the direction that they modulate the response to the orthosteric agonist. For instance, allosteric modulators that increase the functional response to an orthosteric agonist are referred to as “positive allosteric modulators.” In contrast, those that attenuate the functional response to the orthosteric agonist are coined “negative allosteric modulators” (NAMs). If a compound binds to an orthosteric site without inducing effects on the response of the receptor, it is called a “neutral allosteric ligand” (Conn et al., 2009). In functional assays, such as those that measure calcium mobilization or downstream signaling (e.g., cAMP accumulation), the presence of a PAM often induces a leftward shift of the agonist concentration-response curve, whereas a NAM decreases the maximal effect of the response. In addition to this modulatory activity, a subset of allosteric modulators possesses intrinsic activity and can both potentiate agonist responses and directly activate the receptor (ago-PAMs). In sum, allosteric modulators display a number of the following pharmacological properties: 1) efficacy modulation, the signaling capacity (or “intrinsic efficacy”) of an orthosteric agent can be modified via alterations in intracellular responses; 2) affinity modulation, the conformational change induced by binding of the allosteric ligand alters the binding pocket and association/dissociation rates of orthosteric ligands; and 3) agonism/inverse agonism, receptor signaling is altered either positively (agonism) or negatively (inverse agonism) by the allosteric modulator, irrespective of the presence or absence of an orthosteric ligand.

B. Advantages of Metabotropic Glutamate Allosteric Modulators

Allosteric modulators provide several advantages as potential pharmacotherapies and experimental tools in comparison with orthosteric ligands. For example, orthosteric ligand binding sites often possess a high degree of sequence homology, which presents a challenge for the development of receptor subtype-specific ligands. In contrast, allosteric ligand binding sites are often less highly conserved than orthosteric sites, allowing for the development of highly selective allosteric modulators for receptor subtypes that, formerly, have been intractable using traditional approaches (Conn et al., 2009; Nussinov et al., 2011). Additionally, differential receptor cooperativity serves as a means of subtype selectivity. The cooperativity between orthosteric and allosteric sites is not correlated with the affinity of allosteric modulators for their binding sites, and, thus, allows some allosteric modulators to bind to more than one receptor subtype with similar affinities but elicit effects through distinct cooperativity between receptor subtypes (Boehr et al., 2009; Tsai et al., 2009).

One shortcoming of orthosteric agonists targeting mGlu receptors is the risk of receptor overactivation,
which can disrupt brain circuit modulation and result in adverse side effects (Sendt et al., 2012; Rook et al., 2013). This challenge is more effectively circumvented by allosteric modulators with their ability to fine-tune active synapses versus nonphysiological activation of synapses that display low glutamatergic tone. This allows for regulation of receptor responses in brain areas where the endogenous agonist exerts its physiologic effect, reducing the risk for off target or over-activation. For instance, mGlur agonists can induce epileptic seizure activity (Tizzano et al., 1995) This effect is also observed with allosteric agonists but is mitigated by administration of mGlur PAMs that lack agonist activity (Rook et al., 2015; Gould et al., 2016). This requirement for the presence of the endogenous agonist provides spatial and temporal control of allosteric modulators and provide a sizable therapeutic edge by allowing for physiologically appropriate modulation of synaptic signaling and transmission.

In addition to serving as novel treatment strategies, these ligands are essential to driving fundamental investigation into the roles of specific signaling pathways and distinct receptors in modulating identified neural circuits and behavior under physiologic and pathologic conditions. Allosteric modulators of mGlur receptor subtypes are now being pursued as potential drug candidates for numerous neuropsychiatric diseases, including Alzheimer’s disease, Parkinson’s disease, dystonia, schizophrenia, SUDs, and other brain diseases (Conn et al., 2009; O’Brien and Conn, 2016; Foster and Conn, 2017; Maksymetz et al., 2017; Joffé and Conn, 2019; Stansley and Conn, 2019). The present review will summarize the current findings on the efficacy of mGlur receptor subtype PAMs/NAMs for the treatment of neuropsychiatric diseases, with particular focus on schizophrenia and SUDs.

III. Potential of Allosteric Modulators of Metabotropic Glutamate Receptors for Treating Neuropsychiatric Disease

A. Schizophrenia

Schizophrenia is a chronic neuropsychiatric disorder that affects approximately 1% of the world population (GBD 2019 Diseases and Injuries Collaborators, 2020). The disease is characterized by three primary clusters of symptoms: positive (auditory/visual hallucinations), negative (amotivation, anhedonia, social withdrawal), and cognitive deficits (working memory, executive function, attention). Current antipsychotic medications effectively treat the positive symptoms of the disease, such as auditory and visual hallucinations, disorganized thoughts, and delusions; however, they do not improve the negative or cognitive symptoms. Negative symptoms (e.g., flattened affect, social withdrawal) and cognitive symptoms (e.g., deficits in attention, working memory, and cognitive flexibility) are believed to be the best predictors of long-term treatment outcome (Green, 1996; Bobes et al., 2007; McEvoy, 2007). Furthermore, many patients discontinue treatment due to adverse effects, such as extrapyramidal side effects (EPS) (i.e., tardive dyskinesia, tremor, dystonia, and bradykinesia) induced by typical antipsychotics as well as a host of metabolic side effects (i.e., weight gain, hyperlipidosis, and type II diabetes) elicited most commonly by atypical antipsychotics (Lieberman et al., 2005; Meltzer, 2013; Lally and MacCabe, 2015). Therefore, development of improved therapeutic options that mitigate a broader range of symptoms of schizophrenia and are devoid of EPS is of great need.

Our current understanding of the neurochemical alterations driving the symptoms associated with schizophrenia is largely attributed to two major lines of research that are driven by the dopamine and glutamate hypotheses of schizophrenia-related dysfunction (Howes et al., 2015; McCutcheon et al., 2019). The dopamine hypothesis posits that the positive symptoms of the disease are largely driven by aberrant dopamine signaling. This notion is supported by evidence that amphetamine and other dopamine-releasing agents induce symptoms that resemble those of the positive symptoms of schizophrenia (Steeds et al., 2015; Kesby et al., 2018). Further, currently available antipsychotic medications largely target the dopamine system and act, in part, by inhibiting dopamine D2 subtype of dopamine receptors (Meltzer, 2013). In support of this model, in vivo neuroimaging studies show increased subcortical dopamine release after amphetamine challenge in individuals with schizophrenia (Laruelle et al., 1996; Breier et al., 1997; Abi-Dargham et al., 1998). However, since amphetamine exposure exacerbates only the positive symptoms of schizophrenia and dopamine-targeting antipsychotic medications only alleviate positive symptoms, dopaminergic hyperactivity alone cannot account for the negative symptoms or cognitive disturbances observed in patients with schizophrenia (Carlsson, 1988).

Another prominent line of research suggests that disruption of glutamate signaling underlies numerous symptoms of schizophrenia. This notion is derived from extensive evidence that NMDA antagonists, such as PCP, ketamine and dizocilpine (MK-801), induce symptoms closely resembling those of schizophrenia (Javitt and Zukin, 1991). In addition, administration of NMDAR antagonists exacerbates or induces controlled symptoms when administered to schizophrenia patients (Krystal et al., 1994). These findings, in addition to a wealth of preclinical evidence, support the hypothesis that NMDAR hypofunction contributes to the pathophysiology underlying schizophrenia. Thus, pharmacological agents that enhance NMDAR function represent a potential
strategy that could provide therapeutic benefits to patients with schizophrenia. However, a primary obstacle is that overactivation of NMDARs using traditional orthosteric agonists induces adverse effects, such as excitotoxicity and seizures (Zeron et al., 2002; Kaufman et al., 2012; Monaghan et al., 2012; Puddifoot et al., 2012). Importantly, mGlu receptors are critical modulators of NMDAR function and regulate glutamate and GABA neurotransmission throughout the CNS (Niswender and Conn, 2010; Maksymetz et al., 2017). Therefore, pharmacological modulation of mGlu receptors holds the potential to alter NMDAR function and restore excitatory and inhibitory neurotransmission to provide therapeutic benefit in patients with schizophrenia. To this end, allosteric modulators targeting all three groups of mGlu receptors have been pursued as putative targets for novel antipsychotics (Tables 2–4).

I. Group I: Metabotropic Glutamate1/5. The mGlu1 receptor subtype shows promise as a potential therapeutic target for treating schizophrenia. Genetic studies in humans reveal an association of the human gene encoding mGlu1 (GRM1) and, specifically, loss of function single nucleotide polymorphisms in GRM1 with schizophrenia, raising the possibility that mGlu1 signaling is critical to the function of brain circuits underlying symptoms associated with this disorder (Ayalew et al., 2012; Ayoub et al., 2012; Cho et al., 2014). Numerous studies have characterized the pivotal role of mGlu1 in regulating GABA and glutamate signaling in the PFC as well as striatal dopamine dynamics. The development of mGlu1 PAMs and transgenic mouse lines have allowed for these discoveries. Potent first-generation mGlu1 PAMs were developed in the early 2000s; however, they displayed poor pharmacokinetic and metabolic profiles, limiting their use in preclinical studies (Knoflach et al., 2001; Vieira et al., 2005). More recent efforts yielded mGlu1 PAMs, such as VU 6000799, VU6000790, and VU6004909 as potent, highly selective mGlu1 PAMs with improved drug metabolism and pharmacokinetic (DMPK) properties and brain penetrance and are therefore better suited for in vivo studies (Garcia-Barrantes et al., 2015, 2016a,b,c). Recent studies have leveraged these improved mGlu1 PAMs and have yielded promising results. For instance, Yohn et al., showed that activation of mGlu1 negatively regulates striatal dopamine release through an intricate mechanism involving coactivation of

### Table 2

| Receptor Type | Compound | Positive Symptom Models | Negative Symptoms Models | Cognitive Models | References |
|---------------|----------|-------------------------|--------------------------|------------------|------------|
| mGlu1 PAM     | VU0483605 | No effect on AHL        | Attenuates AMPH-          |                 | Cho et al., 2014 |
|               | VU6004909 | induced hyperlocomotion and disruptions in PPI | No effect on PR |                 | Yohn et al., 2020 |
| NAM           | FTIDC    | Attenuates MHL           | PPI deficits             |                 | Satow et al., 2009 |
| CFMTI         |          | Reduced MHL and NMDAR antagonist-induced hyperlocomotion (NMDAR-HL) | Ameliorated METH and ketamine-disrupted PPI | Reversal of MK-801-disrupted social interaction | Satow et al., 2008 |
| mGlu5 PAM     | CDPPB    | Attenuated AMPH-induced hyperlocomotion and deficits in PPI <b>a</b> | Attenuated MK-801-induced decrease in sucrose preference | | (See footnotes.) |
| 5PAM523       |          | Reduced AHL<g>,h and NMDAR-HL<h> | | | (See footnotes.) |
| VU0409551     |          | Reverses MK-801-induced hyperlocomotion | | | Rook et al., 2015 |

AHL, amphetamine-induced hyperlocomotion; AMPH, amphetamine; CF, fear conditioning; DNMTP, delayed nonmatching to position; HL, hyperlocomotion; METH, methamphetamine; MHL, METH-induced hyperlocomotion; NOR, novel object recognition; SR, serine racemase-deficient.

<a>Kinney et al., 2003.</a>
<a>Lindsay et al., 2005.</a>
<a>Vardigan et al., 2010.</a>
<a>Ayala et al., 2009.</a>
<a>Stefani and Moghaddam, 2010.</a>
<a>Horio et al., 2013.</a>
<a>Parmentier-Batteur et al., 2014.</a>
<a>Rook et al., 2015.</a>
muscarinic acetylcholine subtype 4 (M4) receptors and retrograde endocannabinoid signaling (Yohn et al., 2020). Additionally, a recent study has characterized the critical role of mGlu1 receptors located on SST-INs of the prelimbic PFC in regulating inhibitory output onto glutamatergic pyramidal cells, highlighting mGlu1 as a critical determinant of inhibitory/excitatory balance in the PFC (Maksymetz et al., 2021). The ability to normalize both dopamine and GABA dysfunction highlights mGlu1 as a promising target to comprehensively treat symptomology associated with schizophrenia. Importantly, mGlu1 PAMs show robust antipsychotic-like efficacy in rodent models. Specifically, the mGlu1 PAM VU6004909 reverses deficits in spatial working memory induced by NMDAR antagonist, MK-801 (Maksymetz et al., 2021). In addition to the potential of mGlu1 PAMs in treating schizophrenia, mGlu1 NAMs 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2H)-carboxamide (FTIDC) and 2-cyclopropyl-5-[1-(2-fluoro-3-pyridinyl)-5-methyl-1H-1,2,3-triazol-4-yl]-2,3-dihydro-1H-isoindol-1-one (CFMTI) have displayed efficacy in animal models of antipsychotic activity (Table 2), such as decreasing NMDAR antagonist and psychostimulant-induced hyperlocomotion and deficits in PPI as well as reversing social interaction deficits elicited by MK-801, an NMDAR antagonist, in rats (Satow et al., 2008, 2009). The contrasting findings of mGlu1 PAMs and NAMs illustrate the potential complexity of mGlu1-targeting ligands and suggest that mGlu1 PAMs may primarily be effective in patients carrying GRM1 mutations. Considering these exciting findings, continued interrogation is needed to further develop mGlu1-targeting therapeutics.

### Table 3

| Receptor | Type | Compound | Positive Symptom Models | Negative Symptoms Models | Cognitive Models | References |
|----------|------|----------|-------------------------|--------------------------|------------------|------------|
| mGlu2    | PAM  | LY487379 | Reduced NMDAR-HL and AHL but not PCP-disrupted PPI | Reduced PCP-induced deficits in social interaction | Promoted cognitive flexibility in ASST | (See footnotes.) |
| BINA     |      |          |                         | Reduced MK-801-induced increased immobility in the FST |                  | (See footnotes.) |
| TASP0443294 |      | Reduced MHL and NMDAR-HL | | | (See footnotes.) |
| JNJ-4011813/ADX71149 | | Reduced NMDAR-HL; no effect on AHL; inhibited DOM-induced head twitches | | | | Lavreysen et al., 2015 |
| SAR 218645 | | No effect on NMDAR-HL or AHL; no effect on hyperactivity in DAT−/− and NR1neo−/− mice; reduced DOI-induced head twitches | | Reversed MK-801-induced deficits in NOR; attenuated working memory deficits in Y-maze test in NR1neo−/− mice | Griebel et al., 2016 |
| mGlu3    | NAM  | VU0477950 |          | Dose-dependent impairment in extinction learning | Blocked the ability of mGlu2/3 agonists to restore trace fear conditioning after PCP administration | Walker et al., 2015 |
| VU0650786 | |          |          | | | Dogra et al., 2021 |

AHL, amphetamine-induced hyperlocomotion; AMPH, amphetamine; ASST, attentional set-shift task; DAT, dopamine transporter; DOB, dimethoxy-bromoamphetamine; HL, hyperlocomotion; METH, methamphetamine; MHL, METH-induced hyperlocomotion; NOR, novel object recognition; NR1neo, NR1 subunit reduced expression.

*Galici et al., 2005.*
*Harich et al., 2007.*
*Nikiforuk et al., 2010.*
*Galici et al., 2006.*
*Bennyworth et al., 2007.*
*Kawaura et al., 2016.*
*Benneyworth et al., 2007.*
*Hikichi et al., 2015.*
*Lavreysen et al., 2015.*
required to determine the efficacy of mGlu1 PAMs and NAMs in nonhuman primate and clinical studies.

mGlu5 also represents an exciting target for the treatment of schizophrenia and improving cognitive function in multiple brain disorders (Foster and Conn, 2017; Nicoletti et al., 2019). Extensive research supports a bidirectional interaction between mGlu5 and NMDARs, such that activation of mGlu5 receptors facilitates NMDAR function (Doherty et al., 1997; Ugolini et al., 1999; Awad et al., 2000; Attucci et al., 2001; Mannaioni et al., 2001; Pisani et al., 2001), whereas activation of NMDARs amplifies mGlu5 receptor activity by restraining receptor desensitization (Alagarsamy et al., 2005). These observations serve as a foundation for the development of mGlu5 PAMs for the treatment of schizophrenia. A number of mGlu5 PAMs have demonstrated efficacy in rodent models used to predict antipsychotic efficacy and the treatment of cognitive disturbances (Kinney et al., 2003; Lecourtier et al., 2007; Liu et al., 2008; Conn et al., 2009; Stefani and Moghaddam, 2010; Vardigan et al., 2010; Gastambide et al., 2013; Horio et al., 2013; Nicoletti et al., 2019). However, one caveat of mGlu5 PAMs includes excitotoxicity mediated by the enhanced NMDAR activity, as high doses of mGlu5 receptor PAMs have been shown to induce seizures and neurotoxicity in rodents (Parmentier-Batteur et al., 2014; Rook et al., 2015; Conde-Ceide et al., 2016). To circumvent these adverse side effects, biased mGlu5 receptor PAMs have been developed to amplify receptor function without recruiting NMDA receptors (Rook et al., 2013, 2015). Interestingly, these biased mGlu5 PAMs have robust efficacy in animal models without potentiating NMDA receptor signaling (Rook et al., 2015; Gould et al., 2016), suggesting that efficacy of these compounds is not mediated by potentiation of NMDA receptor currents. Thus, leveraging this biased mGlu5 PAM, VU0409551, Rook et al. have shown antipsychotic-like activity and pre-cognitive efficacy without activating NMDA receptors and without inducing the adverse effects of nonbiased mGlu5 PAMs (Rook et al., 2015).

**II. Group II: Metabotropic Glutamate2/3.** Based on evidence that activation of group II mGlu receptor subtypes, mGlu2 and mGlu3, produce robust antipsychotic-like effects in preclinical models (Chaki et al., 2004; Pilc et al., 2008; Conn et al., 2009; Dhanya et al., 2014; Muguruza et al., 2016), longstanding efforts have been aimed at optimizing mGlu 2 and mGlu3 agonists for the treatment of schizophrenia. Despite group II mGlu receptor agonists showing efficacy in improving positive and negative symptoms in an initial phase II trial (Patil et al., 2007), larger clinical studies did not demonstrate significant efficacy of these compounds compared with placebo (Kinon et al., 2011). The development of highly selective allosteric modulators of mGlu2 and mGlu3 have allowed for delineation of the role of these receptor subtypes in the physiologic and behavioral deficits associated with schizophrenia (Table 3).
mGlu receptor agonists are absent in mGlu2, but not mGlu3, receptor knockout mice (Spooren et al., 2000). These observations have been bolstered by the discovery of mGlu2-selective PAMs, which allowed for direct interrogation of this hypothesis. Several mGlu3 PAMs demonstrated antipsychotic-like efficacy as well as preclinical and social effects in multiple preclinical models (Galici et al., 2005, 2006; Harich et al., 2007; Nikiforuk et al., 2010; Griebel et al., 2016). For example, administration of the mGlu2 PAM, potassium 3’-(((2-cyclopentyl-6-7-dimethyl-1-oxo-2,3-dihydro-1H-inden-5-yl)oxy)methyl)biphenyl 1-carboxylate (BINA), reduced dimethoxy-bromoamphetamine-induced head twitches and reversed MK-801-induced immobility in the forced swim test (FST) (Benneyworth et al., 2007; Kawaura et al., 2016). Additionally, (2S)-5-methyl-2-[(4-((1,1,1-trifluoro-2-methylpropan-2-yl)phenoxy)methyl]-2,3-dihydroimidazo[2,1-b][1,3]oxazole-6-carboxamid (TASP0-443294), another mGlu2 PAM, showed efficacy in reducing methamphetamine and NMDAR antagonist-induced hyperlocomotion as well as reversed social memory deficits induced by MK-801 administration in mice (Hikichi et al., 2015; Lavreysen et al., 2015). Two primary mGlu2 PAMs moved forward to clinical testing; however, the mGlu2 PAM AZD 8529 did not significantly improve positive or negative symptoms in patients with schizophrenia when administered as a monotherapy (Litman et al., 2016). Alternatively, another mGlu2 PAM, JNJ-40411813/ADX71149, showed beneficial effects in patients with residual negative symptoms (Hopkins, 2013) and potential efficacy in reversing select cognitive deficits and negative symptoms after administration of ketamine in healthy volunteers (Salih et al., 2015). However, negative phase 2 schizophrenia clinical trials with JNJ-40411813/AZD8529 has tempered expectations on the utility of mGlu2 PAMs for the treatment of schizophrenia. The ongoing efficacy of this compound in large-scale clinical trials remains to be seen.

In addition to the wealth of research on mGlu2-selective PAMs, numerous studies have indicated a relationship between decreased performance PPC-dependent cognitive tasks and single nucleotide polymorphisms in the human gene encoding mGlu3 (GRM3) (Egan et al., 2004; Tan et al., 2007; Harrison et al., 2008), identifying GRM3 as a risk locus for schizophrenia in genome-wide association studies (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). After the discovery of highly selective mGlu3 NAMs, studies in mice revealed that mGlu3 mediates distinct aspects of PPC synaptic plasticity and proognitive effects in rodents (Walker et al., 2015; Dogra et al., 2021). In agreement with these findings, studies in nonhuman primates have demonstrated that mGlu3 agonists elicit postsynaptic effects in the dorsolateral PFC that improve cognitive function (Jin et al., 2017). Together, these studies highlight mGlu3 as a novel regulator of PFC-mediated cognitive processes. Additional investigation using current and next-generation group II mGlu receptor PAMs/NAMs is essential for expanding our understanding of these receptors and their potential utility in treating schizophrenia.

### III. Group III: Metabotropic Glutamate4/6/7/8

Like group II mGlu receptors, the therapeutic promise of group III mGlu receptors arose from evidence of their ability to improve the hyperglutamatergic state believed to take place in schizophrenia and modulate behavioral processes dysregulated in the disease, including cognition and motivation. For instance, mice lacking mGlu4 display impairments in spatial reversal and long-term memory (Gerlai et al., 1998), suggesting a critical role of mGlu4 in cognitive flexibility and associative learning, both of which are known to be impaired in patients with schizophrenia. Previous studies also suggest that mGlu4 activation may elicit antipsychotic-like effects in rodent models. In one study, the group III agonist (1S,3R,4S)-1-aminocyclopentane-1,3,4-tricarboxylic acid (ACPT-I) reduced PCP- and amphetamine-induced hyperlocomotion in addition to head twitching in response to 2,5-Dimethoxy-4-iodoamphetamine (DOI) (Pałucha-Poniewiera et al., 2008). Similar actions of ACPT-I administration are also observed with mGlu4-selective orthosteric agonists, LSP-1-2111 (Wieronska et al., 2012a) and LSP-4-2022 (Woźniak et al., 2016). Activation of mGlu4 receptors with these compounds also improves behavioral deficits associated with negative and cognitive symptoms of schizophrenia (Wieronska et al., 2012a; Woźniak et al., 2016). More recently, mGlu4 PAMs Lu AF 21934 (Bennouar et al., 2013), Lu AF32615 (East et al., 2010), and ADX88178 (Le Poul et al., 2012) have also displayed similar outcomes in models of representing all three symptom clusters of schizophrenia (Table 4). For example, ADX88178, an mGlu4 PAM, was shown to reduce NMDAR antagonist-induced hyperlocomotion and DOI-induced head twitches while also reducing immobility time in FST (Kalinichev et al., 2014). Alternatively, the mGlu4 PAMs, Lu AF21934 and Lu AF32615, showed efficacy in restoring social interaction, reducing hyperlocomotion induced by amphetamine and NMDAR antagonists, and rescuing MK-801-induced deficits in spatial working memory and novel object recognition (Wieronska et al., 2012a; Sławińska et al., 2013a). Together, these studies highlight the potential therapeutic utility of selective mGlu4 activators for schizophrenia.

Despite limited studies focusing on the mGlu4 receptor subtype as a potential therapeutic target for schizophrenia, a polymorphism in the GRM7 gene encoding mGlu7 that decreased transcription in vitro was positively

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*Suggested by the ASPE journal.*
correlated with schizophrenia in a large Japanese cohort (Ohtsuki et al., 2008). These findings suggest that hypofunction of mGlu7 may contribute to the pathophysiology of schizophrenia. Because activation of mGlu7 reduces glutamatergic neurotransmission (Baskys and Malenka, 1991; Ayala et al., 2008) and acts as a heteroreceptor to modulate GABA release and the induction of LTP in brain regions, such as the hippocampus (Klar et al., 2015), it is plausible that selective activators of mGlu7 may enhance aspects of hippocampal-dependent cognitive function. However, some preclinical studies refute this hypothesis such that the mGlu7 allosteric agonist N,N'-dibenzhydrylethane-1,2-diamine dihydrochloride (AMN082) exacerbates MK-801-induced hyperlocomotion (Mitsukawa et al., 2005) and DOI-induced head twitches (Wieronska et al., 2012a). Although these findings may be driven by off-target effects of AMN082 in vivo (Sukoff Rizzo et al., 2011), it has been shown that the psychotic-enhancing effects were occluded in mGlu7 knockout (KO) mice (Wieronska et al., 2012a), implying that they may be mGlu7 receptor-dependent. At this time, future studies must further confirm if the use of selective PAMs may show more promise of mGlu7 activation in schizophrenia-related models.

Although expressed at fairly low levels in the brain, the mGlu8 receptor subtype is expressed in the presynaptic active zone of mainly glutamatergic synapses (Kinoshita et al., 1996a; Shigemoto et al., 1997) where it functions to modulate neurotransmitter release and gates glutamatergic transmission into the hippocampus (Zhai et al., 2002). In line with this function, mGlu8 KO mice display deficits in hippocampal-dependent learning (Gerlai et al., 2002), suggesting that activating mGlu8 with selective ligands could treat the cognitive impairments in patients with schizophrenia. In studies determining the antipsychotic efficacy of mGlu8-targeting ligands, two studies have found that the relatively selective orthosteric mGlu8 agonist (S)-3,4-dichlorophenyl glycine ((S)-3, 4-DCPG) was unable to reverse amphetamine or nicotine, cocaine, and methamphetamine (Addolorato et al., 2012). One of the well-studied candidates, baclofen, a GABAB agonist, has yielded findings in preclinical and clinical studies suggesting its potential utility in treating alcohol use disorder. Baclofen acts presynaptically to hyperpolarize synaptic terminals, inhibits calcium influx, and prevents the release of the excitatory neurotransmitters glutamate and aspartate.

**B. Substance Use Disorders**

SUD is a multifaceted chronically relapsing disorder characterized by excessive drug intake, repeated unsuccessful attempts to reduce or stop drug use, enhanced drug-seeking and self-administration, the emergence of drug tolerance and withdrawal, and continued drug intake despite negative consequences (Koob and Volkow, 2010). SUD represents a serious public health problem with devastating consequences to society. However, there is a lack of pharmacological agents approved to treat the disease. Drugs of abuse exert their effects by altering the signaling of numerous neurotransmitter systems and brain circuits, which serves as a challenge for developing efficacious treatments. Ongoing research highlights detrimental adaptations in GABA and glutamate neurotransmission in SUD (Tzschentke and Schmidt, 2003; Cruz et al., 2008). Drugs of abuse alter glutamate transmission through a diverse array of mechanisms. For example, cocaine increases glutamate transmission indirectly through dopamine transporter-mediated dopamine release (Ritz et al., 1987). Alternatively, it has been reported that alcohol inhibits postsynaptic NMDAR- and non-NMDAR-mediated glutamate transmission and release, possibly via inhibition of GABA interneurons (Lovinger et al., 1989, 1990; Carta et al., 2003; Hendricson et al., 2003, 2004). Notably, imbalance of glutamate and GABA systems in brain regions, such as the PFC, is associated with physiologic and behavioral aspects of SUDs, including impulsivity, reinforcement learning, and executive function. For instance, a recent clinical study using hydrogen (3H)-magnetic resonance spectroscopy revealed significantly higher glutamate levels and lower GABA levels in patients with opioid use disorder compared with healthy controls (Li et al., 2020). Additionally, this study showed that higher impulsivity and cognitive impairment were associated with lower GABA and higher glutamate levels. Furthermore, exposure to pharmacological agents that block glutamate transmission attenuate the reinforcing effects of drugs of abuse. For instance, systemic administration of NMDAR antagonists attenuates self-administration of alcohol (Shelton and Balster, 1997), cocaine (Pierce et al., 1997; Pulvirenti et al., 1997; Hyttia et al., 1999; Blokhina et al., 2005), and nicotine (Kenny et al., 2009). Therefore, restoring GABA and glutamate balance in PFC, among other brain regions, represents a promising therapeutic strategy for treating SUD.

Numerous medications targeting GABA and/or glutamate receptors have been under longstanding investigation for the treatment of SUD, including baclofen, topiramate, and gabapentin. However, to date, there has been mixed evidence for their efficacy in clinical trials for various types of SUDs, including alcohol, nicotine, cocaine, and methamphetamine (Addolorato et al., 2012). One of the well-studied candidates, baclofen, a GABAB agonist, has yielded findings in preclinical and clinical studies suggesting its potential utility in treating alcohol use disorder. Baclofen acts presynaptically to hyperpolarize synaptic terminals, inhibits calcium influx, and prevents the release of the excitatory neurotransmitters glutamate and aspartate.
tion of mGlu receptors, including mGlu5, elicits glutamate release, a potential consequence of direct activation of glutamatergic systems for the treatment of SUDs; however, more effective compounds are required to better achieve this goal.

A wealth of studies using ligands targeting mGlu receptors has demonstrated the utility of targeting these receptors as an alternative approach to mitigate SUD-induced imbalances in glutamate and GABA signaling. For example, systemic administration of LY379268, an mGlu2/3 orthosteric agonist, decreases self-administration of cocaine (Baptista et al., 2004; Adewale et al., 2006; Xi et al., 2010), nicotine (Liechti et al., 2007), and alcohol (Bäckström and Hyytiä, 2005; Sidhpura et al., 2010). However, excessive glutamate release, a potential consequence, directly activates mGlu receptors, including mGlu5, elicits excitotoxicity via NMDAR-mediated mechanisms, limiting the utility of orthosteric mGlu-targeting compounds (Reiner and Levitz, 2018). Thus, allosteric modulators of mGlu receptors represent a novel avenue for restoring homeostasis of GABA and glutamate systems while exerting receptor subtype selectivity and circumventing EPS.

I. Group I: Metabotropic Glutamate1/5

Group I mGlu receptor subtypes, mGlu1 and mGlu5, have been extensively studied in terms of behavioral effects in animal models of SUD. Both mGlu1 and mGlu5 are robustly expressed in brain regions known to be critical to SUD pathophysiology, including the NAc, dorsal striatum, ventral midbrain and PFC (Niswender and Conn, 2010). Repeated exposure to drugs of abuse can dysregulate mGlu1 and mGlu5 expression and function. For example, chronic alcohol consumption in rodents reduces mGlu1/5 mRNA levels in various subregions of the hippocampus and increases mGlu1/5 expression in the NAc core and central nucleus of the amygdala (Simonyi et al., 2004; Obara et al., 2009). Nicotine exposure increases expression of mGlu1 mRNA in the ventral tegmental area (VTA) and amygdala (Kane et al., 2005). Further, repeated cocaine exposure disrupts mGlu1 receptor-mediated signaling in the NAc (Swanson et al., 2001). Finally, prolonged withdrawal from extended-access cocaine self-administration decreases total protein and surface expression levels of mGlu1 in the NAc compared with drug-naïve rats (Loweth et al., 2014). Alternatively, the total and surface levels of mGlu1 are unchanged in the NAc following shorter abstinence periods or during cocaine administration (Ary and Szumlinski, 2007; Loweth et al., 2014). Withdrawal time-dependent reductions in mGlu1/5 expression within ventromedial PFC have also been reported for following extended-access cocaine self-administration following extinction testing compared with drug-naïve animals (Ben-Shahar et al., 2013). Additionally, extinction of cocaine-seeking decreases the surface expression of mGlu1 receptors in the NAc (Knackstedt et al., 2010). Thus, there is extensive evidence that mGlu1 and mGlu5 receptor subtypes may be involved in drug-related behaviors and the pathophysiology of SUDs.

Notably, mGlu1 and mGlu5 have both also been implicated as regulators of drug self-administration behavior. Early studies reported that mice carrying a null mutation for the gene encoding the mGlu5 receptor lack cocaine-induced hyperlocomotion and did not acquire intravenous self-administration of cocaine (Chiamulera et al., 2001). Furthermore, genetic inactivation or pharmacological inhibition of mGlu5 receptors decreases self-administration of alcohol, cocaine, heroin, nicotine, methamphetamine and ketamine and reduces breakpoints for reinforcement for various drugs of abuse in a progressive ratio (PR) paradigm (Cleva and Olive, 2012). mGlu1 antagonist JNJ16259685 reduces alcohol self-administration and breakpoints for alcohol reinforcement under a PR schedule (Besheer et al., 2008a,b). However, other studies have shown that acquisition and fixed ratio operant responding for psychostimulants, such as cocaine and methamphetamine, is intact in mice lacking mGlu5/−/−; however, deletion of mGlu5 enhanced responding on a progressive ratio schedule and impaired extinction of drug-seeking behaviors (Chesworth et al., 2013; Bird et al., 2014). These findings suggest that mGlu5 may play distinct roles in drug reinforcement and instrumental extinction learning. Furthermore, mGlu1 and mGlu5 play an important role in reinstatement of drug seeking. Numerous studies have reported that pharmacological blockade of mGlu5 attenuates the reinstatement of drug-seeking behavior induced by drug-associated cues, stress and drug priming (Bespalov et al., 2005; Bäckström and Hyytiä, 2007; Platt et al., 2008; Schroeder et al., 2008; Kumaresan et al., 2009; Martin-Fardon et al., 2009).
Additionally, mGlu1 antagonism attenuates the reinstatement of nicotine and cocaine-seeking behavior elicited by drug-associated cues (Dravolina et al., 2007). A wealth of additional research supports the roles of mGlu1 and mGlu5 in various other drug-related behaviors, including conditioned place preference and interoceptive drug effects. These findings have been elegantly detailed in reviews elsewhere (Olive, 2009; Cleva and Olive, 2012; Caprioli et al., 2018; Niedzielska-Andres et al., 2021).

The extensive evidence that mGlu1 and mGlu5 play critical roles in behavioral deficits associated with SUD raises the question of whether targeting group I mGlu receptors is a promising therapeutic approach for the treatment of SUD. The development of mGlu1 and mGlu5 allosteric modulators have allowed researchers to directly test this question. Studies have investigated the efficacy of mGlu1 allosteric modulators within the context of SUD (Caprioli et al., 2018). These studies have been summarized in Table 5. Specifically, repeated administration of selective mGlu1 PAMs, SYN119 or Ro0711401, blocks incubation of cue-induced cocaine craving following extended-access cocaine self-administration and prolonged withdrawal in rats (Loweth et al., 2014). Interestingly, administration of mGlu1 PAMs during withdrawal from methamphetamine self-administration did not block incubation of methamphetamine craving. The efficacy of mGlu1 PAMs in reducing incubation of cocaine craving may be driven by mGlu1-mediated blockade of calcium permeable amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor accumulation and synaptic transmission, which is known to be a critical mechanism driving cue-induced drug craving and cocaine seeking (McCUTCHEON et al., 2011; LOWETH et al., 2014; RUAN and YAO, 2021). However, there is evidence that mGlu1 does not regulate calcium permeable amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor accumulation during methamphetamine withdrawal (Murray et al., 2019), further supporting divergent mechanisms underlying incubation of craving for psychostimulants and selective roles of mGlu1. These studies leveraging mGlu1 PAMs provide further support that selective activation of mGlu1 is able to reduce the impact of drug-induced adaptation in mGlu1 function, which drives cocaine-seeking behavior (HALBOUT et al., 2014) and modulates a number of critical forms of synaptic plasticity, such as cocaine-induced plasticity in the VTA (MAMELI et al., 2009) and mGlu1-LTD and synaptic potentiation in the PFC (RUAN and YAO, 2021).

mGlu1 NAMs have been shown to decrease alcohol self-administration in some studies but not others. In alcohol self-administration studies in alcohol-preferring P rats, JNJ16259685, an mGlu1 NAM, decreased responding under fixed ratio and PR schedules (BESHEER et al., 2008a; b), but also decreased locomotor activity and lever-pressing for sucrose, suggesting nonspecific motor effects. Similar observations were reported in additional studies looking at the effect of (−)-ethyl (7E)-7-hydroxyimino-1,7a-dihydrcyclopropa[b]chromene-1a-carboxylate (CPCCOEt), another mGlu1 NAM, on alcohol self-administration in alcohol-preferring (P)-rats or C57BL/6J mice (Casabona et al., 1997; Schroeder et al., 2005; Hodge et al., 2006). In another study, CPCCOEt reduced ethanol reinforcement, consumption, and expression of ethanol conditioned place preference while facilitating the motor-impairing effects of ethanol (Casabona et al., 1997; LominaC et al., 2006). Importantly, CPCCOEt was able to block the acute effects of ethanol on extracellular levels of dopamine and glutamate in the NAc, while potentiating the effects of acute ethanol on extracellular GABA in this region. Leveraging another mGlu1 NAM, JNJ16259685, one study reported decreased psychostimulant (cocaine and methamphetamine) administration under a second-order reinforcement schedule of reinforcement fixed interval 5-10 (FI5-FR10) (Achat-Mendes et al., 2012). In this same study, JNJ16259685 had no effect on food-reinforced responding but exerted motoric effects. The efficacy of mGlu1 NAMs in nicotine use has also been studied. The selective mGlu1 NAM EMQCMC (5 mg/kg) inhibited cue and nicotine-induced reinstatement of nicotine-seeking behavior, albeit when administered at higher doses EMQCMC reduced cue-induced reinstatement of food-seeking, indicating that high doses of this compound may have general inhibitory effects on appetitive responding (Dravolina et al., 2007). Furthermore, EMQCMC has been shown to inhibit the expression of locomotor sensitization to both morphine and cocaine (Dravolina et al., 2006; Kotlinska and Bochenski, 2007). Another study showed that intra-NAc injections of mGlu1 NAM, 6-amino-N-cyclohexyl-N,3-dimethylthiazolo[3,2-a]benzimidazole-2-carboxamide (YM298198), decreases reinstatement of cocaine seeking in rats following cocaine priming (Schmidt et al., 2015). Together, these studies highlight the potential utility of mGlu1 allosteric modulators for the treatment of various SUDs but also highlight the need for continued studies with improved formulations.

mGlu5 allosteric modulators have been of particular interest as therapeutic options for the treatment of SUDs. A summary of these studies has been included in Table 5. Due to the abundance of reports, we have focused on summarizing the effects of mGlu5 allosteric modulators on drug self-administration and reinstatement. Details on the efficacy of mGlu5 PAM/NAMs in other drug-related behaviors have been reviewed previously (Olive, 2009; Cleva and Olive, 2012; Caprioli et al., 2018). A subset of classically studied mGlu5 NAMs include the compounds 2-Methyl-6-(phenylethynyl)pyridine (MPEP) and 3-(2-Methyl-4-thiazolyl)ethyl-nyl)pyridine (MTEP). MPEP and MTEP have been shown to attenuate intravenous self-administration of...
| Receptor Type | Compound | Drug of Abuse | Behavioral Effect | References |
|---------------|----------|---------------|-------------------|------------|
| mGlu1 PAM     | SYN119/Ro0711401 | Cocaine       | Blocks incubation of cue-induced cocaine craving after extended-access self-administration | Loweth et al., 2014 |
| NAM           | JNJ-16259685 | Alcohol       | Decreased responding under FR/PR schedules | Besheer et al., 2008a,b |
|               |           | Cocaine and methamphetamine | Decreased self-administration under a second-order reinforcement schedule of reinforcement (FI5-FR10) | Achat-Mendes et al., 2012 |
| CPCCOEt       | Alcohol   |              | Decreased self-administration under FR and PR schedules of reinforcement in alcohol-prefering P-rats or C57BL/6J mice | ¹ (See footnotes.) |
| EMQMCM        | Nicotine  |              | Inhibited cue and nicotine-induced reinstatement of nicotine-seeking behavior | Dravolina et al., 2007 |
|               | Morphine and cocaine |              | Inhibited the expression of locomotor sensitization | Dravolina et al., 2006; Kotlinska and Bochenski, 2007 |
| YM298198      | Cocaine   |              | Decreased cocaine-primed reinstatement of cocaine seeking | Schmidt et al., 2015 |
| mGlu5 NAM     | MPEP/MTEP | Cocaine, nicotine, heroin, and alcohol | Attenuates self-administration (intravenous or oral) | Ombelet et al., 1994; Kenny et al., 2003, 2005; Paterson et al., 2003; Backström et al., 2004; Tessari et al., 2004; Bespalov et al., 2005; Cowen et al., 2005, 2007; Lee et al., 2005; McMillen et al., 2005; Olive et al., 2005; Hodge et al., 2006; Lominac et al., 2006; Liechti and Markou, 2007; Besheer et al., 2008b; Palmatier et al., 2008; Backström and Hyttia, 2007; Knackstedt et al., 2014; Knackstedt and Schwendt, 2016; Paterson et al., 2003; Besheer et al., 2008b |
|               |           | Cocaine       | Attenuated cue-induced reinstatement of cocaine seeking | ² |
|               |           | Alcohol, cocaine, and nicotine | Reduced breakpoints under PR reinforcement schedule | Gass et al., 2009; Murray et al., 2021 |
|               |           | Methamphetamine | Reduced incubated methamphetamine seeking; decreased self-administration under FR and PR schedules of reinforcement | ³ |
| Partial NAM   | M-5MPEP/Br-5MPEPy | Cocaine | Decreased self-administration and attenuated the discriminative stimulus effects of cocaine | Gould et al., 2016 |

CPP, conditioned place preference; FI, fixed interval; FR, fixed ratio; P, preferring; SYN119, mGlu1 PAM.

¹Casabona et al., 1997.
²Hodge et al., 2006.
³Schroeder et al., 2005.
⁴Lominac et al., 2006.
numerous drugs of abuse, including cocaine, nicotine, methamphetamine, and heroin (Ombelet et al., 1994; Kenny et al., 2003, 2005; Paterson et al., 2003; Tessari et al., 2004; Bespalov et al., 2005; Lee et al., 2005; Liechti and Markou, 2007; Palmatier et al., 2008; Gass et al., 2009) and ethanol in a variety of rodent strains (Bäckström et al., 2004; Cowen et al., 2005; McMillen et al., 2005; Olive et al., 2005; Hodge et al., 2006; Lominac et al., 2006; Cowen et al., 2007; Besheer et al., 2008b) without altering the reinforcing properties of natural reward or food (Paterson et al., 2003; Tessari et al., 2004; Bespalov et al., 2005; Liechti and Markou, 2007; Gass et al., 2009). These compounds have also demonstrated the ability to modulate the motivational properties of many drugs. For instance, MPEP reduces breakpoints for ethanol, cocaine, and nicotine under PR schedules of reinforcement (Paterson et al., 2003; Besheer et al., 2008b). Furthermore, administration of MTEP has been shown to reduce the reinforcing efficacy of methamphetamine as reflected by reduced breakpoints under a PR schedule of reinforcement (Gass et al., 2009). In addition to modulating the rewarding and motivational properties of drugs, MPEP and MTEP have also been shown to prevent cue and drug-induced priming of reinstatement of cocaine, nicotine or ethanol-seeking behavior (Bäckström et al., 2004; Tessari et al., 2004; Lee et al., 2005; Bäckström and Hyytiä, 2006; Iso et al., 2006; Murray et al., 2021). For instance, a recent study showed that systemic administration of MTEP reduces incubated methamphetamine seeking following self-administration and prolonged withdrawal (Murray et al., 2021), elucidating the role of mGlu5 in the incubation of methamphetamine craving and delineating distinct mechanisms from that of incubation of cocaine craving. Interestingly, cue-induced reinstatement of cocaine-seeking behavior is attenuated in response to local infusion of MPEP or MTEP into the NAc (Bäckström and Hyytiä, 2007; Knackstedt et al., 2014), while local infusion of MTEP into the dorsolateral striatum at time of context-induced relapse testing attenuated extinction learning (Knackstedt et al., 2014; Knackstedt and Schwindt, 2016). This finding was paralleled by reduced mGlu5 surface expression and LTD in brain slices of animals during prolonged abstinence from cocaine which could be reversed by bath application of the mGlu5 PAM VU-29, suggesting brain-region specific subpopulations of mGlu5 receptors may play distinct roles in regulating extinction learning and reinstatement of cocaine-seeking. Despite the promising results of these studies, it has also been reported that MPEP and MTEP can attenuate breakpoints for food (Paterson et al., 2003), enhance the sedative properties of ethanol (Sharko and Hodge, 2008) and elicit general behavioral reduction, including decreases in inactive lever responding (Murray et al., 2021). To this end, partial mGlu5 NAMs have been developed, which feature submaximal but saturable levels of blockade and may represent an additional avenue to broaden the therapeutic window of mGlu5 NAMs. One study evaluated the efficacy of partial mGlu5 NAMs, 2-[2-(3-methoxyphenyl)ethyl]-5-methylpyridine (M-5MPEP) and bromo-2-[2-(3-methoxyphenyl)ethyl]-5-methylpyridine (Br-5MPEPy), in comparison with the full mGlu5 NAM MTEP in models of SUD. Gould et al., found that M-5MPEP, Br-5MPEPy, and MTEP dose-dependently decreased cocaine self-administration and attenuated the discriminative stimulus effects of cocaine, suggesting that partial mGlu5 NAM activity is sufficient to elicit therapeutic effects comparable to full mGlu5 NAMs (Gould et al., 2016). In sum, full and partial mGlu5 NAMs may represent a promising therapeutic option for the treatment of numerous SUDs; however, continued exploration of novel mGlu5 NAMs with improved therapeutic window and reduced side effects is required.

II. Group II: Metabotropic Glutamate2/3. Decades of research support a critical role of group II mGlu receptors in SUDs. mGlu2 and mGlu3 regulate neurotransmission in brain regions implicated in SUDs, including the PFC and NAc. In the PFC, mGlu2 receptors are tonically activated by endogenous glutamate and infusion of a selective mGlu2 receptor antagonist, LY341495, increases glutamate levels (Melendez et al., 2005; Xie and Steketee, 2008). Within the NAc, numerous studies suggest that endogenous glutamatergic tone on group II mGlu receptors regulating both glutamate and dopamine levels. In vivo microdialysis studies have demonstrated increased glutamate release after perfusion of selective antagonist LY143495 into the NAc and decreased extracellular glutamate levels in response to agonist (2R,4R)-4-amino-5-(3,4-dicarboxyphenyl)pyrrolidine (APDC) (Xi et al., 2002). Further, electrophysiological recordings from NAc slices reveal that mGlu2 receptors can act as presynaptic autoreceptors to control glutamate release. Specifically, increased paired pulse ratios and reduced miniature EPSC frequency were observed after bath application of selective agonists (S)-4-carboxy-3-hydroxyphenylglycine ((1S,3S)-ACPD) and (2S,1S,2'S)-2-(2'-carboxycyclopropyl)-glycine (L-CCG1) (Manzoni et al., 1997). Evidence also supports that mGlu2 receptors regulate glutamate release in VTA (Manzoni and Williams, 1999), bed nucleus of the stria terminalis (Grueter and Winder, 2005), and hippocampus (Capogna, 2004), among other brain regions within the motivational circuit (Poisik et al., 2005).

The expression and function of mGlu2/3 receptor subtypes are also altered by chronic use of drugs, such as alcohol, cocaine, opioids, and nicotine. The effects of drugs of abuse on mGlu2/3 receptor function have been detailed in greater detail previously (Mousawi and Kalivas, 2010). In brief, it has been reported that ethanol-dependent rats have decreased PFC...
GRM2 mRNA levels and mGlu2/3 autoreceptor function in the NAc shell compared with control rats (Meinhardt et al., 2013). In addition, drinking-induced decreases in mGlu2/3 autoreceptor function have also been observed in the VTA (Ding et al., 2017). Alternatively, enhanced physiologic and behavioral sensitivity to mGlu2/3 agonists is observed in the central nucleus of the amygdala and bed nucleus of stria terminalis of ethanol-dependent rats (Kufahl et al., 2011). In addition to sensitivity to the effects of ethanol, mGlu2/3 receptors are altered by chronic exposure to other drugs of abuse. A recent study revealed that 12 days cocaine self-administration followed by 6 to 10 days of extinction training resulted in a decreased in mGlu2 expression in the NAc core of male and female rats (Logan et al., 2020). Further, the inhibitory effects of mGlu2/3 receptors on excitatory transmission in the VTA and NAc are enhanced in rodents during early withdrawal from chronic morphine (Manzoni and Williams, 1999; Martin et al., 1999). Interestingly, genetic variation in the mGlu2 gene, Grm2, has also been associated with alcohol preference and consumption in rodents, such that the presence of an abnormal stop codon preventing expression of the Grm2 results in increased alcohol preference and intake as measured by a two-bottle choice paradigm (Zhou et al., 2013; Wood et al., 2017).

Notably, mGlu2/3 receptor-dependent plasticity is impaired after exposure to drugs of abuse. For example, chronic morphine impairs mGlu2/3 receptor-induced LTD at excitatory synapses in NAc medium spiny neurons (Robbe et al., 2002) and chronic cocaine exposure impairs mGlu2/3 receptor-dependent LTD in PFC pyramidal cells (Huang et al., 2007). More recently, it was shown that NAc LTP induced by high frequency stimulation of the PFC was abolished after withdrawal from self-administered cocaine via reduced mGlu2/3 receptor stimulation (Moussawi et al., 2009). Given the importance of neuroplasticity in cognition, reinforcement learning, and updating behaviors after changes in environmental contingencies (Malenka and Bear, 2004; Whitlock et al., 2006; De Roo et al., 2008) and evidence of drug-induced plasticity impairments, mGlu2/3 receptors may be a critical target underlying drug-induced deficits in synaptic plasticity. Thus, potentiating the function of mGlu2/3 may represent a promising approach to mitigate drug intake and cognitive deficits in individuals with SUD by restoring neurotransmitter homeostasis and neuroplasticity.

Early studies using the prototypical group II mGlu agonist LY379268 demonstrate decreased reinstatement of alcohol, cocaine, methamphetamine, and heroin seeking induced by cues previously associated with drug self-administration (Acri et al., 2017). Administration of LY379268 has also been shown to decrease cue- and drug priming-induced reinstatement of cocaine self-administration in non-human primates (Adewale et al., 2006; Justinova et al., 2016) and incubation of cocaine, methamphetamine, or nicotine self-administration in rats (Liechti et al., 2007; Crawford et al., 2013). Although less abundant than studies on group I mGlu receptor PAM/NAMs, a few studies have looked at the efficacy of mGlu2/3 allosteric modulators in behavioral models of SUD (Table 6). One study used the selective and brain penetrant mGlu2 PAM BINA in a model of intravenous cocaine self-administration and cocaine-seeking behavior in rats that had short (1 h, ShA) or long (6 h, LgA) access to cocaine. In this study, BINA decreased cocaine self-administration in both ShA and LgA rats, with no effect on food self-administration (Jin et al., 2010). Additionally, this study showed that BINA decreased cue-induced reinstatement of cocaine seeking without altering food seeking, suggesting that mGlu2 allosteric modulators may have potential as treatments for cocaine use disorder and possibly other drugs of abuse.

mGlu2 PAMs, AZD8418 and AZD8529, underwent preclinical and clinical evaluation for their efficacy in nicotine use disorder. Acute treatment with AZD8418 (0.37, 1.12, 3.73, 7.46, and 14.92 mg/kg) and AZD8529 (1.75, 5.83, 17.5, and 58.3 mg/kg) decreased nicotine self-administration and blocked cue-induced reinstatement of nicotine- and food-seeking behavior but did not significantly affect food-maintained responding in rats (Li et al., 2016). Chronic treatment with AZD8418 attenuated nicotine self-administration but resulted in tolerance to this effect. The inhibitory effects of chronic AZD8529 administration on nicotine self-administration persisted throughout the 14 days of treatment; however, chronic treatment with these PAMs inhibited food self-administration. The mGlu2 PAM AZD8529 has since been tested in clinical trials including a 19-week, multicenter, randomized, phase 2 clinical study comparing the efficacy of two different doses of AZD8529 (1.5 and 40 mg) in smoking cessation in female smokers. However, this trial was completed in 2017 and reported only ~10% of either the low- or high-dose AZD8529 groups meeting the primary outcome of abstinence during the course of the 13-week study (Lassi et al., 2016).

III. Group III: Metabotropic Glutamate4/6/7/8

The group III mGlu receptor subtypes have also garnered attention as potential targets for the treatment of SUD. A multitude of studies have reported group III mGlu receptor subtypes are sensitive to the effects of drugs of abuse, including psychostimulants and alcohol. For instance, repeated amphetamine exposure increases mGlu8 mRNA levels in the dorsal striatum and NAc (Parelkar and Wang, 2008). Chronic alcohol consumption has been shown to reduce mGlu7 mRNA levels in numerous hippocampal subregions, whereas
mGlu₈ mRNA levels were unchanged (Simonyi et al., 2004). Additionally, expression levels of the gene encoding the mGlu₇ receptor is associated with higher levels of alcohol consumption (Vadasz et al., 2007). The development of selective compounds targeting group III mGlu receptor subtypes have allowed for delineation of the distinct roles of these receptors in the physiologic and behavioral effects of drugs of abuse. For example, treatment with AMN082, a selective mGlu₇ allosteric agonist, attenuates cocaine-induced decreases of ventral pallidum GABA release in both naïve rats and cocaine self-administering rats (Li et al., 2009). These findings suggest a novel role of mGlu₇ receptors in regulating the effects of cocaine on NAc-ventral pallidum GABA transmission, which is one mechanism proposed to underlie the rewarding and motivational effects of cocaine. Furthermore, microinjection of L-AP4, a nonselective agonist of group III mGlu receptors, into the dorsal striatum reducedamphetamine or cocaine-induced hyperlocomotion in rats (Mao and Wang, 2000). Importantly, L-AP4 attenuatedamphetamine-stimulated dopamine release in the dorsal striatum (Mao et al., 2000), suggesting that group III mGlu receptors may be involved in the acute effects of psychostimulant exposure by inhibiting dopamine release. Striatal glutamate has long been recognized to facilitate dopamine release (Mao et al., 2000). Thus, inhibition of glutamate release by group III autoreceptors may result in this inhibition of dopamine release. A study by Xi et al., showed that L-AP4 decreased extracellular glutamate levels, whereas the group III receptor antagonist (R, S)-a-methylserine-O-phosphate increased extracellular glutamate levels in the NAc of rats, respectively (Xi et al., 2003). In addition to the presynaptic roles of group III mGlu receptors in regulating drug-associated neurotransmission, mGlu₇ is expressed postsynaptically on both striatopallidal and striatonigral medium spiny neurons (Kosinski et al., 1999). To this end, L-AP4 inhibits evoked synaptic responses in the NAc, in part, through a postsynaptic mechanism (Martin et al., 1997). This putative postsynaptic mechanism likely works in concert with group III mGlu receptor subtype-mediated presynaptic modulation to control synaptic responses to drugs of abuse like cocaine.

Importantly, group III mGlu receptors have been shown to be important in drug self-administration in preclinical studies in rodents. For instance, Blednov and Harris demonstrated that mGlu₄ knockout mice showed normal levels of ethanol consumption but are devoid of a locomotor stimulant effect of low doses of alcohol (Blednov and Harris, 2008). Furthermore, administration of the mGlu₄ agonist (S)-3,4-DCPG suppressed alcohol self-administration and cue-induced reinstatement of alcohol-seeking behavior (Bäckström and Hyytiä, 2005). The mGlu₇ subtype has also been established to be critical to drug self-administration and reinstatement of drug-seeking. The development of group III mGlu selective ligands and allosteric modulators has allowed for rigorous characterization of their roles in various SUDs (Table 6). Stimulation of presynaptic mGlu₇ receptors with AMN082 significantly reduced cocaine self-administration under a fixed ratio 2 (FR2) schedule of reinforcement and lowered PR breakpoints for cocaine self-administration in rats (Li et al., 2009, 2013). These effects were replicable when AMN082 was directly infused the NAc or ventral pallidum. Additionally, systemic administration of AMN082 has been shown to attenuate cocaine-primed reinstatement of cocaine-seeking behavior (Li et al., 2010). Consistent with the literature on cocaine self-administration, AMN082 administration has also been shown to significantly inhibit heroin and ethanol self-administration and preference in rodents (Salling et al., 2008; Bahi et al., 2012). Together, these findings provide evidence that the mGlu₇ receptor is a promising target for the treatment of SUD.

### Table 6

| Group | Receptor | Type  | Compound | Drug of Abuse | Behavioral Effect | References          |
|-------|----------|-------|----------|---------------|--------------------|---------------------|
| II    | mGlu₄    | PAM   | BINA     | Cocaine       | Decreased cocaine self-administration in both short-access (1 h) and long-access (6 h) in rats; decreased cue-induced reinstatement of cocaine seeking without altering food seeking | Jin et al., 2010 |
| III   | mGlu₇    | Ago-PAM | AMN082  | Cocaine       | Reduced self-administration under an FR2 schedule of reinforcement; lowered PR breakpoints in rats | Li et al., 2009, 2013 |
|       |          |       |          | Alcohol and heroin | Inhibited self-administration and preference | Salling et al., 2008; Bahi et al., 2012 |

FR, fixed ratio; PR, progressive ratio
C. Stress-Related Disorders

Stress-related disorders, including anxiety, are incredibly pervasive psychiatric conditions and represent an enormous worldwide health concern. Chronic psychosocial stressors have been implicated as some of the most common risk factors for the development of stress-related disorders, which also have high comorbidity with other neuropsychiatric diseases, including depression and SUDs (Risch et al., 2009; Duric et al., 2016). However, our understanding of the mechanisms driving the development and persistence of stress-related disorders is still unclear. Stress-related disorders have been associated with aberrant brain excitability within critical neural circuits and disruption of excitatory and inhibitory transmission has been increasingly implicated as a crucial determinant of the pathophysiology of these diseases (Nuss, 2015; Jie et al., 2018). Mood and stress-related disorders involve both bottom-up and top-down control, primarily by limbic regions of the brain. As such, exposure to various stressors is known to dysregulate transmission of both glutamatergic and GABAergic systems (Nemeroff, 2003; Popoli et al., 2011; Jie et al., 2018). For example, clinical studies using proton magnetic resonance spectroscopy to measure endogenous brain metabolites, such as glutamate, in the brain have demonstrated a positive correlation between frontal cortex glutamate levels and state anxiety levels in healthy subjects (Grachev and Apkarian, 2000). Additionally, patients with social anxiety show higher glutamate levels in brain regions, such as the anterior cingulate cortex, compared with healthy control subjects who positively correlated with the severity of their social anxiety symptoms (Phan et al., 2005). These findings, among many others, suggest that restoring the balance between glutamatergic and GABAergic transmission represents a promising therapeutic strategy for alleviating symptoms of stress-related disorders.

A wide variety of pharmacotherapeutics targeting glutamate and/or GABA systems have been under ongoing investigation for their efficacy in treating stress-related disorders, including ketamine, memantine, gabapentinoids, tiagabine, valproic acid, and...
topiramate (Nasir et al., 2020). For example, the NMDA antagonist ketamine, which first showed efficacy in treating symptoms of depression in 2000 (Berman et al., 2000), has shown some efficacy in the treatment of post-traumatic stress disorder and anxiety disorders (Feder et al., 2014; Liriano et al., 2019; Banov et al., 2020). In addition, benzodiazepines, which act on GABAA receptors, have historically been used for the treatment of anxiety and other stress-related disorders. However, their utility has been limited by adverse side effects and high abuse liability (Tan et al., 2011). Therefore, particular interest has been focused on the development of subtype-selective drugs that will achieve specific therapeutic benefits by balancing glutamate and GABA transmission while limiting undesirable side effects. To this end, the mGlu receptors are in prime locations within these brain regions and neural circuits to normalize excitatory/inhibitory transmission, thus modulating stress responses and serving as a promising therapeutic approach for the treatment of stress-related disorders.

I. Group I: Metabotropic Glutamate1/5. Group I mGlu receptors subtypes have been strongly implicated in the pathophysiology of stress-related disorders. For example, clinical studies leveraging positron emission tomography (PET) imaging have demonstrated a strong association between the mGlu5 receptor subtype and anxiety, obsessive compulsive disorder, and depression (Terbeck et al., 2015). Positive correlations have been reported between mGlu5 binding in regions of the amygdala, anterior cingulate cortex, and medial orbitofrontal cortex and obsessive compulsive disorder severity as assessed by the Yale-Brown Obsessive-Compulsive Scale (Akkus et al., 2014). Furthermore, mice exposed to social isolation stress exhibited selectively reduced mGlu1 levels in the PFC (Ieraci et al., 2016). Prenatal stress models have also shown robust changes in mRNA and protein levels as well as gene methylation levels of mGlu1 and mGlu5 receptor subtypes expressed in the hippocampus of offspring rats that exhibit depression-like behavior (Lin et al., 2018). Several studies leveraging genetic deletion or pharmacological manipulation of group I mGlu receptors subtypes have further substantiated the notion that these receptors may be viable targets for treating stress-related disorders (Li et al., 2006; Shin et al., 2015; Zangrandi et al., 2021). For instance, mGlu5 knockout mice or mice that received the mGlu5 NAM, MTEP, displayed detrimental stress coping mechanisms (Li et al., 2006; Shin et al., 2015; Zangrandi et al., 2021). A recent study showed that mice with conditional knockout of mGlu5 in dopamine receptor D1 neurons demonstrated divergent coping mechanisms in response to acute escapable or inescapable stress compared with littermate controls, such that mGlu5 conditional knockout mice showed enhanced active stress coping upon exposure to escapable stress task and higher levels of passive strategy in response to inescapable stress (Zangrandi et al., 2021). Numerous studies also implicate the mGlu1 receptor subtype in the mechanisms underlying stress and anxiety. One study demonstrated that administration of the mGlu1 antagonist, JNJ16259685, produced an anxiolytic phenotype in mice (Steckler et al., 2005). These findings

| Receptor | Type | Compound | Behavioral Effect | References |
|----------|------|----------|-------------------|------------|
| mGlu4    | PAM  | PHCCC    | Antidepressant-like effects in rats; intra-BLA administration elicits anticonflict effects in rats subjected to the Vogel conflict test | (See footnotes.) |
|          |      | ADX88178 | Reduced duration of immobility in the FST; attenuated conditioned freezing in the acquisition phase of the fear conditioning | Kalinichev et al., 2014 |
|          |      | Lu AF21934 | Anxiolytic, but not antidepressant-like, effects as measured by SH, four-plate, marble-burying, and Vogel’s conflict tests | Ślawińska et al., 2013b |
| mGlu7    | NAM  | ADX71743 | Dose-dependent reduction in the number of buried marbles and increasing open arm exploration in EPM and marble-burying assays | Kalinichev et al., 2013 |

Kłak et al., 2007.
Stachowicz et al., 2004.
BLA, basolateral amygdala
provide strong support for the potential utility of group I mGlue receptor allosteric modulators for the treatment of stress-related disorders.

The development of mGlue1 and mGlue5 allosteric modulators have greatly advanced our understanding of the physiologic and behavioral role of these receptor subtypes in stress and anxiety disorders. For example, intrahippocampal injection of MTEP impairs fear extinction by blocking hippocampal metaplasticity mechanisms that lead to enhanced LTP (Stansley et al., 2018), suggesting a potential utility of mGlue5 PAMs for the treatment of stress-related disorders. As such, preclinical work has demonstrated efficacy of mGlue5 PAM enhancement of fear extinction (Sethna and Wang, 2014). Additionally, MTEP displays antidepressant-like activity in the tail suspension test and FST (Palucha et al., 2005, 2007; Belozertseva et al., 2007). Furthermore, the mGlue5 receptor NAM, DSR-98776, was shown to produce rapid antidepressant-like actions in rats (Kato et al., 2015). Leveraging partial mGlue1 receptor NAMs, studies have also demonstrated antidepressant- and anxiolytic-like effects without inducing sedation (Gould et al., 2016; Nickols et al., 2016). Together, these studies support the possibility that mGlue5 receptor NAMs may provide fast-acting antidepressant activity. Based on extensive preclinical evidence and clinical imaging studies, mGlue5 receptor NAMs were tested in clinical trials for their efficacy to treat depressive symptoms. However, two mGlue5 receptor NAMs, AZD2066 and Basimglurant (RG7090, RO4917523) have been tested in Phase II clinical trials and failed to show any efficacy over the placebo control (ClinicalTrials.gov Identifier: NCT01145755) (Quiroz et al., 2016). Nonetheless, higher doses significantly improved secondary outcomes (Quiroz et al., 2016). In combination with an improved tolerability profile, these findings warrant need for further investigation of mGlue5 receptor NAMs for the treatment of depressive disorders. In contrast to the wealth of literature supporting the utility of mGlue5 receptor allosteric modulators for the treatment of stress-related disorders, little is known about the utility of mGlue1 receptor subtype-targeting allosteric modulators for mitigating stress and anxiety phenotypes. However, several studies using mGlue1 receptor antagonists provide a strong foundation for future studies characterizing mGlue1 allosteric modulators in stress-related disorder. For example, mGlue1 receptor antagonists LY456236, 1-Aminoindan-1,5-dicarboxylic acid (AIDA), and JNJ-16259685 elicit anxiolytic-like effects in rodents (Tatarczyńska et al., 2001; Klodzińska et al., 2004; Varty et al., 2005; Lima et al., 2008; Lavreyesen et al., 2015). In addition to reported anxiolytic-like effects, one study showed antidepressant potential of mGlue1 receptor antagonists such that administration of JNJ-16567083 decreased immobility time in the tail suspension test (Belozertseva et al., 2007). However, further studies are needed to evaluate the potential anxiolytic activity of mGlue1 receptor allosteric modulators in the context of stress physiology.

II. Group II: Metabotropic Glutamate_{2/3} Receptors. Substantial preclinical and clinical evidence supports the role of group II mGlue receptor subtypes in the etiology and maintenance of stress-related disorders (Dogra and Conn, 2021). For example, expression changes in mGlue2 and mGlue3 receptor subtypes are observed in numerous models of anxiety and depression. Elevation in group II mGlue receptor expression in the hippocampus and PFC has been observed in the mice reared under isolated conditions (Kawasaki et al., 2011). Further, increased levels of mGlue2/3 receptors have been observed in the postmortem PFC tissue from patients with depression (Feyissa et al., 2010), providing evidence that increased mGlue2/3 receptor function may contribute to the etiology of depression. To this end, several studies have reported anxiolytic effects with mGlue2/3 agonists (Helton et al., 1998; Shekhar and Keim, 2000; Schoepf et al., 2003; Linden et al., 2005, 2018). A multitude of studies have also leveraged transgenic mouse lines featuring deletion of mGlue2 and/or mGlue3 to parse out the individual contributions of these receptor subtypes in stress-related disorders. However, these studies have yielded mixed results. One study reported that the anxiolytic efficacy of mGlue2/3 agonists is reduced or absent in single GRM2^{-/-} and GRM3^{-/-} mice compared with littermate controls (Linden et al., 2005). Alternatively, several studies have reported that mice lacking either mGlue2 or mGlue3 alone did not display altered anxiety phenotypes (Morishima et al., 2005; Fujioka et al., 2014; De Filippis et al., 2015). Nonetheless, because the anxiolytic efficacy of mGlue2/3 agonists has been observed across a variety of species, including humans and rodents, the lack of an anxiety phenotype in the transgenic mice may be due to species differences or compensatory changes.

As such, selective mGlue2 and mGlue3 receptor allosteric modulators have been investigated for their efficacy in treating symptomology of stress-related disorders (Table 7). mGlue3 receptor NAMs have been shown to elicit antidepressant-like and anxiolytic-like effects as measured by FST and marble-burying tests, respectively (Engers et al., 2015). Further, recent studies show that mGlue2 and mGlue3 receptor NAMs reverse passive coping behavior in the FST (Joffe et al., 2020) and mGlue3 receptor NAMs reverse motivational deficits and changes in the amygdalo-cortical plasticity induced by acute stress (Joffe et al., 2019a). Other studies using mGlue2 receptor PAMs, BINA, N-(4-(2-(trifluoromethyl)-3-hydroxy-4-(iso- butyryl)phenoxy)methyl)benzyl)-1-methyl-1H-imidazole-4-carboxamide, and LY487379, have demonstrated anxiolytic-like efficacy in multiple assays of rodent stress
response and also displayed antidepressive-like efficacy (Galici et al., 2006; Fell et al., 2011; Wieronska et al., 2012b). A recent study also reported that administration of the selective mGlu4 NAM VU0650786 blocked the LY379268-induced trace fear conditioning enhancement in mice (Dogra et al., 2021). Together, these studies provide strong evidence that mGlu4 and mGlu8 allosteric modulators may also be a promising novel treatment strategy for stress-related disorders.

### III. Group III: Metabotropic Glutamate4/6/7/8

Group III mGlu receptor subtypes have garnered attention as potential therapeutic targets for the treatment of stress-related disorders. Initial studies using nonselective group III mGlu receptor agonists have aimed to determine the role of these receptors in stress-related phenotypes. For instance, Tatarczyńska et al. showed that intrahippocampal administration of the group III mGlu receptor agonist ACPT-I elicits anxiolytic-and antidepressant-like effects in mice (Tatarczyńska et al., 2001). Other studies also found anxiolytic-like effects of ACPT-I as measured by stress-induced hypothermia (SIH), elevated plus-maze (EPM) tests in mice, and the Vogel test in rats (Palucha et al., 2004; Stachowicz et al., 2009). However, until recently, a lack of selective compounds has largely limited our current understanding of the specific contribution of each group III mGlu receptor subtype in the pathophysiology of stress-related disorders. The development of transgenic rodent models and selective pharmacological agents has allowed us to gain insights on the role of group III mGlu receptor subtypes in stress-related phenotypes. For example, mGlu7-selective antagonist 7-hydroxy-3-(4-iodophenoxy)-4H-chromen-4-one, which inhibits lateral amygdala LTP, reduces innate anxiety and freezing during acquisition of Pavlovian fear in mice (Gee et al., 2014). Additionally, mGluR7−/− mice displayed anxiolytic efficacy in a battery of behavioral tasks, including the staircase test, EPM, light-dark box, and SIH (Cryan et al., 2003). Interestingly, mGluR7−/− mice also show an increase in glucocorticoid-dependent feedback suppression of the hypothalamic–pituitary–adrenal axis and increases hippocampal brain-derived neurotrophic factor (BDNF) levels compared with wild-type littermate controls (Mitsukawa et al., 2006), further bolstering the hypothesis that mGlu7 receptors are critical in stress physiology. In support of this notion, a wealth of literature reports anxiolytic-and antidepressant-like efficacy of the mGlu7 agonist, AMN082 (Palucha et al., 2007; Palazzo et al., 2008; Palucha-Poniewiera et al., 2010, 2014; Bradley et al., 2012; O’Connor and Cryan, 2013; Palucha-Poniewiera and Pilc, 2013). Alternatively, mice lacking the mGlu4 receptor subtype exhibited increased anxiety in the open-field and EPM test as well as improvements in cued-fear conditioning (Davis et al., 2013). Studies using the orthosteric mGlu4 agonist, DCPG, showed that mGlu4 stimulation reduced anxiety-like behavior in open field and EPM tests while also attenuating the expression of contextual fear (Fendt et al., 2013). Together, these studies have begun to elucidate the role of each receptor subtype in the pathophysiology of stress-related disorders.

Based on this evidence, studies have focused on the potential utility of allosteric modulators of group III mGlu receptor subtypes for the treatment of stress-related disorders (Table 8). Studies have leveraged mGlu4 PAMs and shown anxiolytic-and antidepressant-like activity in rodents. For example, a study by Klak et al. reported that administration of the mGlu4-selective PAM 7-hydroxyiminono-N-phenyl-1,7 adhydrocyclopropa[b]chromene-1a-carboxamide (PHCCC) in combination with a subeffective dose of (1S,3R,4S)-1-aminocyclopentane-1,3,4-tricarboxylic acid produced antidepressant-like effects in rats (Klak et al., 2007). Intra-basolateral amygdala (BLA) administration of PHCCC was reported to elicit anticonflict effects in rats subjected to the Vogel conflict test (Stachowicz et al., 2004). Another mGlu4 PAM, ADX88178, dose-dependently reduced duration of immobility in the forced swim test and attenuated conditioned freezing in the acquisition phase of the fear conditioning test without altering freezing propensity in the expression phase of the task (Kalinichev et al., 2014). Furthermore, Slawinska et al. also showed that administration of the mGlu4 PAM, Lu AF21934, produced anxiolytic- but not antidepressant-like effects as measured by SIH, four-plate, marble-burying, and Vogel’s conflict tests (Slawinska et al., 2013b). In addition to promising evidence of the anxiolytic efficacy of mGlu4 allosteric modulators, another study reported an anxiolytic-like profile of mGlu7 NAMs. Specifically, the mGlu7 NAM, ADX71743, produced a robust anxiolytic-like phenotype as evidenced by dose-dependent reduction in the number of buried marbles and increasing open arm exploration in EPM and marble burying assays, respectively (Kalinichev et al., 2013).

### IV. Future Directions/Concluding Remarks

A wealth of preclinical literature over the past decade supports the potential utility of allosteric modulators of mGlu receptors as promising therapeutic options to treat multiple neuropsychiatric diseases, including schizophrenia, SUDs, and stress-related disorders, which currently have limited or no effective treatments. Thus far, investigation of mGlu receptor allosteric modulators has yielded important insights into the neuropharmacology of these diseases, and surely more discoveries are yet to be discovered. However, many outstanding questions remain that the field is primed to address and that will propel research on allosteric modulators forward. One important outstanding question involves the utility of mGlu allosteric modulators as novel cognitive enhancers. Neuropsychiatric conditions, such as schizophrenia, SUDs, and stress-related disorders, are known to produce marked deficits in cognitive behavior (Gould et al., 2003). Continued investigation of the role of group III mGlu receptor subtypes in stress-related phenotypes may also provide important insights into the development of novel therapeutic strategies.
et al., 2012; Robinson et al., 2013; Tripathi et al., 2018; Lukasik et al., 2019). Extensive literature supports the notion that many cognitive deficits associated with neuropsychiatric diseases (working memory, attention, executive function, etc.), such as schizophrenia and SUD, are driven by aberrant glutamate and GABA signaling and associated detrimental effects in synaptic plasticity (Logue and Gould, 2014; Guidi et al., 2015). Based on the well-established and critical role of mGlu receptor subtypes in regulating glutamate/GABA transmission and synaptic plasticity (see Metabotropic Glutamate Receptor Regulation of Neurotransmission and Synaptic Plasticity), allosteric modulators of mGlu receptors are promising targets to reverse disease-related cognitive deficits, a major unmet need of numerous neuropsychiatric diseases. For example, by leveraging mGlu5-mediated regulation of cortical and hippocampal plasticity, mGlu5 PAMs show excellent potential as cognitive enhancers. Recent work demonstrates that mGlu1 PAMs regulate spatial working memory via regulation of PFC SST-IN function in mice (Maksymetz et al., 2021). Furthermore, mGlu5 PAM VU0092273 enhances trace fear conditioning in wild-type mice but not in mGlu5-CA1-KO mice (Xiang et al., 2019). Further supporting this notion, the biased mGlu5 PAM VU0409551 demonstrated robust cognition enhancement as measured by enhancement of contextual fear conditioning acquisition and an increase in recognition memory in the novel object recognition task in rats, both commonly used rodent learning and memory assays dependent upon hippocampal function (Rook et al., 2015). In the same study, VU0409551 also enhanced working memory performance in rats, such that systemic administration increased accuracy in a delayed non-match-to-sample task.

Several studies have also provided evidence for the cognitive-enhancing abilities of group II mGlu receptor subtype allosteric modulators. For example, mGlu1 PAM SAR 218645 improved learning and memory in rodent models of schizophrenia (Griebel et al., 2016). In line with their ability to enhance hippocampal LTP, selective activation of mGlu1 has been shown to improve cognition in hippocampal dependent temporal associative tasks in rodents (Stansley and Conn, 2019). Interestingly, the functional interplay between mGlu3 and mGlu5 receptor subtypes and the involvement of mGlu3 in cortical plasticity further suggests that mGlu3 PAMs may also exert cognition-enhancing effects. Lastly, group III mGlu receptors may be promising cognition-enhancing approach to mitigate neuropsychiatric-related deficits. This notion is further evidenced by preclinical cognition-enhancement observed with mGlu7 NAMs. Specifically, the mGlu7 NAM, ADX71743, reverses MK-801-induced deficits in novel object recognition and delayed spatial alternation in mice (Cieslik et al., 2018). Ongoing studies are required to evaluate the procognitive utility of mGlu receptor allosteric modulators for other cognitive processes and neuropsychiatric disease models.

Lastly, questions remain about pharmacological refinement of next-generation allosteric modulators of mGlu receptor subtypes. Allosteric-related factors, such as heterodimer engagement and signal bias, are essential to the translation of these compounds into the clinical setting. Expanding our understanding of mGlu7 receptor signal biases and interaction with heterodimer complexes, for example, will allow us to optimize treatments to restore normal physiology within the circuits underlying specific disease states. Several compounds targeting mGlu receptor subtypes display biased allosteric modulation or “functional selectivity” (Zhang et al., 2005; Sheffler and Conn, 2008; Rook et al., 2015). For example, the gadinolinum ion, an allosteric modulator of the mGlu1z receptor subtype, inhibits Gzα11-linked Ca2+ mobilization but also stimulates Gzα2-mediated cAMP production when administered with glutamate (Tateyama and Kubo, 2006). Recent reports have also shown that signal bias can have crucial implications for therapeutic efficacy, as evidenced by β-arrestin-biased mGlu5 NAMs in models of Fragile X (Stoppel et al., 2017) or mGlu5 PAMs biased away from NMDAR modulation for schizophrenia (Rook et al., 2015; Gould et al., 2016). Moving forward, efforts will determine how to translate these pharmacological properties to emerging drug candidates through different systems and species to avoid context-dependent pharmacology that has the potential to hinder efficacy. Accounting for these pharmacological factors, among others, throughout model systems will likely increase the competitiveness and effectiveness of future drug candidates for the treatment of neuropsychiatric disease.

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