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# Allosteric modulation of GPCRs: new insights and potential utility for treatment of schizophrenia and other CNS disorders

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## Abstract

G-protein coupled receptors (GPCRs) play critical roles in regulating brain function. Recent advances have greatly expanded our understanding of these receptors as complex signaling machines that can adopt numerous conformations and modulate multiple downstream signaling pathways. While agonists and antagonists have traditionally been pursued to target GPCRs, allosteric modulators provide several mechanistic advantages including the ability to distinguish between closely related receptor subtypes. Recently, the discovery of allosteric ligands that confer bias and modulate some, but not all, of a given receptor's downstream signaling pathways can provide pharmacological modulation of brain circuitry with remarkable precision. In addition, allosteric modulators with unprecedented specificity have been developed that can differentiate between subpopulations of a given receptor subtype based on the receptor's dimerization state. These advances are not only providing insight into the biological roles of specific receptor populations, but hold great promise for treating numerous CNS disorders.

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All major functions of the central nervous system require rapid and precise communication between neurons that are organized in circuits that range from simple di-synaptic feedback loops to large networks of interconnected brain nuclei that regulate complex CNS functions. These networks are driven by activation of excitatory and inhibitory ligand-gated ion channels and can be modulated by G protein-coupled receptors (GPCRs; also known as 7 transmembrane spanning receptors). GPCRs represent a large family of receptors that regulate multiple intracellular signaling pathways either through the activation of various G-proteins, or through interactions with β-arrestin and other regulatory proteins (Kristiansen, 2004). GPCRs are critical for fine-tuning transmission through CNS networks and modulate several key neuronal functions such as neurotransmitter release, neuronal excitability, action potential firing patterns, and gene transcription. Given their central role in regulating CNS function, it is not surprising that GPCRs have been among the most successful targets for developing drugs for treatment of CNS disorders (Wise et al., 2002). However, ligands that

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selectively activate a single receptor subtype exist for only a small fraction of GPCRs and it has been difficult to develop highly selective ligands for most GPCR subtypes. Historically, efforts to develop ligands that target GPCRs have focused on agonists and antagonists that interact with the orthosteric neurotransmitter binding site to either mimic or block the action of the endogenous neurotransmitter. While this strategy has been fruitful, the high conservation of orthosteric binding sites across related receptors can make development of subtype-selective orthosteric ligands challenging.

In recent years, allosteric modulators of GPCRs have emerged as a promising new approach for developing highly selective ligands and potential therapeutic agents for treatment of CNS disorders. Allosteric modulators of GPCRs bind to sites that are often less highly conserved than orthosteric sites (Conn et al., 2009a) and this has allowed optimization of highly selective allosteric modulators of some GPCR subtypes that have been intractable using traditional approaches. Allosteric modulators bind to sites that are topographically distinct from the orthosteric neurotransmitter binding site and can alter receptor signaling (See Table 1). Positive allosteric modulators (PAMs) increase responses to the orthosteric agonist, whereas negative allosteric modulators (NAMs) inhibit responses to orthosteric agonists. PAMs and NAMs often act by modulating the affinity of an orthosteric ligand. Thus, a GPCR PAM can increase the affinity of the endogenous neurotransmitter whereas a NAM may reduce the affinity. However, allosteric modulators can also modulate GPCR coupling to downstream signaling mechanisms independent of the affinity of the orthosteric agonist (Wootten et al., 2013). Major advances have improved our understanding of how allosteric modulators interact with receptors, driven by crystal structures of receptors bound to allosteric modulators (Dore et al., 2014; Kruse et al., 2013; Oswald et al., 2016; Thal et al., 2016; Wu et al., 2014). These studies not only provide detailed information on the molecular interactions between allosteric modulators and their binding sites, but also provide invaluable information on how conformational changes at allosteric sites mechanistically alter orthosteric signaling (Staus et al., 2016). The effects of allosteric ligands on receptor function have been outlined in detail in multiple excellent reviews and can be described and quantified by equations including an “operational model of allosterism” that describes both allosteric modulation of affinity and efficacy (Gregory et al., 2010; May et al., 2007) that has been highly useful in quantifying different aspects of allosteric modulator function.

The unique characteristics of allosteric modulators make this mode of GPCR regulation extremely attractive for developing agents with which to interrogate the physiological roles of individual GPCR subtypes and for developing novel treatment strategies for CNS disorders. Allosteric modulators of GPCRs are now being pursued as potential drug candidates for Alzheimer's disease, dystonia, Parkinson's disease, schizophrenia, and other brain disorders (for reviews see Conn et al., 2009a; Kruse et al., 2014). In addition to providing potential new treatment strategies, these compounds are helping drive fundamental insights into the roles of various receptors and specific signaling pathways in modulating identified brain circuits and animal behavior under both physiological and pathological conditions.

## Potential antipsychotic and cognition-enhancing effects of $mGlu_5$ receptor PAMs

In recent years, the metabotropic glutamate (mGlu) receptor subtype 5 ($mGlu_5$) has emerged as an exciting new target for the treatment of schizophrenia and improving cognitive function in multiple brain disorders (Nickols and Conn, 2014; Nicoletti et al., 2015). Multiple $mGlu_5$ PAMs have robust efficacy in rodent models used to predict antipsychotic efficacy and the treatment of cognitive disturbances (Conn et al., 2009b; Liu et al., 2008; Parmentier-Batteur et al., 2014; Rook et al., 2013). Interestingly, $mGlu_5$ PAMs enhance induction of both long-term potentiation (LTP) and long-term depression (LTD) at excitatory synapses in the hippocampus and other brain regions (Ayala et al., 2009; Rook et al., 2015; Sarihi et al., 2008; Sun et al., 2016) and can restore deficits in animal models in which synaptic plasticity and cognitive function are impaired (Bhardwaj et al., 2015; Lin et al., 2014; Waung and Huber, 2009; Won et al., 2012).

In addition to potential symptomatic effects, early studies raise the possibility that $mGlu_5$ PAMs could reduce developmental changes that underlie specific deficits in schizophrenia. Deletion of $mGlu_5$ from parvalbumin expressing neurons is sufficient to induce cognitive and sensorimotor gating deficits in rodents (Barnes et al., 2015), and administration of $mGlu_5$ PAMs in adolescence prevented the appearance of delayed cognitive deficits in a developmental model of schizophrenia (Clifton et al., 2013). These findings suggest the exciting possibility of a preventative role for $mGlu_5$ PAM treatment in the development of schizophrenia, and further work will be important to evaluate these findings.

### Maintenance of activity-dependence may be important for efficacy of $mGlu_5$ PAMs

An important attribute of pure PAMs is that these compounds have no intrinsic efficacy, but act to enhance activation of the receptor by the endogenous agonist, a property that fundamentally differentiates PAMs from traditional agonists. Recent studies suggest that the ability of $mGlu_5$ PAMs to maintain the spatial and temporal patterning of receptor activation (Figure 1), provides these compounds with the unique ability to enhance both LTP and LTD without altering the afferent activity patterns required for induction of these two forms of synaptic plasticity (Ayala et al., 2009). The strict dependence of LTP and LTD on defined patterns of afferent activity is important for appropriately regulating different domains of cognitive function (see Mockett and Hulme, 2008 for review). The unique ability of $mGlu_5$ PAMs to maintain appropriate activity-dependent plasticity provides an excellent demonstration of the advantages afforded by strictly potentiating responses to endogenous neurotransmitter release compared to use of an agonist. Consistent with the effects of $mGlu_5$ PAMs on synaptic plasticity, systemic administration of selective $mGlu_5$ PAMs improves performance in a broad range of animal models of cognitive function that are dependent on intact function of the hippocampus or prefrontal cortex (PFC) and are disrupted in schizophrenia patients (Bhardwaj et al., 2015; Gilmour et al., 2013; Homayoun et al., 2004; Horio et al., 2013; Moghaddam, 2004; Uslaner et al., 2009). Thus, $mGlu_5$ PAMs have the potential to improve both positive symptoms and cognitive disturbances in schizophrenia patients.

## Minimizing Ago-PAM activity reduces the side-effect liability of $mGlu_5$ PAMs

The combined effects of $mGlu_5$ PAMs in preclinical models raise the possibility that these compounds could provide a fundamental advance in treatment of schizophrenia and other disorders that impair cognitive function. However, some $mGlu_5$ PAMs induce severe seizure activity (Bridges et al., 2013; Rook et al., 2013) and excitotoxicity leading to cell death in the forebrain (Parmentier-Batteur et al., 2014; Rook et al., 2015). Interestingly, recent studies suggest that a major factor contributing to the adverse effect liability of some $mGlu_5$ PAMs is the ability of some PAMs to also directly activate the receptor. While prototypical or pure PAMs do not alter receptor activity on their own, some allosteric modulators do possess intrinsic efficacy (Conn et al., 2009a). Allosteric modulators that possess both intrinsic efficacy and potentiate responses to orthosteric agonists are referred to as Ago-PAMs (Table 1). While both pure PAMs and Ago-PAMs can induce a shift in the concentration curve of an orthosteric agonist, only an Ago-PAM will induce receptor activation in the absence of orthosteric agonist. These differences in mechanism of action can profoundly impact both the side-effect profiles and therapeutic potential of a given compound.

Interestingly, systematic comparison of structurally related $mGlu_5$ PAMs that possess Ago-PAM activity, relative to pure PAMs that do not possess agonist activity, revealed that $mGlu_5$ Ago-PAMs induce seizures and behavioral convulsions, whereas closely related pure PAMs do not (Rook et al., 2013). Furthermore, appropriate activity-dependence of LTP and LTD was maintained with pure $mGlu_5$ PAMs but not with Ago-PAMs (Rook et al., 2013). Based on the striking impact of Ago-PAM activity, it is critical to optimize $mGlu_5$ compounds for clinical development that strictly avoid Ago-PAM activity. Based on the propensity of over-activation of $mGlu_5$ to induce seizures and excitotoxicity, recent efforts have focused on developing $mGlu_5$ PAMs that have the minimal positive cooperativity with glutamate required for achieving efficacy (Parmentier-Batteur et al., 2014; Rook et al., 2013). However, avoiding high cooperativity and Ago-PAM activity is not likely to be a universal guideline for all allosteric modulators and should be evaluated independently for each target as well as disease state. For example, in disease states where endogenous neurotransmitter levels are sufficiently attenuated, a PAM may lack sufficient efficacy and optimizing Ago-PAMs may be preferred.

## Novel $mGlu_5$ PAMs that induce stimulus bias reduce adverse effect liability

In recent years it has become increasingly clear that agonists can stabilize multiple active states of GPCRs that can engage different signaling pathways (Digby et al., 2010; Kenakin and Christopoulos, 2013). In the simplest case, an allosteric modulator would induce similar inhibition or amplification of all signaling pathways that are activated by an agonist. In this case, the allosteric modulator would not induce a qualitative change in receptor signaling but would potentiate or inhibit all responses that are normally induced by activation of the receptor. However, some allosteric modulators can selectively modulate the ability of agonists to stabilize specific active conformations of the receptor and thereby introduce a “stimulus bias” that differentially alters the effects of the endogenous agonist on specific signaling pathways (Figure 2). Recent studies provide exciting new insights into the

potential for optimizing $mGlu_5$ PAMs that display stimulus bias for achieving robust efficacy in the absence of adverse effect liability.

While the presence of Ago-PAM activity at $mGlu_5$ is known to lead to severe adverse effects, avoiding Ago-PAM activity does not completely eliminate adverse effect liability of these compounds. For instance, an $mGlu_5$ PAM termed 5PAM-523 lacks agonist activity but can induce seizure activity and excitotoxicity after chronic administration (Parmentier-Batteur et al., 2014). While the adverse effects are less pronounced than those seen with $mGlu_5$ Ago-PAMs, this finding suggests that other aspects of $mGlu_5$ signaling can contribute to the adverse effect liabilities. Interestingly, $mGlu_5$ is a close signaling partner with the N-Methyl-D-aspartate subtype of glutamate receptor (NMDAR; Attucci et al., 2001; Awad et al., 2000; Doherty et al., 2000; Ehlers, 1999; Mannaioni et al., 2001; Marino and Conn, 2002; Ugolini et al., 1999) and the ability of $mGlu_5$ to potentiate NMDAR currents in forebrain regions has been commonly viewed as a key mechanism by which $mGlu_5$ PAMs exert their efficacy in rodent models (Darrah et al., 2008; Kinney et al., 2005; Niswender and Conn, 2010; Stefani and Moghaddam, 2010; Won et al., 2012). However, it is well established that excessive activity of NMDARs can induce seizures and excitotoxicity raising the possibility that potentiation of NMDAR signaling could contribute to the adverse effect liability of some $mGlu_5$ PAMs. To directly evaluate the importance of potentiation of NMDAR currents for the observed *in vivo* efficacy of $mGlu_5$ PAMs, a novel $mGlu_5$ PAM (VU0409551) was developed that displays stimulus bias and potentiates $mGlu_5$ coupling to $G_{\alpha q}$ and related signaling pathways but does not enhance $mGlu_5$ modulation of NMDAR currents (Rook et al., 2015). Interestingly, VU0409551 produces robust antipsychotic-like and cognition-enhancing effects in rodent models (Balu et al., 2016; Conde-Ceide et al., 2015; Rook et al., 2015), suggesting that the efficacy of $mGlu_5$ PAMs in these models does not require potentiation of NMDAR currents. Furthermore, chronic administration of VU0409551 had no adverse effects at doses over 100× those required to achieve *in vivo* efficacy (Rook et al., 2015). These studies raise the exciting possibility that it will be possible to develop $mGlu_5$ PAMs that have robust efficacy but are devoid of adverse effects that can be associated with modulation of NMDAR currents. However, these findings also raise the question of what signaling pathways and physiological responses are critical for specific *in vivo* actions of $mGlu_5$ PAMs. As new tools are further developed that selectively modulate specific signaling pathways by $mGlu_5$, they will provide opportunities to develop a full understanding of the specific signaling pathways that are critical for mediating the efficacy of $mGlu_5$ PAMs in preclinical models of numerous CNS disorders including schizophrenia, fragile X syndrome, Rett syndrome, autistic spectrum disorders, obsessive compulsive disorder, Parkinson's disease, substance abuse (Ade et al., 2016; Gass et al., 2016; Gogliotti et al., 2016; Gould et al., 2016; Michalon et al., 2012; Rylander et al., 2010; Vicidomini et al., 2016), and others.

In addition to discovery of biased $mGlu_5$ PAMs, examples are beginning to emerge in which allosteric modulators induce biased signaling for multiple other GPCR subtypes. These include selective PAMs for multiple mGlu receptor subtypes (Noetzel et al., 2013; Rook et al., 2015; Sheffler and Conn, 2008; Zhang et al., 2005), muscarinic acetylcholine receptors (mAChRs; Leach et al., 2010; Leach et al., 2007; Marlo et al., 2009), dopamine receptors (Free et al., 2014), and cannabinoid receptors (Ahn et al., 2012). In addition, biased or

functionally selective NAMs have been developed for $mGlu_7$ (Niswender et al., 2010), prostaglandin $D_2$ CHTH2 receptors (Mathiesen et al., 2005), and neurokinin NK2 receptors (Maillet et al., 2007). While the ability of orthosteric agonists to induce biased GPCR signaling is well established (Digby et al., 2010; Furness et al., 2016), the unique potential of GPCR NAMs to selectively inhibit specific signaling pathways is not shared by orthosteric antagonists and provides an exciting opportunity to develop biased NAMs that target pathways that are most critical for achieving a therapeutic effect. As these new tools continue to emerge, this will provide unprecedented opportunities to develop a more complete understanding of the specific signaling pathways responsible for modulation of different physiological responses in identified neuronal populations and brain circuits.

## Potential utility of $mGlu_1$ PAMs for treatment of schizophrenia

In addition to $mGlu_5$, the closely related $mGlu_1$ receptor may also be a potential therapeutic target for treating schizophrenia. Genetic studies have identified multiple mutations in the $mGlu_1$ gene (*Grm1*) in schizophrenic patients (Ayoub et al., 2012). Interestingly, a recent study revealed that each of the mutations associated with schizophrenia leads to a loss of $mGlu_1$ signaling and that highly selective $mGlu_1$ PAMs can potentiate signaling through these mutant receptors (Cho et al., 2014). At present, the functional impact of these mutations in circuits that may be relevant for the pathophysiology of schizophrenia are not understood. However, $mGlu_1$ regulates many of the same circuits that are relevant for potential $mGlu_5$– mediated antipsychotic efficacy, including modulation of NMDAR signaling (Benquet et al., 2002; Heidinger et al., 2002) and hippocampal plasticity (Aiba et al., 1994). In addition, $mGlu_1$ knock out mice show disrupted prepulse inhibition similar to that seem with $mGlu_5$ knock out mice (Brody et al., 2003; Brody et al., 2004). Future studies with newly developed $mGlu_1$ tools (Cho et al., 2014; Lovell et al., 2013) will provide critical insights into the biological roles and therapeutic potential of $mGlu_1$ in treating schizophrenia, and other disorders in which $mGlu_1$ has been implicated, including ataxia, substance abuse, and autism spectrum disorders (Bariselli et al., 2016; Lum et al., 2014; Power et al., 2016).

## Selective $mGlu_2$ and $mGlu_3$ PAMs for treatment of schizophrenia

Over the past two decades there have been intensive efforts targeting Group II mGlu receptors ($mGlu_2$ and $mGlu_3$) for the treatment of schizophrenia. Orthosteric agonists that activate both $mGlu_2$ and $mGlu_3$ have robust antipsychotic-like effects in preclinical models (Muguruza et al., 2016; Niswender and Conn, 2010). Unfortunately, while group II mGlu receptor agonists showed significant improvements in both positive and negative symptoms in an initial phase II trial (Patil et al., 2007), subsequent larger clinical studies failed to demonstrate significant efficacy of these agents compared to placebo (Kinon et al., 2011). However, preclinical studies have demonstrated that the antipsychotic-like activity of group II mGlu receptor agonists are absent in $mGlu_2$, but not $mGlu_3$, receptor knock out mice, suggesting that $mGlu_2$ activation may be sufficient to provide therapeutic benefit (Spooren et al., 2000). The discovery of $mGlu_2$-selective PAMs allowed direct testing of this hypothesis and several of these compounds demonstrated antipsychotic-like efficacy and pro-cognitive effects in multiple preclinical models (Galici et al., 2005; Galici et al., 2006; Griebel et al.,

2016). However, a recent report revealed that the $mGlu_2$ PAM AZD8529 had no significant effects on positive or negative symptoms in schizophrenic patients when administered as a monotherapy (Litman et al., 2016). Another $mGlu_2$ PAM, JNJ-40411813/ADX71149, had promising beneficial effects in patients with residual negative symptoms (Hopkins, 2013) and showed potential efficacy in improving some aspects of cognition and reducing negative symptoms after administration of ketamine in healthy volunteers (Salih et al., 2015). However, it is not yet known whether this compound will show efficacy in larger trials in schizophrenia patients. As discussed below several factors including disease etiology, disease progression, and prior medication history may be critical determinants of what compounds are most likely to provide therapeutic benefit.

## mGlu2 PAMs may be effective in select patient subpopulations

While the underpinnings of schizophrenia are diverse, great strides have been made in identifying genetic and environmental risk factors as well as understanding the clinical and physiological correlates associated with progression of the disease (Millan et al., 2016). In addition to disease progression, it is important to consider the potential impact of prior medication when assessing a given therapy. Interestingly, some recent studies suggest that group II mGlu agonists, or $mGlu_2$ PAMs, may have a higher likelihood of providing significant efficacy in patients in which treatment is initiated soon after diagnosis and prior to long-term exposure to atypical antipsychotics (Kinon et al., 2015), which may repress activity of the $mGlu_2$ gene promoter (Kurita et al., 2012). Thus, it is possible that stratification of patients will help in identifying patients that would be most responsive to $mGlu_2$ PAMs. In addition, recent advances in stratifying patients using biomarkers, such as PET or functional imaging, could aid in identifying patients that could be most responsive to $mGlu_2$ PAMs or agonists. Such approaches have been effective in correlating treatment responses of atypical antipsychotics with $D_2$ receptor occupancy (Kapur et al., 2000), and similar strategies including genetic and functional screening have been applied with great success with regards to cancer treatment (Vargas and Harris, 2016). While future work is needed to determine the utility of similar strategies in treating schizophrenia and other CNS disorders it is possible that stratification of patient populations could help identify what patients are most likely to respond to a given therapy.

## Potential role of heterodimers in mediating $mGlu_2$-mediated antipsychotic efficacy

In addition to the potential importance of segregating patient populations, it is also important to consider the possibility that $mGlu_2$ heterodimers could impact the *in vivo* effects of $mGlu_2$ PAMs. Recent studies suggest that $mGlu_2$ forms functional heterodimers with $mGlu_4$ that are expressed in the CNS and that some PAMs can selectively activate homomeric relative to heteromeric forms of the receptor (Figure 3; Kammermeier, 2012; Niswender et al., 2016; Yin et al., 2014). Interestingly some $mGlu_4$ PAMs have antipsychotic-like effects in rodent models (Kalinichev et al., 2014; Slawinska et al., 2013), including Lu AF21934, which acts as a robust PAM of $mGlu_{2/4}$ heterodimers (Niswender et al., 2016; Yin et al., 2014), raising the possibility that $mGlu_{2/4}$ heterodimers could play a key role in mediating the antipsychotic efficacy seen with $mGlu_4$ and $mGlu_2$ PAMs. Furthermore, $mGlu_2$ and $mGlu_3$ readily form $mGlu_2/mGlu_3$ heterodimers in cell lines (Levitz et al., 2016) and these receptors are highly co-expressed in the PFC and other brain regions (Petralia et al., 1996).

Interestingly, while neither $mGlu_2$ nor $mGlu_3$ knock out mice show overt behavioral deficits, dual $mGlu_2/_3$ knock out mice display blunted responses to amphetamine and cognitive deficits (Lyon et al., 2011), raising the possibility that $mGlu_{2/2}$, $mGlu_{3/3}$, and $mGlu_{2/3}$ complexes could all play roles in regulating schizophrenia-associated circuitry. Finally, recent studies revealed that both $mGlu_2$-mediated signaling in the cortex (Moreno et al., 2016) and the antipsychotic-like effects of Group II mGlu receptor agonists (Fribourg et al., 2011) are absent in $5HT_{2A}$ knock-out mice, suggesting an important role for cross-talk between these receptors in mediating antipsychotic effects. This cross talk has been postulated to be mediated by formation of $mGlu_2/5HT_{2A}$ heterodimers and to be critical for the antipsychotic-like effects of $mGlu_2$ and $5HT_{2A}$ ligands. Thus, in future studies, it will be important to consider the possibility that regulating signaling through certain heterodimers of $mGlu_2$ could be crucially important in determining antipsychotic-like or cognition-enhancing effects of these compounds. With the exception of PAMs that are known to be active at $mGlu_{2/4}$ heterodimers, there are no studies detailing if specific pharmacological compounds can distinguish between different Group II mGlu receptor complexes. Furthering our understanding regarding the importance of these various group II receptor complexes to regulating signaling in both physiological and pathological contexts could provide important insights that could translate into improved therapeutics targeting these receptors.

The discovery of allosteric modulators that differentiate between $mGlu_{2/4}$ relative to $mGlu_4$ and $mGlu_2$ raises the exciting potential of developing allosteric modulators for homomeric versus heteromeric forms of other GPCR subtypes. GPCRs belonging to each major subclass form functional homomeric as well as heteromeric complexes (Oldham and Hamm, 2008; Smith and Milligan, 2010). However, the majority of studies of GPCR heterodimers have been performed in cell lines and the extent to which many GPCRs function as heterodimers in native systems is not yet clear (Milligan, 2013). Discovery of additional selective modulators could provide critical information on the molecular determinants of signaling through homo- vs heterodimer complexes and pave the way for understanding the unique roles of specific heteromeric GPCR complexes in regulating CNS function. However, at present, it is still unclear how allosteric modulators differentiate between these complexes. The $mGlu_{2/4}$ allosteric modulators outlined above appear to act at a conserved binding site on a single protomer ($mGlu_2$ or $mGlu_4$) of the $mGlu_{2/4}$ complex (Niswender et al., 2016). Future studies will be needed to fully understand why some modulators that bind at a single protomer modulate $mGlu_{2/4}$ signaling, whereas others do not. In addition, other approaches using bivalent compounds to target heterodimer complexes have shown promise (Hubner et al., 2016), and it is possible that allosteric modulators could be developed that bind at the interface between the two subunits. Future work will be needed to determine the structural and molecular mechanisms through which allosteric modulation of heterodimer complexes occurs. However, the approach of developing compounds that not only target a given receptor subtype but specifically target a given heterodimeric complex (Figure 3), has the potential to allow incredibly precise pharmacological modulation of neuronal communication.

### Selective $mGlu_3$ PAMs for improving PFC-dependent cognitive function

In addition to potential antipsychotic effects of $mGlu_2$-selective PAMs, recent studies also point to the potential utility of $mGlu_3$ PAMs in improving cognitive function in schizophrenia and other brain disorders. Several studies have identified single nucleotide polymorphisms (SNPs) in the human gene encoding $mGlu_3$ (*GRM3*) that are associated with poor performance on cognitive tests that are dependent on function of the prefrontal cortex (Egan et al., 2004; Harrison et al., 2008; Tan et al., 2007), and *GRM3* has been identified as a risk locus for schizophrenia in genome wide association studies (Schizophrenia Working Group of the Psychiatric Genomics, 2014). The recent discovery of selective $mGlu_3$ NAMs enabled studies in mice that revealed that $mGlu_3$, but not $mGlu_2$, mediates clear postsynaptic effects in the PFC and could regulate synaptic plasticity as well as mediate pro-cognitive effects (Walker et al., 2015). This is consistent with recent studies in non-human primates demonstrating that $mGluR_{2/3}$ agonists have unexpected postsynaptic actions in the dorsolateral PFC that strengthen synaptic connections and improve cognitive function (Jin et al., 2016). Collectively, these studies suggest that $mGlu_3$ plays a key role in regulating PFC-dependent cognition. Future studies with current and next generation group II modulators will broaden our understanding of these receptors and their potential utility in treating numerous CNS disorders such as schizophrenia, substance abuse, depression (Chaki et al., 2004; Dhanya et al., 2014), and others.

## Potential utility of muscarinic receptor PAMs for treatment of schizophrenia

Preclinical and clinical studies suggest that potentiation of specific mAChR subtypes can improve symptoms in patients suffering from schizophrenia and Alzheimer's disease (AD). A large multicenter trial examined the effects of the $M_1/M_4$-preferring mAChR agonist xanomeline in patients suffering from AD. While the primary endpoint was improved cognitive function, secondary measures surprisingly revealed that this compound had robust efficacy in reducing psychotic symptoms such as suspiciousness, delusions, and hallucinations in AD patients (Bodick et al., 1997). A subsequent study revealed that xanomeline induced robust improvements in positive symptoms, negative symptoms, and specific domains of cognitive function in patients suffering from schizophrenia (Shekhar et al., 2008). Despite these exciting advances, the clinical utility of xanomeline and other mAChR agonists is restricted by dose-limiting adverse effects (bradycardia, GI distress, salivation, sweating) that are mediated by activation of peripheral $M_2$ and $M_3$ mAChRs (Bymaster et al., 2003). Interestingly, $M_1$ and $M_4$ are the primary mAChR subtypes thought to be involved in the therapeutic effects of mAChR agonists in schizophrenia patients (Langmead et al., 2008). Thus, it is possible that highly selective activators of $M_1$ and/or $M_4$ could provide therapeutic efficacy in these patients in the absence of the peripheral adverse effects associated with less selective mAChR agonists.

### $M_4$ PAMs reduce dopamine release and have antipsychotic-like effects in animal models

Despite major investments, previous efforts to develop highly selective agonists of $M_1$ or $M_4$ mAChRs have failed due to high conservation in the orthosteric ACh site across subtypes. However, more recent efforts to develop subtype-selective mAChR PAMs have been highly successful and have yielded multiple selective PAMs for $M_1$ and $M_4$ mAChRs that have

excellent pharmacokinetic (PK) profiles and brain penetration, providing excellent tools for evaluating the effects of $M_1$ and $M_4$ PAMs in preclinical animal models for numerous CNS disorders (Conn et al., 2014; Kruse et al., 2014).

The discovery of two structurally distinct $M_4$ PAMs (VU10010 and LY2033298) represented a milestone in the development of $M_4$-selective molecules (Chan et al., 2008; Shirey et al., 2008). Since then, medicinal chemistry efforts have provided compounds with improved brain exposure and properties that are ideal for *in vivo* use. Studies utilizing these novel $M_4$–selective PAMs have demonstrated robust effects, similar to those seen with xanomeline, in multiple animal models used to predict antipsychotic-like activity including reversal of psychostimulant-induced changes in conditioned avoidance responding, prepulse inhibition, and locomotor activity (Bubser et al., 2014; Chan et al., 2008; Leach et al., 2010; Suratman et al., 2011). Importantly, $M_4$ PAMs do not display any of the detrimental peripheral effects that are seen after administration of non-selective mAChR compounds (Bubser et al., 2014), suggesting that $M_4$ PAMs may provide a novel strategy for treating positive symptoms in schizophrenia patients.

Psychotic symptoms associated with schizophrenia are thought to be intimately associated with hyperactive dopaminergic signaling in the striatum and nucleus accumbens and all currently available antipsychotics act as antagonists of dopamine (DA) receptors (Sawa and Snyder, 2003). Interestingly, $M_4$ PAMs decrease amphetamine-induced DA levels in the dorsal striatum and nucleus accumbens and induce a profound reduction in amphetamine-induced activation of forebrain regions *in vivo* as assessed by fMRI (Byun et al., 2014), raising the possibility that $M_4$ PAMs mediate their effects through modulating DA release. Cholinergic regulation of DA signaling is complex and cholinergic interneurons in the striatum can exert bidirectional control over dopaminergic signaling through both nicotinic acetylcholine receptors (nAChR) and mAChRs (Rice et al., 2011). Studies using fast scan cyclic voltammetry demonstrated that $M_4$ knock out mice display attenuated mAChR agonist-induced reductions in DA release (Threlfell et al., 2010) and selective $M_4$ PAMs induce a robust inhibition of DA release in striatal slices that persists well after receptor activation (Foster et al., 2016). This sustained inhibition of DA release following $M_4$ activation was distinct from the acute inhibition observed following application of mAChR agonists, which is mediated by primarily by mAChR autoreceptors expressed on cholinergic interneurons (Shin et al., 2015). $M_4$ is not expressed on DA neurons (Weiner et al., 1990), but is highly expressed on $D_1$-containing direct pathway spiny projection neurons (D1-SPNs; Ince et al., 1997). Interestingly, selective deletion of $M_4$ from $D_1$-expressing neurons ($D_1$-$M_4^{-/-}$ mice) eliminated the sustained reductions in DA release and antipsychotic-like effects induced by $M_4$ PAMs and xanomeline (Dencker et al., 2011; Foster et al., 2016), suggesting that $M_4$ expressed on $D_1$-containing neurons mediate these effects. The finding that $M_4$ PAMs act at $D_1$-SPNs to inhibit DA release suggests that $M_4$ activation must act by inducing release of a local messenger that acts on neighboring DA terminals to inhibit DA release. Interestingly, $M_4$-mediated effects on DA release are blocked by a $CB_2$ endocannabinoid (eCB) receptor antagonist, absent in $CB_2$ knock out mice, and are occluded by inhibition of the eCB synthetic enzyme diacylglycerol lipase (Foster et al., 2016). Taken together, these data suggest that the effects of $M_4$ PAMs on DA release in the striatum are

mediated by activation of eCB synthesis in $D_1$-SPNs and activation of $CB_2$ receptors, possibly expressed on neighboring DA terminals.

The ability of $M_4$ to reduce DA through the local release of eCBs provides a mechanism that may afford a spatially restricted modulation of DA signaling in the limbic forebrain. This could provide a major advantage over clinically available antipsychotics that act as DA receptor antagonists in that it may allow reduced DA signaling in striatal regions that are thought to be important for antipsychotic efficacy, without reducing DA signaling in the hippocampus and cortical regions that may impair cognitive function (Davis et al., 1991; Reilly et al., 2007; see Figure 4). Consistent with this, early reports suggest that $M_4$ PAMs can improve some aspects of cognitive function in animal models that are impaired by DA receptor blockade (Bubser et al., 2014).

In addition to regulation of DA release, recent studies reveal that $M_4$ PAMs can decrease glutamatergic transmission at cortico-striatal synapses (Pancani et al., 2014) and induce spike-timing dependent LTD on $D_1$-SPNs (Shen et al., 2016). $M_4$ receptors are present on cortico-striatal terminals, and activation of these presynaptic $M_4$ receptors may contribute to $M_4$ PAM effects at these excitatory synapses (Pancani et al., 2014). However, as with the effect of $M_4$ PAMs on DA release, the ability of $M_4$ PAMs to induce LTD in $D_1$-SPNs is absent in $D_1$-$M_4^{-/-}$mice and, surprisingly, was blocked by a $CB_1$ receptor antagonist (Shen et al., 2016), suggesting $M_4$ PAM-induced eCB release from $D_1$-SPNs can inhibit excitatory transmission in the striatum. Extensive studies in rodents and non-human primates suggest that these actions of $M_4$ PAMs on striatal plasticity could provide therapeutic benefits in treating L-DOPA-induced dyskinesia in Parkinson's disease (Shen et al., 2016). Furthermore, $M_4$ PAMs can normalize excessive excitatory transmission at cortico-striatal synapses in rodent models of Huntington's disease and chronic administration of $M_4$ PAMs prevents the appearance of motor deficits in these animals (Pancani et al., 2015). Finally, the ability of $M_4$ PAMs to reduce behavioral effects of cocaine suggest that these agents may be useful for the treatment of substance abuse disorders (Dencker et al., 2012). Thus, recent optimization of highly selective $M_4$ PAMs is providing important insights into the roles of this receptor in regulating CNS function and may lead to novel treatment strategies for multiple CNS disorders.

## $M_1$ PAMs may enhance specific domains of cognitive function and reduce negative symptoms

In addition to a potential role for $M_4$ in regulating dopaminergic systems that are relevant for positive symptoms in schizophrenia, the $M_1$ receptor may be important for reducing cognitive impairments and negative symptoms in AD and schizophrenia patients. Cholinergic signaling is disrupted in the prefrontal cortex (PFC) of schizophrenia patients (Berman et al., 2007; Raedler et al., 2007) and a subset of schizophrenia patients display profound decreases in $M_1$ levels in PFC, hippocampus, and other forebrain regions (Dean et al., 2002; Scarr et al., 2013). The recent discovery of highly selective $M_1$ PAMs enabled studies that reveal that these agents can enhance both PFC- and hippocampal-dependent forms of cognitive function in rodents (Chambon et al., 2012; Digby et al., 2012; Gould et al., 2015; Ma et al., 2009; Shirey et al., 2009) and non-human primates (Lange et al., 2015;

Vardigan et al., 2015) which is consistent with a large literature suggesting that selective $M_1$ receptor activation can have cognition-enhancing effects.

Recent studies are providing important new insights into the possible mechanisms by which $M_1$ PAMs could provide specific benefits to some schizophrenia patients. An emerging body of clinical and preclinical research has led to the hypothesis that deficits in LTD at hippocampo-PFC synapses (Strube et al., 2015; Thomases et al., 2014) and excessive activation of the PFC by excitatory projections from the hippocampus (Jodo, 2013; Woodward et al., 2013) contribute to the cognitive deficits and negative symptoms observed in schizophrenia patients. This is especially interesting in light of the findings that $M_1$ plays a major role in induction of long-term depression (LTD) at hippocampo-PFC synapses (Caruana et al., 2011; Ghoshal et al., 2016), and that $M_1$ knock-out mice display deficits in forms of cognitive function that involve interactions between the hippocampus and PFC (Anagnostaras et al., 2003). Interestingly, $M_1$ receptor-mediated LTD at the hippocampo-PFC synapse is completely lost in rodent models that pharmacologically or genetically inhibit NMDAR function during juvenile development, and $M_1$ PAMs can restore deficits in synaptic plasticity, cognitive function and social interaction in these rodent models (Ghoshal et al., 2016; Grannan et al., 2016). Taken together with multiple studies demonstrating robust effects of $M_1$ PAMs on other aspects of cognitive function, these studies support the exciting possibility that highly selective $M_1$ PAMs may provide a novel approach for reducing cognitive deficits and negative symptoms associated with changes in cortical plasticity in schizophrenia patients. Furthermore, while $M_4$ PAMs are likely to have more robust antipsychotic-like effects, $M_1$ PAMs can augment the antipsychotic-like effects of atypical antipsychotics in wild type, but not $M_1$ knock-out mice (Choy et al., 2016). Accordingly, $M_1$ PAMs either alone or in combination with current antipsychotics, have the potential to provide comprehensive relief across symptom clusters.

Surprisingly, a recent study suggested that some $M_1$-selective Ago-PAMs can induce cholinergic side-effects in animal models that are typically associated with $M_2/M_3$ activation (Alt et al., 2016; Davoren et al., 2016). However, several studies with other $M_1$-selective PAMs did not observe any of the side-effects seen with broad spectrum cholinergic mimetics (Chambon et al., 2012; Jones et al., 2008; Vardigan et al., 2015), suggesting that it is possible to develop $M_1$ PAMs that have a desirable side-effect profile. The exact mechanism underlying the adverse effects of $M_1$ Ago-PAMs are not entirely clear. However, it is possible that subtle variations in properties of different $M_1$–selective compounds could influence their *in vivo* effects in a manner similar to that outlined above for $mGlu_5$ PAMs. $M_1$ PAMs have been reported to possess distinct differences in signaling bias and allosteric agonist activity, and the impact of these differences has not been fully explored. For instance, while $M_1$ PAMs can potentiate ACh-induced activation of both phospholipase D (PLD) and $Ca^{2+}$ mobilization in cell lines, a novel $M_1$ PAM (VU0029767) selectively potentiates $M_1$-induced $Ca^{2+}$ mobilization but has no effect on $M_1$-mediated activation of PLD (Marlo et al., 2009). $M_1$-mediated activation of phospholipase C and $Ca^{2+}$ mobilization involves signaling through $G_{\alpha q}$, whereas PLD typically involves the activation of $G_{\alpha 12}$ or small G-proteins such as R-Ras (Lopez De Jesus et al., 2006). Thus, it is possible that VU0029767 stabilizes a conformation of $M_1$ that couples to $G_{\alpha q}$ but not $G_{\alpha 12}$ or small G-proteins. While the precise roles of these signaling pathways in different physiological and

behavioral responses to $M_1$ activation are not known, closely related $M_1$ PAMs that display such striking differences in their effects on $M_1$ signaling could have fundamentally different effects on animal behavior greatly modifying their potential therapeutic efficacy or adverse effect liability.

The robust pro-cognitive effects of $M_1$–selective PAMs could prove useful for treatment of multiple disorders in which cognitive function is impaired. Acetylcholinesterase inhibitors, which boost cholinergic signaling, have well-established efficacy in improving cognitive function in patients with early to moderate AD and other neurodegenerative disorders. Current evidence suggests that the $M_1$ receptor plays a vital role in mediating cholinergic pro-cognitive effects. In addition to the $M_1$ PAM efficacy observed in healthy rodents and schizophrenia models discussed above, $M_1$ PAMs have pro-cognitive effects in numerous rodent models of AD (Puri et al., 2015; Shirey et al., 2009), and can reverse cognitive deficits and prolong survival in a mouse model of prion disease that shows AD-like pathology (Bradley et al., 2016). As discussed above in the context of $mGlu_2$ PAMs for schizophrenia, it is possible that careful patient stratification will be critical for evaluating the potential efficacy of $M_1$ PAMs in patient populations. For instance, developing an appropriate PET ligand or other approaches for identifying and recruiting the subset of schizophrenia patients that display decreases in cortical $M_1$ levels (Dean et al., 2002; Scarr et al., 2013) could enrich for patients that would receive the greatest benefit from $M_1$ PAMs. Collectively, these studies call attention to the potential utility of $M_1$ PAMs in correcting cognitive and attentional deficits in a wide range of neurological disorders including schizophrenia, attention deficit hyperactivity disorder, AD, Parkinson's disease, and others.

## Allosteric modulation of dopamine receptors as potential treatments of schizophrenia

Since all currently available antipsychotics act via downregulation of $D_2$ signaling it is tempting to hypothesize that a $D_2$-selective NAM (or partial NAM) that possessed desirable signal bias, or selectively modulates only certain $D_2$ dimer complexes, could provide antipsychotic efficacy with reduced cognitive and motor side-effects. The discovery of the first NAM of the $D_2$ receptor represented a breakthrough in efforts to achieve allosteric regulation of this target (Lane et al., 2014). Interestingly, this $D_2$ NAM (SB269652) possessed a bitopic mode of action, binding to both the orthosteric and an allosteric site, and only displayed NAM activity at functional $D_2$ receptor homodimers. Excitingly, medicinal chemistry efforts have recently succeeded at fragmenting SB269652 and have resulted in the first purely allosteric compound possessing NAM activity at the $D_2$ receptor (Mistry et al., 2015). While the hypothesis that a $D_2$ NAM could provide efficacy with a preferred side-effect profile has yet to be tested, these advances suggest that such approaches could be testable in the near future.

Conversely, in preclinical animal models $D_1$ agonists have been shown to mediate cognitive enhancing effects, although with an inverted U-shaped dose-response curve suggesting that too little or too much $D_1$ activation can be detrimental (Arnsten et al., 2016). However, a proof of principle study demonstrated that administration of the $D_1$-preferring agonist

dihydrexidine improved working memory in patients with schizotypal personality disorder (Rosell et al., 2015). Unfortunately dihydrexidine, which is only moderately selective for $D_1$ over $D_5$, has poor bioavailability and is rapidly metabolized, limiting the clinical utility of this compound. Excitingly, new $D_1$-selective PAMs have recently been developed that demonstrate enhanced specificity over $D_5$ receptors (Lewis et al., 2015). $D_1$ PAMs, due to their mechanism of action, have the potential to avoid the adverse effects seen excessive $D_1$ receptor activation. Consistent with this $D_1$ PAMs, unlike $D_1$ agonists, induced changes in locomotion in a humanized mouse line that plateaued at high doses without inducing stereotypies (Svensson et al., 2016). The recent discovery of both $D_2$- and $D_1$-selective allosteric scaffolds represents an important advance and hopefully will lead to optimized compounds that can provide therapeutically desirable outcomes with fewer side-effects than those observed with DA receptor agonists and antagonists.

## Conclusions and Future Directions

The discovery of allosteric modulators initially provided the ability to target binding sites on specific receptor subtypes that were less conserved than the neurotransmitter binding site allowing allosteric compounds to act with unprecedented selectivity. Given the vast number of possible allosteric binding sites there is reason to believe that many of these sites have not been identified or targeted from a medicinal chemistry perspective. Numerous examples have been detailed where multiple distinct allosteric binding sites have been found for a single GPCR subtype (Gregory and Conn, 2015), and the recent discovery of a cytoplasmic allosteric binding site in chemokine CCR9 represents one example of a previously unappreciated binding site that could allow therapeutic modulation of a previously intractable target (Oswald et al., 2016). As we continue to advance our understanding of the mechanisms underlying allosteric modulation of GPCRs, we have come to appreciate the immense number of conformations and higher-order complexes that these receptors can adopt (Changeux and Christopoulos, 2016; Latorraca et al., 2016), and are beginning to elucidate how these conformations differentially regulate various signaling pathways. In addition to differences in terms of stimulus bias, activity at heterodimers, and presence or absence of ago-PAM activity, it has been possible to develop NAMs that possess weak negative cooperativity can act as partial NAMs that only partially inhibit the response to an orthosteric agonist (Kenakin, 2004; Nickols et al., 2016; Rodriguez et al., 2010; Rodriguez et al., 2005). These partial NAMs could provide a key mechanistic advantage that cannot be achieved using orthosteric antagonists and have the potential to maintain efficacy similar to full NAMs in animal models, but have fewer adverse effects than agents that completely block GPCR signaling (Gould et al., 2016).

Other recent advances include the use of allosteric modulators in combination with optogenetic and chemogenetic technologies to allow selective modulation of specific receptor subpopulations with unprecedented spatial and temporal specificity. For instance, engineered chimeras of GPCRs with rhodopsin can adopt active conformations in the presence of light, allowing optical control over receptor activity (Airan et al., 2009). In addition, introduction of targeted mutations into GPCRs allows for labeling of receptor populations using coordination chemistry approaches that introduce either metal binding domains (Kiyonaka et al., 2016), or photoswitchable tethered ligands (Levitz et al., 2013).

By utilizing mutated GPCRs that can be expressed in specific neuronal populations, it is possible to assess the physiological and behavioral consequences of activating a particular receptor subtype in a specific location. More recently, this approach has refined through the use of non-tethered photoswitchable allosteric ligands that possess the properties of an allosteric modulator, but adopt inactive conformations when exposed to light. Discovery of photoswitchable allosteric modulators for the $mGlu_5$ (Pittolo et al., 2014) and $mGlu_4$ receptors (Rovira et al., 2016) allows fast and direct modulation of endogenous receptor subtypes in a particular brain region. Collectively, these tools have the promise to provide unprecedented insights into the biology and circuitry underlying numerous CNS diseases. These discoveries will inform drug discovery efforts on how to optimally steer receptor signaling in a given patient population to provide maximal efficacy with minimal side-effects and provide exciting opportunities for the treatment of CNS orders.

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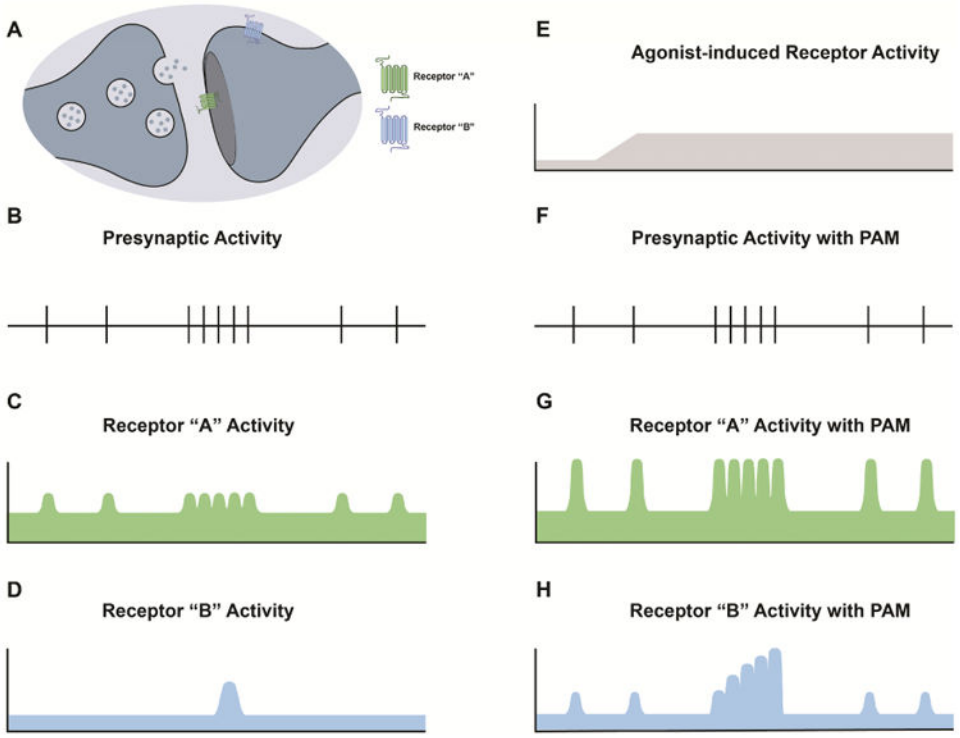


**Figure 1. Positive allosteric modulators modulate receptor activity while maintaining spatial and temporal dynamics associated with neurotransmitter release**

Communication between neurons is commonly encoded by neurotransmitter release events emanating from presynaptic terminals resulting in postsynaptic receptor activation (**A**). Presynaptic activity patterns (**B**), and the proximity of a receptor from a neurotransmitter release site, both play a key role in determining postsynaptic receptor activity patterns. In receptor populations that are present in the synaptic cleft, the site of neurotransmitter release is sufficiently proximal to the receptor such that each release event may induce receptor activation (**C**). Receptor populations that are expressed in extrasynaptic or perisynaptic areas, which are further removed from neurotransmitter release sites, may not be exposed to sufficient neurotransmitter levels following a single release event to become active. However these receptors may be activated following bursts of high-frequency activity when neurotransmitter levels are sufficiently elevated to spill out from the synapse (**D**). Exogenously applied agonists activate receptors with a temporal profile (**E**) that is very different from presynaptic activity patterns (**B**) and will activate receptors regardless of their proximity to presynaptic inputs. In the simplest case, positive allosteric modulators (PAMs) of post-synaptic receptors do not affect presynaptic firing rates (**F**), but potentiate responses to the endogenous neurotransmitter while maintaining temporally and spatially coded information with respect to receptor activity patterns (**G** and **H**). This activity-dependence of allosteric modulators can avoid detrimental effects due to excessive receptor activation and preserve complex physiology such as spike-timing dependent plasticity.

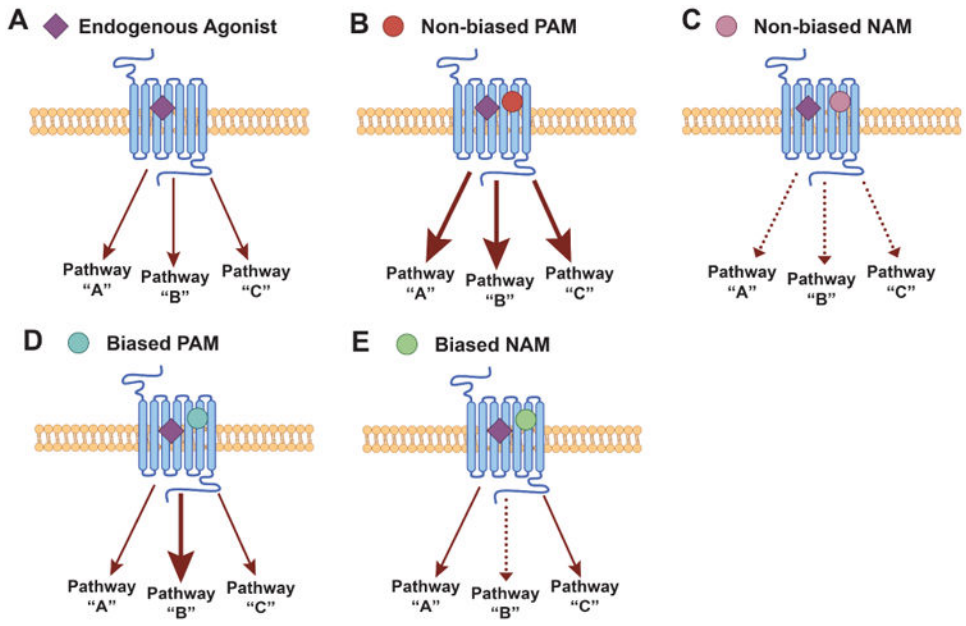


**Figure 2. Ability of allosteric modulators to confer bias of GPCR signaling**

GPCRs can adopt multiple conformations upon neurotransmitter binding that can lead to activation of numerous signaling pathways (**A**). Non-biased allosteric modulators equally potentiate (**B**) or inhibit (**C**) all the signaling pathways that are activated by an agonist. However, some PAMs and NAMs can confer bias to GPCR signaling and selectively modulate coupling of GPCRs to specific signaling pathways while having little or no effect on others (**D** and **E**). These tools are affording the opportunity to determine the outcome of modulating a specific receptor-mediated signaling pathway, and hold great therapeutic potential by allowing receptor activation to be steered in maximally beneficial directions.

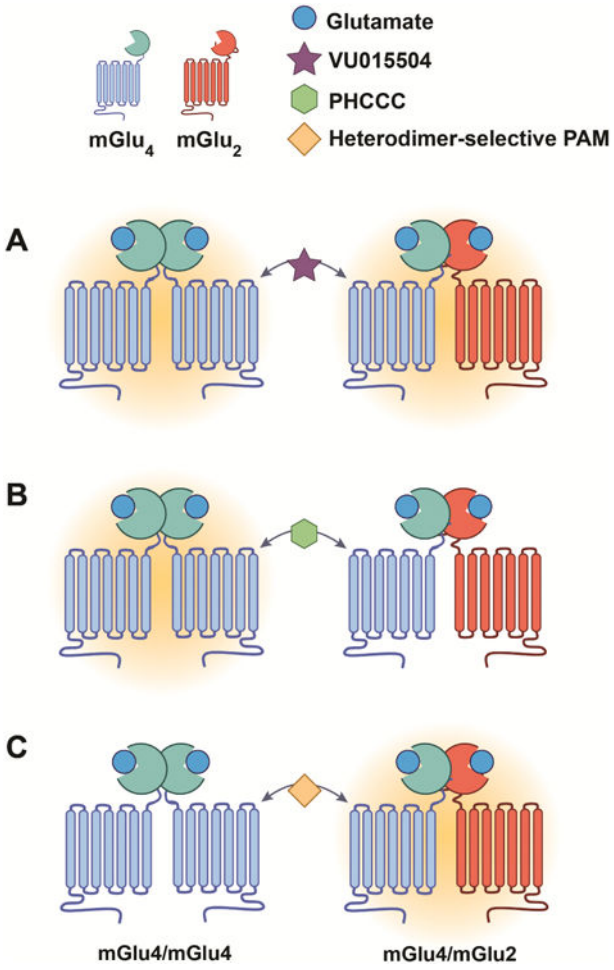


**Figure 3. Allosteric modulators can differentially modulate receptor populations based on their dimerization**

Many GPCRs can form oligomers either as homodimers (containing two receptors of the same subtype) or heterodimers (containing two different receptor subtypes). As depicted above using $mGlu_4$ and $mGlu_2$ receptors as an example, some PAMs (such as VU0155041) are active at both $mGlu_4/mGlu_4$ homodimer and $mGlu_4/mGlu_2$ heterodimer complexes (**A**; Potentiation of receptor activity is depicted by orange shading). Other PAMs (such as PHCCC) selectively activate $mGlu_4$ homodimers but are inactive at $mGlu_4/mGlu_2$ heterodimers (**B**). Furthermore, while no examples have been described to date, it is possible that other classes of PAMs could be active at the heterodimer, but inactive at the homodimer (**C**). These novel tools that can distinguish between receptor complexes will shed light onto the physiological significance of homodimer and heterodimer complexes and could provide therapeutic benefits through incredibly precise modulation of circuitry that targets not only a given receptor subtype, but can target specific receptor complexes.

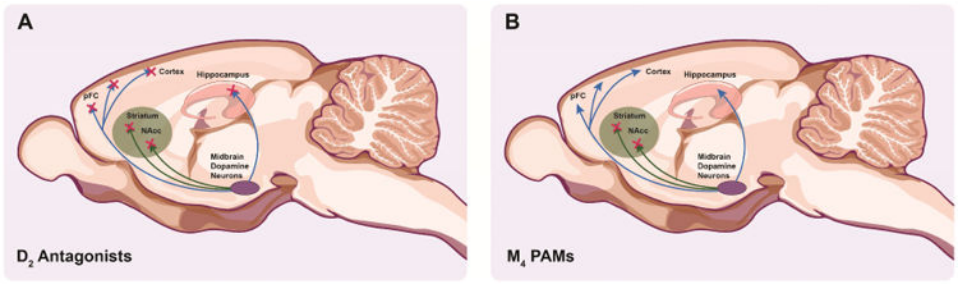


**Figure 4. The potential therapeutic benefits of selectively regulating dopaminergic signaling in the basal ganglia**

Midbrain dopamine (DA) neurons project to several nuclei including the striatum, nucleus accumbens (NAcc), prefrontal cortex (PFC), cortex, and hippocampus. DA signaling is dysregulated in schizophrenia manifesting in excessive DA release in the striatum and NAcc that is associated with the positive symptoms such as hallucinations and delusions (depicted as green shaded areas). However, DA signaling is not hyperactive in all brain regions and hypoactive disruptions in cortical and hippocampal DA signaling are thought to contribute to the negative symptoms such as anhedonia as well as the cognitive deficits. Currently available antipsychotics act by blocking DA $D_2$ receptors across all brain regions, including the areas that are already DA deficient, potentially worsening negative and cognitive symptoms (**A**). By depressing DA release through the release of local messengers in the hyperactive limbic brain regions, $M_4$ PAMs have the potential to correct hyperactive DA signaling without further depressing DA signaling in other areas (**B**), demonstrating how circuit-selective therapeutics have the potential to provide efficacy with reduced adverse effect liability.

## Table 1
### Mechanisms of action observed by allosteric modulators

| | |
|---|---|
| **Orthosteric Agonist** | Binding of orthosteric agonists (which can be either endogenous neurotransmitters or synthetic agonists) to the neurotransmitter binding site induces a change in receptor confirmation and stabilizes an active state of the receptor. |
| **Positive Allosteric Modulator (PAM)** | PAMs bind to an allosteric site on the receptor that is distinct from the orthosteric binding pocket and increases the potency and/or efficacy of orthosteric agonists resulting in enhanced receptor activation when an orthosteric agonist is present. |
| **Ago-PAM** | Binding of Ago-PAMs alone is sufficient to induce receptor activation. In addition, these compounds increase the potency and/or efficacy of orthosteric agonists. |
| **Orthosteric Antagonist** | Binding of orthosteric antagonists have no effect on receptor activity. These compounds prevent activation of the receptor by preventing neurotransmitter binding. |
| **Negative allosteric modulator (NAM)** | NAMs bind to an allosteric site distinct from the orthosteric or neurotransmitter binding site and inhibit receptor activation via negative cooperativity to reduce the affinity and/or efficacy of orthosteric agonists. |
| **Partial NAM** | Partial NAMs do not completely block receptor activation, often due to weak negative cooperativity with respect to agonist binding. Accordingly, even when the allosteric binding pocket is fully occupied, partial NAMs will only partially reduce agonist responses. |