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The crosstalk between 5-HT_{2A}R and mGluR2 in schizophrenia

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Abstract

Schizophrenia is a severe brain disorder that usually produces a lifetime of disability. First generation or typical such as haloperidol and second generation or atypical such as clozapine and risperidone remain the current standard for schizophrenia treatment. In some patients with schizophrenia, antipsychotics produce complete remission of positive symptoms, such as hallucinations and delusions. However, antipsychotic drugs are ineffective against cognitive deficits and indeed treated schizophrenia patients have small improvements or even deterioration in several cognitive domains. This underlines the need for novel and more efficient therapeutic targets for schizophrenia treatment. Serotonin and glutamate have been identified as key parts of two neurotransmitter systems involved in fundamental brain processes. Serotonin (or 5-hydroxytryptamine) 5-HT_{2A} receptor (5-HT_{2A}R) and metabotropic glutamate 2 receptor (mGluR2) are G protein-coupled receptors (GPCRs) that interact at epigenetic and functional levels. These two receptors can form GPCR heteromeric complexes through which their pharmacology, function and trafficking becomes affected. Here we review past and current research on the 5-HT_{2A}R-mGluR2 heterocomplex and its potential implication in schizophrenia and antipsychotic drug action.

Keywords

Serotonin 5-HT_{2A} receptor; metabotropic glutamate 2 receptor; G protein-coupled receptor (GPCR); GPCR dimerization; psychedelics; antipsychotics; schizophrenia; epigenetics

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Somdatta Saha: Conceptualization, Writing – original draft. **Javier González-Maeso:** Conceptualization, Writing – review and editing, Supervision, Funding acquisition.

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Introduction

Schizophrenia is a chronic debilitating mental disorder that affects approximately 1% of the general population (Sawa and Snyder, 2002; Freedman, 2003). The symptoms generally include positive such as hallucinations and delusions, negative such as deficits of motivation and social withdrawal, and cognitive such as attention deficit and impaired memory. Although currently used antipsychotic medications may reduce hallucinations and delusions in certain groups of schizophrenia patients, their clinical efficacy in negative and cognitive symptoms remains modest (Lieberman *et al.*, 2008; Ebdrup *et al.*, 2011; Miyamoto *et al.*, 2012; Meltzer, 2013). Identifying and testing new molecular targets for the treatment of schizophrenia is therefore crucial to optimally manage this and other psychiatric disorders.

Over the past several decades, the most common neurotransmitter potentially linked to schizophrenia has been the monoaminergic dopamine, and particularly a potential excess of dopamine transmission in certain subcortical limbic areas such as the nucleus accumbens (NAc), amygdala and hippocampus (Seeman and Lee, 1975; Meltzer and Stahl, 1976; Seeman *et al.*, 1976). This notion stemmed from pharmacological evidences that demonstrated that drugs such as phenothiazine, which decrease dopamine activity, showed antipsychotic properties, whereas drugs such as amphetamine, which increase dopamine release, were considered as psychotomimetic (Meltzer and Stahl, 1976). However, more recent findings have implicated additional neurotransmitter systems in psychotic disorders and their treatment. As an example, psychosis in patients with Parkinson's disease (PD) has been proposed to be caused by a serotonin-dopamine imbalance (Stahl, 2016), and accordingly, the relatively selective serotonin (or 5-hydroxytryptamine) 5-HT_{2A} receptor (5-HT_{2A}R) antagonist pimavanserin, which also shows a negligible affinity against the dopamine D_2 receptor (Casey *et al.*, 2022), has proven antipsychotic activity in PD patients without an effect on motor function when compared with placebo (Meltzer *et al.*, 2010).

The glutamate theory of psychosis, and particularly the N-methyl-D-aspartate (NMDA) receptor hypoactivity theory, proposes that hypofunction of the NMDA receptor in brain regions such as the prefrontal cortex may be responsible for some of the behavioral deficits observed in schizophrenia patients (Moghaddam, 2004; Stahl, 2018). In healthy volunteers, non-competitive NMDA antagonists such as phencyclidine (PCP) (Morris *et al.*, 2005), also known as the angel dust, impair cognitive functioning in a manner similar to that encountered in schizophrenia patients (Kristiansen *et al.*, 2007). Preclinical studies in rodent models suggest that targeting two subtypes of metabotropic glutamate receptors (mGluRs) has the capability to improve cognitive deficits resulting from dysfunction of NMDA receptor. These include mGluR5, which can directly modulate the function of NMDA channel, and mGluR2/3s, which regulate the release of glutamate principally via presynaptic mechanisms (Moghaddam, 2004).

The potential involvement of serotonin in schizophrenia was hypothesized in the 1950s. It was based on the structural similarities between serotonin and psychedelics such as lysergic acid diethylamide (LSD) and psilocin (active metabolite of psilocybin), and the findings that psychedelics caused hallucinations and emotional disturbances similar to those observed in schizophrenia patients (Woolley and Shaw, 1954). Several clinical studies indicate that

a portion of schizophrenia patients respond to selective blockade of 5-HT_{2A}Rs (Geyer and Vollenweider, 2008; Meltzer, 2013). Psychosis caused by agonism at 5-HT_{2A}Rs upon psychedelic administration resembles some of the symptoms in first-episode schizophrenia patients (Vollenweider *et al.*, 1998; Schmid *et al.*, 2015). Additionally, the *5-HT_{2A}R* (*HTR2A*) gene ranks in the top 12 of the more than 1,000 genes tested for genetic association with schizophrenia, and genome-wide association studies focused on the *de novo* mutations showed that the *HTR2A* gene might potentially be implicated in schizophrenia-related networks (Fromer *et al.*, 2014).

This review attempts to explore recent advances in the interactions between serotonergic and glutamatergic systems, particularly 5-HT_{2A}R and mGluR2, in preclinical models of psychosis, neuropsychiatric disorders and antipsychotic drug action.

Structure and function of GPCRs

G protein-coupled receptors (GPCRs) comprise the largest superfamily of membrane proteins in the mammalian genome (Thomsen *et al.*, 2005; Rosenbaum *et al.*, 2009; Katritch *et al.*, 2013; Lee *et al.*, 2018), and are targets of around 700 approved drugs (Sriram and Insel, 2018). They enable communication between the extracellular and intracellular milieu of the cell through mediators that include a large variety of hormones, neurotransmitters, metabolites, cytokines, photons, ions and other stimuli essential for normal physiological functions (Rosenbaum *et al.*, 2009). Based on function and primary sequence, the GPCR superfamily is broadly classified into six families, namely class A – rhodopsin-like receptors, class B – secretin family, class C – metabotropic glutamate receptors, class D – fungal mating pheromone receptors, class E – cAMP receptors, class F – frizzled (FZD) and smoothened (SMO) receptors. This receptor superfamily comprises of more than 800 genes which modulate several signaling pathways involved in regulation of biological processes that include blood pressure, behavior, cognition, immune response, smell, taste and mood, among many others. All members of this superfamily share several structural motifs, mainly the seven transmembrane domains interconnected by loops with extracellular N-terminus and intracellular C-terminus. However, they have low sequence identity, and possess different extracellular N-terminal domains and diverse ligand-binding pockets. For class A GPCRs, the ligand-binding site is within the transmembrane (TM) region. For class B GPCRs, ligand binding involves both extracellular and TM domains. For class C GPCRs, the ligand-binding pocket is found in the extracellular domain (ECD) that contains a Venus flytrap (VFT) domain. In the case of class F GPCRs, their ECD comprises of an extracellular cysteine-rich domain (CRD) and an ECD linker domain (Basith *et al.*, 2018).

GPCRs were traditionally thought to transduce extracellular stimuli to intracellular second messengers by acting as ligand-regulated guanine nucleotide exchange factors for heterotrimeric (α-, β-, and γ-subunit) GTP-binding proteins (G proteins) (Gilman, 1987). According to this model, an agonist-bound GPCR adopts an active conformation that causes exchange of GDP for GTP on the Gα-subunit. This causes dissociation of Gα from Gβγ subunit. Subsequently, the activated G protein subunits are capable of either activating or inhibiting downstream targets such as ion channels, phospholipases and adenylyl cyclases (ACs). Regulation of GPCR signaling is accomplished by a conserved two-step mechanism

that involves receptor phosphorylation by GPCR kinases (GRKs) followed by arrestin recruitment to phosphorylated receptors (Gurevich and Gurevich, 2019). More recent studies highlighted the ability of GPCRs to signal via G protein-independent mechanisms like the β-arrestin signaling pathway. They also act as scaffolds for a large number of GPCR-interacting proteins which in turn regulate their function, localization and trafficking (Magalhaes *et al.*, 2012).

GPCR complexes (dimers and oligomers)

Initially, GPCRs were considered to function as monomeric structural units. However, in the last three decades, several techniques including crystallographic studies have provided substantial evidence supporting the existence of GPCR homomers and/or heteromers as functional signaling units (Gonzalez-Maeso, 2011; Gonzalez-Maeso, 2014; Gaitonde and Gonzalez-Maeso, 2017; Ferre *et al.*, 2022). The concept of receptor dimerization/oligomerization traces back to the 1980/90s, and these include the study that reported reduction in the affinity of dopamine D_2 agonist binding in plasma membrane preparations of rat striatal neurons upon stimulation of adenosine A_{2A} receptors (Ferre *et al.*, 1991). However, it was only in 1993 that the first insight into the role dimerization in GPCR function was established at the molecular level (Maggio *et al.*, 1993). They generated chimeras of the muscarinic M_3 and α_{2C}-adrenergic receptors and tested the functional response elicited by these chimeric constructs: chimeric M_3/α_{2C} receptor was comprised of TM domains 1–5 of the muscarinic M_3 receptor and the last two TM domains of the α_{2C}-adrenergic receptor, whereas chimeric α_{2C}/M_3 receptor was generated by fusing TM domains 1–5 of the α_{2C}-adrenergic receptor and the last two TM domains of the muscarinic M_3 receptor. In these two chimeric constructs, the intracellular loop 3 of the muscarinic M_3 receptor was kept unaltered because this region mediates G protein coupling. In the presence of the muscarinic receptor agonist carbachol, signaling was abolished when either of these two chimeric constructs were expressed alone. However, when the α_{2C}/M_3 and M_3/α_{2C} chimeric constructs were co-expressed together, carbachol-induced signaling was rescued.

The first example that heterodimerization was fully required for class C GPCR function was that of the $GABA_B$ receptor. Expression of a fully functional receptor required heterodimerization between two distinct and separate gene products, namely, $GABA_B$-R1 and $GABA_B$-R2 (Jones *et al.*, 1998; Kaupmann *et al.*, 1998; White *et al.*, 1998). Another key example of how molecular proximity affects functional and ligand binding properties is the heteromerization of the δ and κ opioid receptors (Jordan and Devi, 1999). More recently, fluorescence resonance energy transfer (FRET) studies demonstrated that within an heteromeric complex formed between α_{2A}-adrenergic and μ-opioid receptors, binding of morphine to the μ-opioid receptor triggers a conformational change in the α_{2A}-adrenergic receptor bound to norepinephrine that inhibits G_i protein-dependent signaling and subsequently the downstream MAPK signaling cascade (Vilardaga *et al.*, 2008).

It has also been proposed the existence of GPCR-effector macromolecular membrane assemblies (GEMMAs) comprised of specific GPCR combinations, G proteins, effectors and other associated plasma membrane-localized proteins (Ferre *et al.*, 2022). GEMMAs harbor distinct functional and pharmacological characteristics thereby making them unique

drug targets. For example, mutants of the small GTPase Sar1 or Rab1 prevented anterograde trafficking to the plasma membrane and diminished plasma membrane localization of β_2-adreneregic receptor but did not interfere with the interactions between β_2-adrenergic receptor and $G_{\beta1}/G_{\gamma2}$ subunits, AC2 and β_2-adrenergic receptor or AC2 and $G_{\beta1}$ or $G_{\gamma2}$ fused to bioluminescence resonance energy transfer (BRET) chromophores (Ebdrup *et al.*, 2011), indirectly pointing towards the pre-assembly of β_2-adrenergic receptor $G_{\beta1\gamma2}$-AC2 complexes.

Serotonin and 5-HT_{2A}Rs

Serotonin is one of the main neurotransmitters that has been detected in the nervous systemsof virtually all metazoan phyla. It functions as a crucial regulator of memory, cognition, sleep, mood, appetite, learning, social behaviors and whole-body homeostasis. In humans, it exerts its physiological effects through 14 distinct receptors, out of which 13 are GPCRs, and one is a ligand-gated cation channel (McCorvy and Roth, 2015; Sharp and Barnes, 2020). The International Union of Basic and Clinical Pharmacology (IUPHAR) has classified serotonin receptors into 5-HT_1 (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1e}, and 5-HT_{1F}), 5-HT_2 (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}), 5-HT_3, 5-HT_4, 5-HT_5 (5-HT_{5A}, 5-ht_{5b}), 5-HT_6, and 5-HT_7 receptors (McCorvy and Roth, 2015; Barnes *et al.*, 2021). This classification was based on their primary sequence, intracellular transduction mechanisms, selective agonists and antagonists, and ligand binding affinities criteria. It is currently accepted that the CNS expresses seven types of serotonin receptors (5-$HT_{1–7}$). However, 5-ht_{1e} and 5-ht_{5b} retain low case letters since their functional response remains unknown. All 5-HT receptor subtypes have been reported in both brain and peripheral tissues with the exception of 5-ht_{1e}, 5-HT_{2C} and 5-HT_6 which show limited expression outside the CNS. However, 95% of 5-HT in human body is synthesized, stored and released in the gut, where its function is focused on either contraction of smooth muscles or depolarization of the enteric nerve (McCorvy and Roth, 2015).

The serotonin 5-HT_{2A}R is the main excitatory GPCR of the 5-HT receptor family. In humans and rodent models, this receptor subtype is primarily expressed in the frontal cortex, whereas it is also detectable at relatively lower densities in other brain regions such as hippocampus, thalamus and basal ganglia. Within the cortex, they are densely populated on the dendrites of excitatory glutamatergic pyramidal neurons, particularly in layer V (Lopez-Gimenez *et al.*, 1997; Jakab and Goldman-Rakic, 1998; Lopez-Gimenez *et al.*, 2001).

The three 5-HT_2R subtypes, namely, 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R, couple to $G_{q/11}$ proteins and activate a cytoplasmic protein phospholipase C (PLC). Activated PLC cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2), a phospholipid of the plasma membrane, followed by generation of diacylglycerol (DAG) and inositol triphosphate (IP_3). Ultimately, IP_3 induces release of Ca^{2+} from intracellular stores (Leysen, 2004). These three 5-HT_2R subtypes can also activate phospholipase A2 (PLA2), which thereby causes the release of arachidonic acid (AA). Interestingly, previous studies have shown that 5-HT_{2A}R-induced PLA2 activation does not depend upon PLC activation (Kurrasch-Orbaugh *et al.*, 2003b). Rather, it has been observed that NIH3T3 cells stably expressing 5-HT_{2A}R causes PLA2 activation though a complex signaling mechanism involving both $G_{i/o}$-associated $G_{\beta\gamma}$-

mediated activation of extracellular signal-regulated kinase, p44/p42 (ERK1,2) and $G\alpha^{12/13}$-coupled, Rho-mediated p38 activation (Kurrasch-Orbaugh *et al.*, 2003a).

Apart from endogenous 5-HT, several antipsychotics, psychedelics and antidepressants function at least in part by targeting the 5-HT_{2A}Rs. Preclinical and clinical studies suggest that 5-HT_{2A}R antagonists, such as mirtazapine, mianserin and trazodone, have antidepressant properties (Marek *et al.*, 2005), whereas its agonists possess cognition-enhancing and psychedelic characteristics. Abnormality in 5-HT_{2A}R activity is associated with a number of psychiatric conditions, including schizophrenia, depression, anxiety, and substance use disorders. Pharmacological studies indicate that high-affinity antagonists of 5-HT_{2A}Rs such as clozapine and risperidone are effective atypical antipsychotics (Hanks and Gonzalez-Maeso, 2013; Hanks and González-Maeso, 2016b; Hanks and González-Maeso, 2016a; Lopez-Gimenez and Gonzalez-Maeso, 2018; Jaster *et al.*, 2021).

Classical psychedelics are compounds that evoke profound alterations in perception, cognition and mood, and have been used recreationally and for religious and spiritual purposes for centuries (Nichols, 2016). Richard Glennon, Milt Titeler and their teams were among the first to propose the potential role of 5-HT_2Rs in the mechanism of action of psychedelics (Glennon *et al.*, 1984). Later, experiments with knockout (KO) mice (Gonzalez-Maeso *et al.*, 2003; Gonzalez-Maeso *et al.*, 2007) and 5-HT_{2A}R antagonists such as M100907 (volinanserin) (Halberstadt and Geyer, 2018; de la Fuente Revenga *et al.*, 2019) corroborated the role of 5-HT_{2A}R-dependent signaling in the cellular and behavioral effects of psychedelics. These studies in rodent models were further supported by data from humans which demonstrated that the psychosis-like effects of psilocybin and LSD in healthy volunteers were reversed by the 5-HT_2R antagonist ketanserin (Vollenweider *et al.*, 1998; Schmid *et al.*, 2015; Preller *et al.*, 2016; Preller *et al.*, 2017).

All psychedelics such as LSD, psilocin and mescaline are potent 5-HT_{2A}R agonists but not all 5-HT_{2A}R agonists are psychedelics – these include non-psychedelic 5-HT_{2A}R agonists such as lisuride and ergotamine which are currently used in the treatment of neuropsychiatric conditions such as PD or migraine, respectively (Silbergeld and Hruska, 1979; Mokler *et al.*, 1983; Adams and Geyer, 1985; Marona-Lewicka *et al.*, 2002). One of the potential mechanisms behind the behavioral differences between psychedelic and non-psychedelic 5-HT_{2A}R agonists is the concept of biased agonism, which describes the ability of ligands acting at the same GPCR to elicit distinct cellular signaling profiles (Urban *et al.*, 2007; Gonzalez-Maeso and Sealfon, 2009a; Gonzalez-Maeso and Sealfon, 2009b). Recent findings in rodent models suggests that psychedelic 5-HT_{2A}R agonists may represent a new approach to treat psychiatric conditions including depression and substance use disorders (Ly *et al.*, 2018; de la Fuente Revenga *et al.*, 2021; Jaster *et al.*, 2021; Shao *et al.*, 2021; Cameron *et al.*, 2023), an effect that is also induced by the non-psychedelic 5-HT_{2A}R agonist lisuride (Nakamura *et al.*, 1989; Qu *et al.*, 2022). Although this topic goes beyond the scope of this review, psychedelic and non-psychedelic 5-HT_{2A}R agonists are currently being used as a pharmacological tool to investigate the signaling mechanisms that allow chemically similar agonists to target the same receptor population but induce different signaling and, potentially, therapeutic-like effects in rodent models of depression and substance use disorder (Cameron *et al.*, 2021; Cao *et al.*, 2022; Kaplan *et al.*, 2022).

An interesting question for additional studies seems to be how both receptor activation via psychedelic 5-HT_{2A}R agonists and receptor blockade via either traditional antidepressants such as mirtazapine (see above) or more selective 5-HT_{2A}R (M100907 and ketanserin) and 5-HT_{2C}R (SB242084) antagonists lead to antidepressant-like effect in rodents (Kaplan *et al.*, 2022; Pacheco *et al.*, 2023).

Previous review articles have emphasized that LSD, a classical psychedelic, appears to exert its psychoactive properties principally via the 5-HT_2-like receptors, particularly through the 5-HT_{2A}R (Hanks and Gonzalez-Maeso, 2013; Hanks and González-Maeso, 2016b; Hanks and González-Maeso, 2016a; Lopez-Gimenez and Gonzalez-Maeso, 2018; Jaster *et al.*, 2021). The first attempt to provide structural insights into the binding mode of LSD to a 5-HT_2R was the crystal structure of the human 5-HT_{2B}R bound to LSD, which was resolved at 2.9 Å (Wacker *et al.*, 2017). This study shows that LSD has an exceptionally slow dissociation rate from both 5-HT_{2B}R and 5-HT_{2A}R. Molecular dynamics (MD) simulation studies revealed that LSD's slow binding kinetics might be due to the formation of a "lid" by the extracellular loop 2 (EL2) at the entrance of the binding pocket. Substitution of a key residue (L209) in EL2 increases the mobility of this lid and augments LSD's binding kinetics. This selectively hampers LSD-mediated β-arrestin-2 recruitment yet negligibly affects G_q signaling. The crystal structures of the human 5-HT_{2A}R complexed with the antipsychotic drugs risperidone and zotepine were resolved at 3.0/3.6 Å and at 2.9/3.3 Å resolution, respectively (Kimura *et al.*, 2019). Several other structural analyses were conducted to gain molecular insights into actions of psychedelics at the serotonin receptors. The active-state structure of the human 5-HT_{2A}R bound to 25-CN-NBOH (a prototypical psychedelic and selective 5-HT_{2A}R agonist) complexed with an engineered $G\alpha_q$ heterotrimer was determined by cryo-electron microscopy (cryo-EM) at a resolution of 3.27 Å. X-ray crystal structures of the human 5-HT_{2A}R complexed with LSD or the inverse agonist methiothepin were obtained at a resolution of around 3.4 Å (Kim *et al.*, 2020). These findings could accelerate the discovery of more selective 5-HT_{2A}R agonists and antagonists for the treatment of a variety of psychiatric conditions.

Related to the effect of psychedelic drug administration on gene expression, previous studies using traditional gene-expression assays such as DNA microarrays reported that a single administration of psychedelic and non-psychedelic 5-HT_{2A}R agonists induced the expression of *INBD* and *c-Fos* genes in brain regions such as the mouse somatosensory cortex (Nichols and Sanders-Bush, 2002; Gonzalez-Maeso *et al.*, 2003; Nichols *et al.*, 2003; Gonzalez-Maeso *et al.*, 2007). However, expression of *Egr-1* and *Egr-2* was only escalated upon administration of psychedelics such as DOI, DOM, DOB, mescaline, LSD, and psilocin, an effect that was not observed by non-psychedelic 5-HT_{2A}R agonists such as lisuride and ergotamine. Also, with the exception of *INBD*, the effect of psychedelic and non-psychedelic 5-HT_{2A}R agonists on gene expression was abolished in 5-HT_{2A}R-KO mice. These alterations in gene expression showed maximal changes at ~60 min and returned to basal levels at ~120 min after drug exposure. one marks and DNA methylation affect chromatin organization and DNA accessibility (Ibi and Gonzalez-Maeso, 2015). Recent findings reported long-lasting (up to 7 days) changes on chromatin organization in mouse frontal cortex, particularly at enhancer regions of genes involved in synaptic plasticity, following a single administration of the psychedelic DOI (de la Fuente Revenga *et al.*,

2021). Repeated LSD administration (7 days) also affects DNA methylation in CpG sites of mouse frontal cortex samples (Inserra *et al.*, 2022). Additional investigation is required to characterize the functional relevance of these prolonged transcriptomic and epigenomic alterations induced by classical psychedelics.

Metabotropic glutamate receptors and mGluR2

Glutamate is one of the most functionally relevant neurotransmitters of the CNS. It executes its functions principally via two main classes of receptors: ionotropic glutamate receptors, which are multimeric ion channels conducting fast synaptic responses, and mGluRs, which are GPCRs modulating slow synaptic transmission via intracellular second messengers (Hollmann and Heinemann, 1994). There are 8 members within the mGluR family which have been subdivided into 3 groups on the basis of G protein coupling, sequence homology and ligand selectivity. Group I includes mGluRs 1 and 5, group II includes mGluRs 2 and 3, and group III includes mGluRs, 4, 6, 7, and 8 (Conn and Pin, 1997; Niswender and Conn, 2010; Nicoletti *et al.*, 2011).

Generally, group I mGluRs activate PLCβ by coupling to $G_{q/11}$ proteins. Apart from this signaling pathway, group I mGluRs can also activate a plethora of downstream effectors, including phospholipase D, cyclin-dependent protein kinase 5, casein kinase 1, c-Jun kinase, components of MAPK/ERK pathway, and the MTOR/p70 S6 pathway. Group II and III mGluRs are predominantly coupled to $G_{i/o}$ proteins, and also couple to other signaling pathways, including activation of phosphatidyl inositol 3-kinase PI3 kinase and MAPK pathways (Conn and Pin, 1997; Niswender and Conn, 2010; Nicoletti *et al.*, 2011).

Several members of the mGluR family undergo alternative splicing. mGluR1 has 5 splice variants mGluR1a, b, c, d, and e. mGluR5 has two main splice variants, namely, mGluR5a and mGluR5b. Amongst group II mGluRs, mGluR2 does not have any reported splice variant but mGluR3, mGluR6, mGluR7 and mGluR8 have 4, 3, 5 and 3 splice variants, respectively (Conn and Pin, 1997; Niswender and Conn, 2010; Nicoletti *et al.*, 2011).

Members of the class C GPCR tend to form constitutive homo and heterodimers amongst themselves as well as with other GPCR classes, and dimerization is a prerequisite process for glutamate-induced signaling through the ECD to the mGluR TM core (Conn and Pin, 1997; Niswender and Conn, 2010). It has been demonstrated that heterodimerization between members of the mGluR family is selective. Thus, group I members only heterodimerize with members belonging to their group, whereas group II and III members can form heterodimers with each other (Doumazane *et al.*, 2011). Examples of mGluR heterodimers include mGluR2/mGluR4, mGluR2/mGluR3, mGluR2/mGluR7, and mGluR1/mGluR5 (Levitz *et al.*, 2016; Pandya *et al.*, 2016; Habrian *et al.*, 2019; Lee *et al.*, 2020).

Related to their functional properties, although binding of one agonist to either of the subunits in an mGluR homodimer is able to induce G protein coupling, agonist occupation of both ECDs is required for full receptor activation (Kniazeff *et al.*, 2004). Ligand binding to mGluR homodimers recruits G protein to only one of the two subunits (Hlavackova *et al.*, 2005). Assays combining single-molecule pull-down (SiMPull) with single-molecule

total internal reflection fluorescence (smTIRF) microscopy revealed that homo- and heteromerization of mGluRs is primarily mediated via interactions at a hydrophobic interface in the upper lobe of the ligand binding domain (LBD), followed by secondary sites of interaction that include the inter-subunit disulfide bridge and TM domains (Levitz *et al.*, 2016). FRET measurements also revealed that conformational rearrangements underlying activation of mGluRs may be controlled by interactions between ligand binding domains (Levitz *et al.*, 2016).

More recent cryo-EM studies determined the structures of mGluR2 homodimers complexed with G_{i1}, and mGluR4 homodimers complexed with G_{i3} at resolutions of 3.1 Å (extracellular domains of mGluR2) and 3.5 Å (G_{i1} and the TM domains of mGluR2), and 3.1 Å (extracellular domains of mGluR4) and 4.0 Å (mGluR4–G_{i3}). In both mGluR2 and mGluR4, the C-terminal region of the $G_{\alpha i}$ subunit binds to a groove formed by ICL1, ICL2 and ICL3 and the intracellular regions of TM 3 and 5. In the mGluR2–G_{i1} complex, the mGluR2 positive allosteric modulator (PAM) JNJ-40411813 binds at a site formed by TMs 3, 5, 6 and 7 of the G protein-bound subunit. In the inactive state, the interface for homodimerization is TM6. Agonist binding induces a conformational change that alters the dimerization interface to TM6. Additionally, in order to facilitate coupling of G proteins, the TM domains undergo a clockwise rotation and formation of an asymmetric interface between TM6 of the mGluR2-G protein-bound subunit and TM7 of the mGluR2-G protein free subunit (Lin *et al.*, 2021).

Another study solved the cryo-EM structures of inactive mGluR2, inactive mGluR7, heterodimer of mGluR2–mGluR7 and the PAM- and agonist-bound mGluR2 structures at overall resolutions of 3.6 Å, 4.0 Å, 3.9 Å and 3.1 Å, respectively. The extracellular domains of mGluR7 and the mGluR2–mGluR7 heterodimer were further resolved to 3.6 Å and 3.5 Å, respectively. The crystal structures of the transmembrane domains of mGluR2 bound to two negative allosteric modulators (NAM), namely, NAM563 and NAM59710, were resolved at 2.5 Å and 2.7 Å, respectively (Du *et al.*, 2021). This study confirmed TM4 as the symmertric dimeric interface between two mGluR2 subunits in inactive state and TM6 as the interface when bound to an agonist and PAM. However, in the case of the inactive mGluR7 homodimer and mGluR2-mGluR7 heterodimer, the interface is formed by TM5 (Du *et al.*, 2021). These studies also suggest that it is the TM4 domain as the major responsible for the difference in dimerization between full length mGluR2 and mGluR3, given that the sequence indentity between these two receptors is nearly 70%. Othosteric agonist activation of group II mGluRs is influenced by inter-TM4 interactions. Ligand-coupled SiMPull assays strongly support a rearrangement from TM4 to TM6, with agonists and PAMs favoring a TM6-TM6 interface while antagonists and NAMs favoring a TM4-TM4 interface (Thibado *et al.*, 2021). Some of these cryo-EM and SiMPull employed mGluR2 constructs with a C121A mutation, which may have an impact on the relative location of the two subunits within the mGluR2 homodimer. This is because previous studies suggested that the mGluR2-C121A construct alters homodimerization and dynamics (Levitz *et al.*, 2016; Koehl *et al.*, 2019; Shah *et al.*, 2020).

5-HT_{2A}R-mGluR2 heteromeric complex

Class A $G_{q/11}$-coupled 5-HT_{2A}R and class C $G_{i/o}$-coupled mGluR2 are able to interact physically to form a GPCR complex (Gonzalez-Maeso *et al.*, 2008). Assembly of 5-HT_{2A}R and mGluR2 as a GPCR complex has been validated by several independent *in vitro* methods, such as co-immunoprecipitation, FRET, BRET, bimolecular fluorescence complementation (BiFC), and proximity ligation assay (PLA) (Gonzalez-Maeso *et al.*, 2008; Moreno *et al.*, 2012; Moreno *et al.*, 2016; Toneatti *et al.*, 2020). Most of these studies were conducted *in vitro* in HEK293 cells.

Three amino acids located at the intracellular region of the TM4 of mGluR2, namely, Ala-677, Ala-681, and Ala-685, have been proposed to be necessary for the association with 5-HT_{2A}R as a GPCR heterocomplex (Gonzalez-Maeso *et al.*, 2008; Moreno *et al.*, 2012). Substitution of these three residues in mGluR2 with the corresponding residues from mGluR3 disrupted 5-HT_{2A}R-mGluR2 heteromerization. By introducing the photoactivatable unnatural amino acid p-azido-L-phenylalanine (azF) at selected individual positions along the TM segments of mGluR2, it has also been suggested that 5-HT_{2A}R crosslinked with azF incorporated at the intracellular end of mGluR2's TM4 (Shah *et al.*, 2020). It remains unknown whether 5-HT_{2A}R assembly affects the structural arrangement of the two protomers within the mGluR2 homodimer under apo- and agonist-bound states, and further cryo-EM and X-ray assays are needed to provide additional structural insights into the interaction of 5-HT_{2A}R and mGluR2.

Orthosteric agonists usually present a higher affinity to the active conformation of the GPCR, whereas neutral antagonists cannot distinguish between the active and inactive structural states (Strange, 1998; Strange, 1999). Consequently, competitive binding displacement of a radiolabeled antagonist by an unlabeled (cold) agonist usually leads to a biphasic displacement curve with high affinity ($K_{i\text{-}high}$) and low affinity ($K_{i\text{-}low}$) binding sites. This particular pharmacological profile has been reported in HEK293 cells expressing 5-HT_{2A}R alone (displacement of [^{3}H]ketanserin by DOI and other 5-HT_{2A}R agonists) and mGluR2 alone (displacement of [^{3}H]LY341495 by LY379268 and other mGluR2/3 agonists) (Gonzalez-Maeso *et al.*, 2007; Gonzalez-Maeso *et al.*, 2008; Moreno *et al.*, 2012). Related to the allosteric crosstalk between 5-HT_{2A}R and mGluR2, it was also shown that the biphasic displacement curve of [^{3}H]ketanserin by DOI in cells expressing 5-HT_{2A}R alone became monophasic in cells co-expressing 5-HT_{2A}R and mGluR2, and that the mGluR2/3 agonist LY379268 concentration-dependently increased the affinity of DOI displacing [^{3}H]ketanserin in cells co-expressing 5-HT_{2A}R and mGluR2. As expected, cold GTPγS shifted the displacement curve of [^{3}H]ketanserin with DOI to the right. Importantly, it was reported that this allosteric crosstalk between 5-HT_{2A}R and mGluR2 required GPCR heteromerization since it was absent in cells co-expressing 5-HT_{2A}R and mGluR3, and prevented in cells co-expressing 5-HT_{2A}R and mGluR2ΔTM4 (an mGluR2/mGluR3 chimeric construct that, according to previous *in vitro* findings, is not able to from the 5-HT_{2A}R-mGluR2 complex) (Gonzalez-Maeso *et al.*, 2007; Gonzalez-Maeso *et al.*, 2008; Moreno *et al.*, 2012).

Previous data using orthosteric agonists and/or antagonists suggested a functional crosstalk that requires assembly of 5-HT_{2A}R and mGluR2 as a GPCR heterocomplex. As one example, it was demonstrated that stimulation of cells expressing 5-HT_{2A}R-mGluR2 heteromers with an mGluR2 agonist led to activation of $G_{q/11}$ proteins by the 5-HT_{2A}R in HEK293 cells (Moreno *et al.*, 2016). Additionally, in *Xenopus* oocytes, using inhibition of the Kir2.3 channel current as a readout of G_q activity together with activation of Kir3.4 channel current as a readout of G_i activity, it was observed that co-expression of 5-HT_{2A}R and mGluR2 reduced G_q activity induced by the endogenous neurotransmitter 5-HT to almost half of the activity of the 5-HT_{2A}R construst when expressed alone (Fribourg *et al.*, 2011). In contrast to G_q activity, the G_i activity induced by the endogenous neurotransmitter glutamate was increased in cells co-expressing mGluR2 and 5-HT_{2A}R. This gave rise to the concept of balance index (BI), which measures the change in activity of G_i and G_q such that BI= ΔG_i - ΔG_q. Methysergide, DOI and clozapine function as antagonist, agonist and inverse agonist of the 5-HT_{2A}R, respectively. Upon co-expression of both receptors and in the presence of glutamate, methysergide had no effect on G_i signaling, DOI decreased G_i activity, while clozapine significantly escalated G_i signaling. (2S)-α-Ethylglutamic acid (eGlu), LY379268 and LY341495 function as antagonist, agonist and inverse agonist of mGluR2, respectively (Conn and Pin, 1997; Niswender and Conn, 2010; Nicoletti *et al.*, 2011). Upon expression of the heteromer and in the presence of serotonin, eGlu did not affect serotonin-elicited G_q signaling, LY379268 decreased G_q signaling to around 35%, while LY341495 increased it to around 83% (Fribourg *et al.*, 2011). This cross-signaling between 5-HT_{2A}R and mGluR2 was also observed in mammalian HEK293 cells stably transfected with these two receptors (Baki *et al.*, 2016).

Published reports by other groups have validated the formation of the 5-HT_{2A}R-mGluR2 complex *in vitro* (Rives *et al.*, 2009; Delille *et al.*, 2012; Murat *et al.*, 2019) and *ex vivo* (Hamor *et al.*, 2018; Olivero *et al.*, 2018; Banerjee and Vaidya, 2020; Taddeucci *et al.*, 2022). However, some (Olivero *et al.*, 2018; Murat *et al.*, 2019; Poulie *et al.*, 2020; Taddeucci *et al.*, 2022) but not all (Delille *et al.*, 2012) of the previous studies reported functional crosstalk between these two receptors. A potential explanation of this lack of functional crosstalk in cells co-expressing 5-HT_{2A}R and mGluR2 may be related to variations in densities of 5-HT_{2A}R and/or mGluR2, as it has been suggested in HEK293 cells either co-transfected with different ratios of plasmids encoding 5-HT_{2A}R and mGluR2 (Moreno *et al.*, 2016), or stably expressing various levels of the two GPCRs (Baki *et al.*, 2016).

Some of the conclusions obtained from *in vitro* biochemical and biophysical studies have been supported by assays in whole animal models. As an example, 5-HT_{2A}R and mGluR2 are part of the same protein complex in native tissue since co-immunoprecipiation has been reported in mouse (Fribourg *et al.*, 2011; Hamor *et al.*, 2018) rat (Taddeucci *et al.*, 2022) and postmortem human brain frontal cortex samples (Gonzalez-Maeso *et al.*, 2008), as well as in synaptosomal lysates from rat spinal cord (Olivero *et al.*, 2018). Head-twitch behavior – a mouse behavioral proxy of human psychedelic activity (Hanks and Gonzalez-Maeso, 2013) – was significantly diminished in mGluR2-KO mice (Moreno *et al.*, 2011). This intriguing phenotype has been validated by other groups (Benvenga *et al.*, 2018). Using a virally (HSV)-mediated over-expression approach, it was also demonstrated that this behavior

phenotype is rescued in mGluR2-KO mice stereotaxically injected with HSV-mGluR2 in the frontal cortex (Moreno *et al.*, 2012). This, however, did not occur upon HSV-mediated expression of mGluR2ΔTM4. The ability of clozapine to reduce hyperlocomotor activity induced by dissociative drugs such as MK801 and PCP was also diminished in mGluR2-KO as compared to wild-type controls (Hideshima *et al.*, 2018). These findings suggest a potential role of this GPCR heterocomplex in the cortical processes affected by psychedelics and antipsychotic medications.

A key molecular event affecting the crosstalk between 5-HT_{2A}R and mGluR2 was found to be the phosphorylation of Ser843 in mGluR2 in HEK293 cells co-expressing 5-HT_{2A}R (Murat *et al.*, 2019). Only in cells co-expressing these two receptors, phosphorylation of Ser843 in mGluR2 was augmented upon application of mGluR2 agonist LY379268. This phenonemom was corroborated in frontal cortex samples from wild-type mice, and absent in the same tissue samples of 5-HT_{2A}R-KO animals. Increase in Ser843 phosphorylation was also observed upon 5-HT_{2A}R stimulation by 5-HT in cells co-expressing both mGluR2 and 5-HT_{2A}R. In both mouse frontal cortex tissue samples and HEK293 cells, the mGluR2 antagonist LY341495 prevented Ser843 phosphorylation elicited by 5-HT_{2A}R stimulation, while the LY379268-induced effect was also blocked by the 5-HT_{2A}R receptor antagonist M100907. Additionally, G protein-dependent signaling elicited by mGluR2 or 5-HT_{2A}R receptor stimulation in cells co-expressing both receptors was strongly reduced upon mutation of Ser843 into Ala.

One of the most commonly abused synthetic drugs worldwide is methamphetamine. Its chronic usage often turns into a compulsive drug-taking disorder accompanied by cognitive deficits and recurring psychosis. Recent findings reported dysregulation of 5-HT_{2A}R and mGluR2 expression in brain regions including medial prefrontal cortex, dorsal hippocampus, and perirhinal cortex upon 14 days of repeated treatment with methamphetamine as compared to vehicle and 7 days of withdrawal (Hamor *et al.*, 2018). As compared with other psychostimulants, these findings also reported that there were alterations in the expression ratio of 5-HT_{2A}R and mGluR2 in medial prefrontal cortex (methamphetamine, PCP and MK801), dorsal hippocampus (methamphetamine and PCP), and perirhinal cortex (MK801). Repeated administration of methamphetamine also led to an increase in G_q activity and a decrease in G_i activity in the medial prefrontal cortex. These data suggest that alrerations in G protein-coupling via cortical 5-HT_{2A}R vs mGluR2 may be reponsible for some of the common signaling mechanisms underlying psychosis and cognitive alterations in subjects with methamphetamine use disorder.

Cellular and sub-cellular colocalization of 5-HT_{2A}R and mGluR2

Autoradiography with [^{3}H]LY354740 (a selective mGluR2/3 receptor agonist) and [^{125}I]DOI (a potent partial 5-HT_{2A}R agonist) demonstrated an overlapping distribution of mGluR2/3 and 5-HT_{2A}R in rat medial prefrontal cortex samples (Marek *et al.*, 2000). Subsequent FISH assays were used to suggest co-localization of 5-HT_{2A}R and mGluR2 in mouse frontal cortex both at cellular (FISH and immunocytochemistry assays) (Gonzalez-Maeso *et al.*, 2008; Fribourg *et al.*, 2011) and sub-cellular (electron microscopy) (Moreno *et al.*, 2012) levels. This co-localization occurred particularly within layer V cortical pyramidal

neurons (Gonzalez-Maeso *et al.*, 2008). However, in frontal cortex pyramidal neurons, the expression of *mGluR3* mRNA was modest and it barely co-localized with *5-HT$_{2A}$R* mRNA (Gonzalez-Maeso *et al.*, 2008). Using a subcellular fractionation approach to purify fractions enriched in presynaptic active zone (PAZ) and post-synaptic density (PSD) proteins in mouse frontal cortex samples, it was observed that 5-HT$_{2A}$R was detected in the PSD, whereas mGluR2 was detected in both the PSD and PAZ fractions (Moreno *et al.*, 2016). This suggests that the 5-HT$_{2A}$R-mGluR2 complex is located mostly postsynaptically in frontal cortex pyramidal neurons. However, the potential role of presynaptic 5-HT$_{2A}$R modulating plasticity via presynaptic thalamocortical circuits has also been suggested (Marek *et al.*, 2001; Barre *et al.*, 2016). As an example, there is a presynaptic crosstalk between 5-HT$_{2A}$R and mGluR2/3 in the frontal cortex where the 5-HT$_{2A}$R antagonist MDL11939 augments the inhibitory effect induced by LY379268 through a mechanism that may not require GPCR heteromerization, since it was reported that 5-HT$_{2A}$R and mGluR2 co-immunoprecipitate in homogenates but not in subcellular fractions isolated from synaptic terminals in rat frontal cortex (Taddeucci *et al.*, 2022).

Presynaptic mGluR2/3 autoreceptors control glutamate exocytosis in spinal cord endings. Interestingly, recent findings reported that glutamatergic presynaptic terminals of rat spinal cord show strong colocalization of 5-HT$_{2A}$R and mGluR2/3, where they crosstalk with each other in an antagonistic fashion. Thus, antagonists of the 5-HT$_{2A}$R act as indirect positive allosteric modulators of mGluR2/3 autoreceptors and regulate the exocytosis of glutamate (Olivero *et al.*, 2018). Further studies are required to better understand the pattern of localization and co-localization of 5-HT$_{2A}$R and mGluR2 in different central and peripheral tissues as well as the mechanisms that regulate their function.

GPCR activity is regulated by membrane trafficking processes (Dong *et al.*, 2007; Magalhaes *et al.*, 2012; von Zastrow and Williams, 2012; Pavlos and Friedman, 2017; Thomsen *et al.*, 2018). In mammalian HEK293 cells, 5-HT$_{2A}$Rs affected the trafficking and localization of mGluR2 via a mechanism that required their assembly as a GPCR heteromeric complex. In resting state (*i.e.*, absence of agonists), the 5-HT$_{2A}$R was mainly localized in intracellular vesicles whereas mGluR2 showed surface localization (Toneatti *et al.*, 2020). However, co-expression of both receptors increased the intracellular localization of the otherwise membrane-localized mGluR2. Co-localization of mGluR2 with Rab5, Rab7 and transferrin receptor (all markers of endocytic vesicles) increased upon co-expression with 5-HT$_{2A}$R. Additionally, the presence of DOI as a 5-HT$_{2A}$R agonist or LY379268 as an mGluR2/3 agonist differentially affected the density and localization of mGluR2 with endosomal markers in cells expressing mGluR2 alone or co-expressing mGluR2 and 5-HT$_{2A}$R. The antipsychotic clozapine but not the 5-HT$_{2A}$R antagonist M100907 reduced the sub-cellular localization of 5-HT$_{2A}$R and mGluR2 through a mechanism that required their molecular proximity. *Ex vivo* in mouse frontal cortex samples, expression of 5-HT$_{2A}$R also augmented the intracellular localization of mGluR2. Thus, there was a significantly greater proportion of mGluR2 at the plasma membrane in neurons from 5-HT$_{2A}$R-KO mice as compared to wild-type controls (Toneatti *et al.*, 2020). Together, these data indicate that heteromerization of 5-HT$_{2A}$R-mGluR2 plays a plausible role in receptor trafficking and sorting.

Epigenetic crosstalk between 5-HT_{2A}R and mGluR2

Expression of 5-HT_{2A}R indirectly affects *mGluR2* transcription since 5-HT_{2A}R-KO animals exhibited reduced mGluR2/3 binding and *mGluR2*, but not *mGluR3*, mRNA expression in frontal cortex samples (Gonzalez-Maeso *et al.*, 2008; Kurita *et al.*, 2013). Additionally, Cre-mediated *5-HT_{2A}R* transcription in the frontal cortex of flox-STOP-flox mice restored *mGluR2* expression to control levels (Gonzalez-Maeso *et al.*, 2008). Covalent histone modifications and DNA methylation act cooperatively to regulate gene expression in a dynamic and reversible fashion (Ibi and Gonzalez-Maeso, 2015). Chromatin immunoprecipitation (ChIP) followed by qPCR techniques revealed that acetylation of histone H3 (H3ac) and histone H4 (H4ac), markers of transcriptional activation, were diminished at the promoter region of the *mGluR2* gene in the frontal cortex of 5-HT_{2A}R-KO mice (Kurita *et al.*, 2013). Neither methylation of histone H3 at lysine 4 (H3K4me1/2/3) nor trimethylation of histone H3 at lysine 9 (H3K9me3) were affected. However, trimethylation of histone H3 at lysine 27 (H3K27me3), a marker for transcriptional repression, was considerably augmented at the *mGluR2* promoter in the frontal cortex of 5-HT_{2A}R-KO mice as compared to wild-type controls. Similar ChIP-qPCR assays also revealed that binding of the transcription factor Egr1 at the *mGluR2* promoter was reduced in the frontal cortex of 5-HT_{2A}R-KO mice. Additionally, immunoreactivity of mGluR2 was increased upon viral (HSV)-mediated over-expression of Egr1 in the mouse frontal cortex (Kurita *et al.*, 2013). These findings suggesting that 5-HT_{2A}R-dependent signaling epigenetically affects *mGluR2* transcription have been supported by studies testing the effect of chronic antipsychotic treatment.

Thus, chronic treatment with the atypical antipsychotic clozapine and risperidone, but not with the typical antipsychotic haloperidol, strongly decreased H3ac binding at the *mGluR2* promoter in both mouse and human frontal cortex (Kurita *et al.*, 2012; de la Fuente Revenga *et al.*, 2018a). A similar epigenetic phenotype was observed in schizophrenia subjects since H3ac binding at the *mGluR2* promoter was reduced in frontal cortex samples of antipsychotic treated, but not antipsychotic free, schizophrenic subjects (Kurita *et al.*, 2012). This epigenetic modification at the *mGluR2* promoter region was 5-HT_{2A}R-dependent since it was absent in 5-HT_{2A}R-KO mice, and was associated with an increased binding of histone deacetylase 2 (HDAC2) to the *mGluR2* promoter. Conversely, co-administration of the class I and II HDAC inhibitor SAHA (vorinostat) prevented the antipsychotic profile of the mGluR2/3 agonist LY379269 and downregulation of frontal cortex mGluR2/3 density via HDAC2 (de la Fuente Revenga *et al.*, 2018a).

Based on this preclinical work, it was proposed that repeated administration of atypical antipsychotics would consequently restrict the clinical effects of the antipsychotic LY2140023 (pomaglumetad), an oral prodrug of the mGluR2/3 agonist LY404039 (Patil *et al.*, 2007). Importantly, this hypothesis was validated by clinical results from a post-hoc analysis where the antipsychotic effects of LY2140023 were comparable to those induced by risperidone in schizophrenia patients previously treated with typical antipsychotics (i.e., haloperidol) whereas previous exposure to atypical antipsychotics (*e.g.*, clozapine and olanzapine) led to an effect of LY2140023 that did not separate from placebo (Kinon *et al.*, 2015).

Antipsychotic drugs including both typical (*e.g.*, haloperidol) and atypical (*e.g.*, clozapine and risperidone) were serendipitously discovered in the mid-twentieth century (Crilly, 2007). Although chronic administration of these agents produces significant reduction or even complete remission of psychotic symptoms, such as hallucinations and delusions (Lieberman *et al.*, 2008; Ebdrup *et al.*, 2011; Miyamoto *et al.*, 2012; Meltzer, 2013), the majority of schizophrenia patients have cognitive deficits that often do not improve upon antipsychotic drug treatment – some patients even deteriorate (Goldberg *et al.*, 1993; Husa *et al.*, 2014; Fervaha *et al.*, 2015; Nielsen *et al.*, 2015; Husa *et al.*, 2016). This decline in cognitive abilities upon long-term exposure to antipsychotics has been corroborated in animal models (Rosengarten and Quartermain, 2002; Schroder *et al.*, 2005). Related to this, it was reported that chronic treatment with clozapine decreases the density of 5-HT_{2A}R in the frontal cortex, which in turn reduces the impact of 5-HT_{2A}R-dependent signaling on the MAPK-ERK cascade (Ibi *et al.*, 2017). This leads to downregulation of the NF-κB repressor IκBα, which consequently augments both nuclear translocation of NF-κB and its binding to the *Hdac2* promoter in frontal cortex pyramidal neurons – an atypical antipsychotic-induced epigenetic event that occurred in association with up-regulation of HDAC2 in CaMKIIα-positive frontal cortex pyramidal neurons (Fig. 1). As previous data also suggested that chronic treatment with the atypical antipsychotic clozapine reduces the density of mature spines, and impairs cognition through a HDAC2-dependent mechanism (Ibi *et al.*, 2017; de la Fuente Revenga *et al.*, 2018b), together, these observations suggest that the NF-κB→HDA2 pathway via 5-HT_{2A}R may be responsible for the deleterious effects of these drugs in terms of synaptic frontal cortex synaptic remodeling and cognitive function, and that HDAC2 may represent a novel target to improve synaptic plasticity and cognition in treated schizophrenia patients.

Bivalent ligands for the 5-HT_{2A}R-mGluR2 complex

An attractive strategy to enable selective targeting of a GPCR heteromeric complex over its two parent receptors is the design of a heterobivalent ligand, which comprises two receptor-selective “head” groups separated by a chemical spacer of suitable length and flexibility (Berque-Bestel *et al.*, 2008; Shonberg *et al.*, 2011; Huang *et al.*, 2021a). In theory, bivalent ligands successfully bridging two binding sites of adjacent protomers should confer extremely high affinity (resulting from the total binding energy of two recognition elements) and thus selectivity for the GPCR heteromer. The bivalent ligand strategy was pioneered by Portoghese and colleagues in their development of selective ligands for studies of opioid receptor dimerization (Erez *et al.*, 1982; Portoghese *et al.*, 1982; Portoghese *et al.*, 1986). Because of their selective recognition properties, bivalent ligands could be eventually used for a tissue-specific targeting of cells expressing an individual GPCR dimer. Toward this goal, numerous studies have explored the binding and signaling properties of new bivalent ligands targeting putative GPCR heterodimers/heterocomplexes, providing valuable insights into the quaternary structure of a GPCR dimer and the functional relevance of GPCR dimerization (Akgun *et al.*, 2013; Le Naour *et al.*, 2013; Hubner *et al.*, 2016; Poulie *et al.*, 2020; Huang *et al.*, 2021b; Romantini *et al.*, 2021; Pulido *et al.*, 2022).

Among these, synthesis of a series of heterobivalent ligands targeting the heteromeric 5-HT_{2A}R-mGluR2 complex based on the 5-HT_{2A}R antagonist M100907 and the mGluR2

PAM JNJ-42491293 deserved special attention (Poulie *et al.*, 2020). This combination of an orthosteric 5-HT_{2A}R antagonist with an mGluR2 PAM was selected because of the distance between the orthosteric binding sites within the VFT domain of the mGluR2 and the TM core of the 5-HT_{2A}R. In this paper, the authors showed a functional crosstalk between the two receptors in HEK293 cells co-expressing 5-HT_{2A}R and mGluR2. This was validated in cells co-expressing 5-HT_{2A}R and mGluR2 and the chimeric Gα protein G_{qo5} that directs $G\alpha_{i/o}$-signaling of mGluR2 into the $G\alpha_{q/11}$-dependent pathway and intracellular Ca^{2+} mobilization. However, as for the seven bivalent ligands designed for the putative 5-HT_{2A}R-mGluR2 complex, the authors did not observe a definitive correlation between the functional potency and spacer length of the ligands (Poulie *et al.*, 2020). The most obvious molecular reason behind this lack of simultaneous interaction with the two different binding sites is their use of short spacer with lengths ranging from 3 to 12 polyethylene glycol (PEG) units. It also remains unknown whether these bivalent ligands truly act by simultaneously targeting binding sites in both receptors of the 5-HT_{2A}R-mGluR2 heteromer, or whether they have preference for the orthosteric 5-HT_{2A}R and/or the allosteric mGluR2 sites and thus mediate their functional outcomes exclusively through binding to one of the two receptors. An additional alternative molecular mechanism to explain crosstalk phenotypes observed upon bivalent ligand administration in cells co-expressing two GPCRs may not require GPCR heteromerization but binding of each of the two pharmacophoric heads of the bivalent ligand to two GPCRs that are not in close molecular proximity. This is an important point to address experimentally since, as an example, previous findings reported that agonists targeting $GABA_BR$ or mGluR1 lead to crosstalk between these two class C GPCR through a mechanism that does not require their heteromerization (Rives *et al.*, 2009).

5-HT_{2A}R-mGluR2 in postmortem schizophrenia brain samples

Several previous studies have tested the level of expression and density of the 5-HT_{2A}R in postmortem human brain samples of schizophrenia subjects and controls, particularly in the frontal cortex. Most of these assays were carried out with radioligands including the 5-HT_{2A}R antagonist [^{3}H]ketanserin (Reynolds *et al.*, 1983; Mita *et al.*, 1986; Laruelle *et al.*, 1993; Burnet *et al.*, 1996; Dean and Hayes, 1996; Dean *et al.*, 1996; Dean *et al.*, 1998; Dean *et al.*, 1999; Marazziti *et al.*, 2003; Matsumoto *et al.*, 2005; Dean *et al.*, 2008; Gonzalez-Maeso *et al.*, 2008; Kang *et al.*, 2009; Muguruza *et al.*, 2013) and the 5-HT_{2A}R agonist [^{125}I]LSD (Bennett *et al.*, 1979; Whitaker *et al.*, 1981; Joyce *et al.*, 1993; Gurevich and Joyce, 1997). More recent findings also evaluated 5-HT_{2A}R density by positron emission tomography (PET) with [^{18}F]altanserin in drug-naïve first-episode schizophrenia patients (Rasmussen *et al.*, 2010). Intriguingly, there are obvious differences in the results obtained: some studies suggested up-regulation of 5-HT_{2A}R density whereas other groups proposed absence of alterations or down-regulation in the number of 5-HT_{2A}R binding sites. One of the principal reasons behind these differences may be related to demographic conditions such as age, since 5-HT_{2A}R density correlates negatively with aging (Gonzalez-Maeso *et al.*, 2008; Muguruza *et al.*, 2013), and some of the studies in postmortem human brain samples included control subjects that were not individually matched by age with the schizophrenia group. Additionally, chronic treatment with atypical antipsychotics down-regulates 5-HT_{2A}R receptor density in brain regions such as the frontal cortex (Kurita *et al.*, 2012; Moreno *et*

al., 2013), and some of the studies in postmortem human brain samples did not evaluate the presence of antipsychotic medications in postmortem toxicological analysis as an independent experimental variable (for a review article, see (Gonzalez-Maeso and Sealfon, 2009b)). In suicide victims with other psychiatric conditions including dysthymia, alcohol use disorder and anorexia nervosa, 5-HT_{2A}R density did not differ as compared to controls (Muguruza *et al.*, 2013)

Consequently, a potential pharmacological explanation related to these differences in 5-HT_{2A}R density in frontal cortex samples from schizophrenia subjects as compared to controls is related to the functional properties of the radioligand used to assess 5-HT_{2A}R. As mentioned above, agonists present a higher affinity for the active conformation of the receptor, whereas inverse agonists bind with higher affinity to the inactive conformation of the receptor. Recent findings tested 5-HT_{2A}R density in the same cohort of postmortem human frontal cortex samples of schizophrenia subjects and controls individually matched by sex and age using three separate 5-HT R radioligands: [^{18}F]altanserin (inverse agonist), [^{3}H]LSD (agonist) and [^{3}H]MDL100907 (antagonist) (Diez-Alarcia *et al.*, 2021). The authors reported that 5-HT_{2A}R density (B_{max}) was reduced, increased, and unaffected in schizophrenia subjects when binding assays were carried out with [^{18}F]altanserin, [^{3}H]LSD, and [^{3}H]MDL100907, respectively (Fig. 2). Based on these findings, it was proposed that the fraction of the active conformation of the 5-HT_{2A}R could be increased in the frontal cortex of schizophrenic subjects as compared to controls, which would also provide the basis for the differences in 5-HT_{2A}R densities upon binding with radioligands of different functional characteristics. This concept of alterations in the functional properties of the 5-HT_{2A}R in schizophrenia subjects is further supported by findings suggesting a higher stimulation of G proteins by the 5-HT_{2A}R agonist DOI (Garcia-Bea *et al.*, 2019) (Fig. 3), as well as the increase in the fraction of high-affinity binding sites of DOI displacing [^{3}H]ketanserin binding in postmortem frontal cortex samples of antipsychotic-free schizophrenia subjects (Muguruza *et al.*, 2013).

Potential alterations in the 5-HT_{2A}R-mGluR2 complex have also been observed in postmortem frontal cortex samples of schizophrenia subjects. The authors compared the effect of DOI on displacement of [^{3}H]LY341495 binding by LY379268 in postmortem frontal cortex samples of schizophrenia subjects and controls (Moreno *et al.*, 2012). As expected, competition of[^{3}H]LY341495 binding by LY379268 was best described by a two-site model. Additionally, and supporting previous findings in vitro in tissue culture and in mouse frontal cortex samples (Gonzalez-Maeso *et al.*, 2008), DOI decreased the high affinity component of LY379268 displacing [^{3}H]LY341495 in both schizophrenia subjects and controls. Interestingly, it was also reported that the difference between the high affinities of LY379268 in the presence and in the absence of DOI was increased in postmortem frontal cortex samples of schizophrenia subjects as compared to controls (Moreno *et al.*, 2012) (Fig. 4). Related to the functional crosstalk between 5-HT_{2A}R and mGluR2 in native tissue, it was previously reported that LY379268 was able to activate both $G_{q/11}$ and $G_{i1,2,3}$ proteins in mouse frontal cortex samples. However, when this functional outcome was tested in the frontal cortex of 5-HT_{2A}R-KO animals, LY379268 induced [^{35}S]GTPγS binding via $G_{i1,2,3}$ whereas the effect of LY379268 on $G_{q/11}$-dependent[^{35}S]GTPγS binding was eliminated (Moreno *et al.*, 2016). These findings suggest that mGluR2 activates $G_{q/11}$-dependent

signaling pathways via 5-HT_{2A}R. This signaling crosstalk between mGluR2 and 5-HT_{2A}R was also observed in frontal cortex samples of controls subjects – LY379268 induced both $G_{q/11}$ - and $G_{i1,2,3}$-dependent [S]GTPγS binding. However, the effect of LY379268 on $G_{q/11}$ activation was reduced in frontal cortex samples from schizophrenia subjects, whereas the LY379268-dependent effect on $G_{i1,2,3}$ coupling was unaffected (Moreno *et al.*, 2016). These findings could be relevant for the elucidation of mechanisms underlying psychosis and antipsychotic treatment response in schizophrenia patients.

Concluding remarks and future directions

Several lines of evidence suggest the existence of a close functional interplay between 5-HT_{2A}R and mGluR2 receptors (Fig. 5). They regulate each other at an epigenetic level and via postsynaptic mechanisms. The heteromeric GPCR complex formed by 5-HT_{2A}R and mGluR2 has also been involved in signaling and neural circuit processes related to clinically relevant effects of psychedelics and antipsychotics. Several questions remain open for further study including the molecular stability of these two GPCRs as a heterocomplex, and the subcellular location where the two protomers of the 5-HT_{2A}R-mGluR2 complex come to a stage of physical proximity. Similarly, additional investigation will be required to determine the potential role of the 5-HT_{2A}R-NF-κB-HDAC2 pathway in the epigenetic control of mGluR2-dependent clinical outcomes. The majority of the preclinical studies focused on 5-HT_{2A}R-dependent mechanisms were carried out almost exclusively in male rodent models, yet recent findings indicate the importance of studying sex as a biological variable (Vohra *et al.*, 2021; Jaster *et al.*, 2022; Sierra *et al.*, 2022; Alper *et al.*, 2023). Although it is beyond the scope of this review, it is worth mentioning that accumulating data mainly from the area of placebo analgesia within the opioid-induced anti-nociception field clearly shows that the effectiveness of a placebo is often related to a subject's expectations for pain relief (Pecina and Zubieta, 2015). This placebo effect has also been observed in the effect of the deliriant drug scopolamine on disruption of learned behavior in rats (Herrnstein, 1962). The lack of a valid placebo group in all the clinical studies testing the effects of classical psychedelics in patients with psychiatric conditions particularly depression – together with expectancy effects (Butler *et al.*, 2022) and the wish of some of the participants to experience a psychedelic trip – leaves open the question of the effects of psychedelics by themselves on therapeutic outcomes.

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Highlights:

- 5-HT_{2A}R-mGluR2 heteromeric complex
- 5-HT_{2A}R-mGluR2 in postmortem schizophrenia brain samples
- Epigenetic crosstalk between 5-HT_{2A}R and mGluR2
- Bivalent ligands for the 5-HT_{2A}R-mGluR2 complex

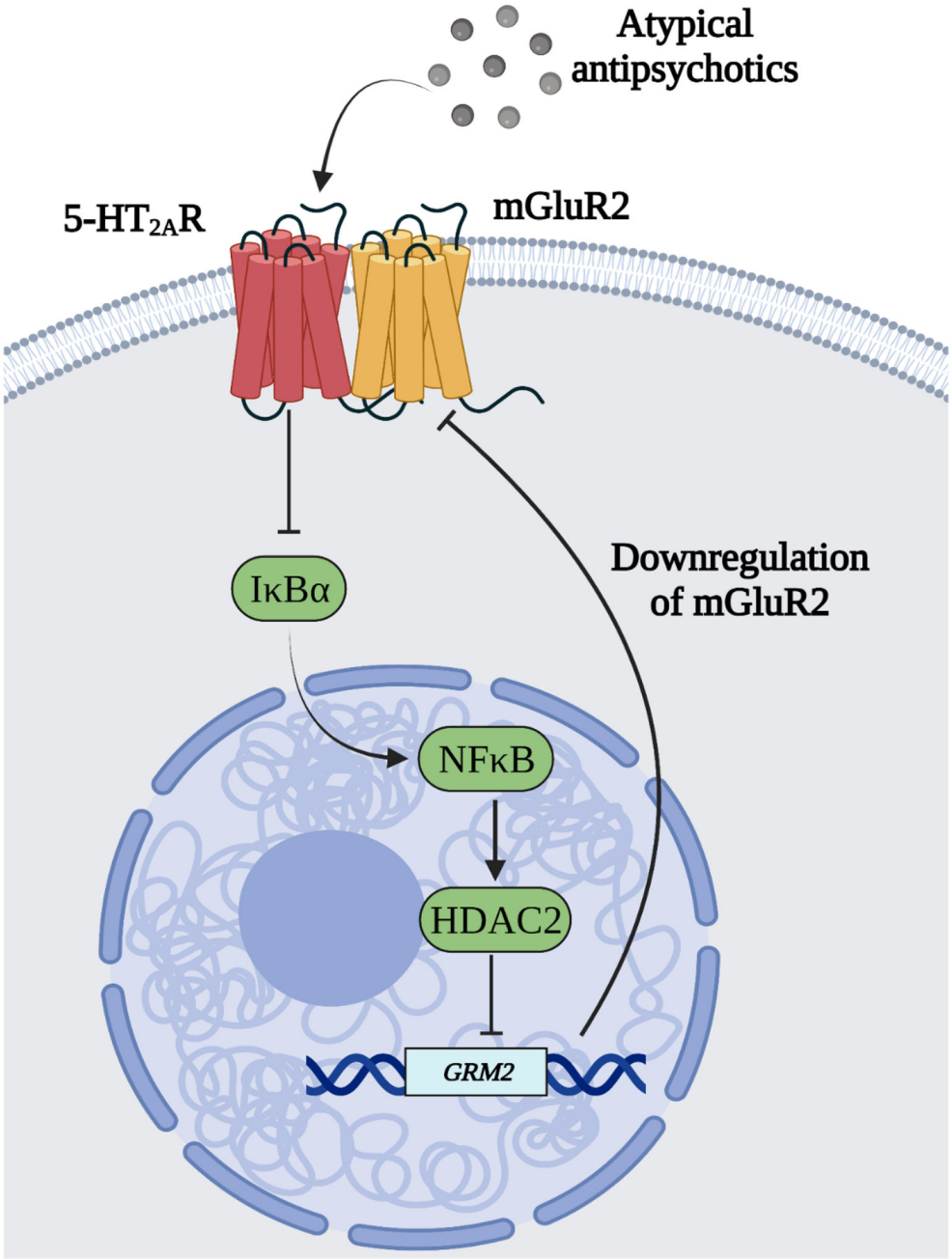

Fig. 1.

HDAC2-dependent regulation of *mGluR2* transcription upon chronic atypical antipsychotic treatment. Chronic atypical antipsychotic treatment decreases density of 5-HT_{2A}R in the frontal cortex, which also leads to down-regulation of the NF-κB repressor IκBα. This pathway induces NF-κB-dependent augmentation of HDAC2 expression and, consequently, increased binding of HDAC2 to the *mGluR2* promoter. Together, these findings suggest that disinhibition of the NF-κB pathway by chronic treatment with atypical, but not typical, antipsychotics induces HDAC2-dependent repressive histone modifications at the *mGluR2* promoter, which consequently restricts the antipsychotic effects of mGluR2/3 agonists.

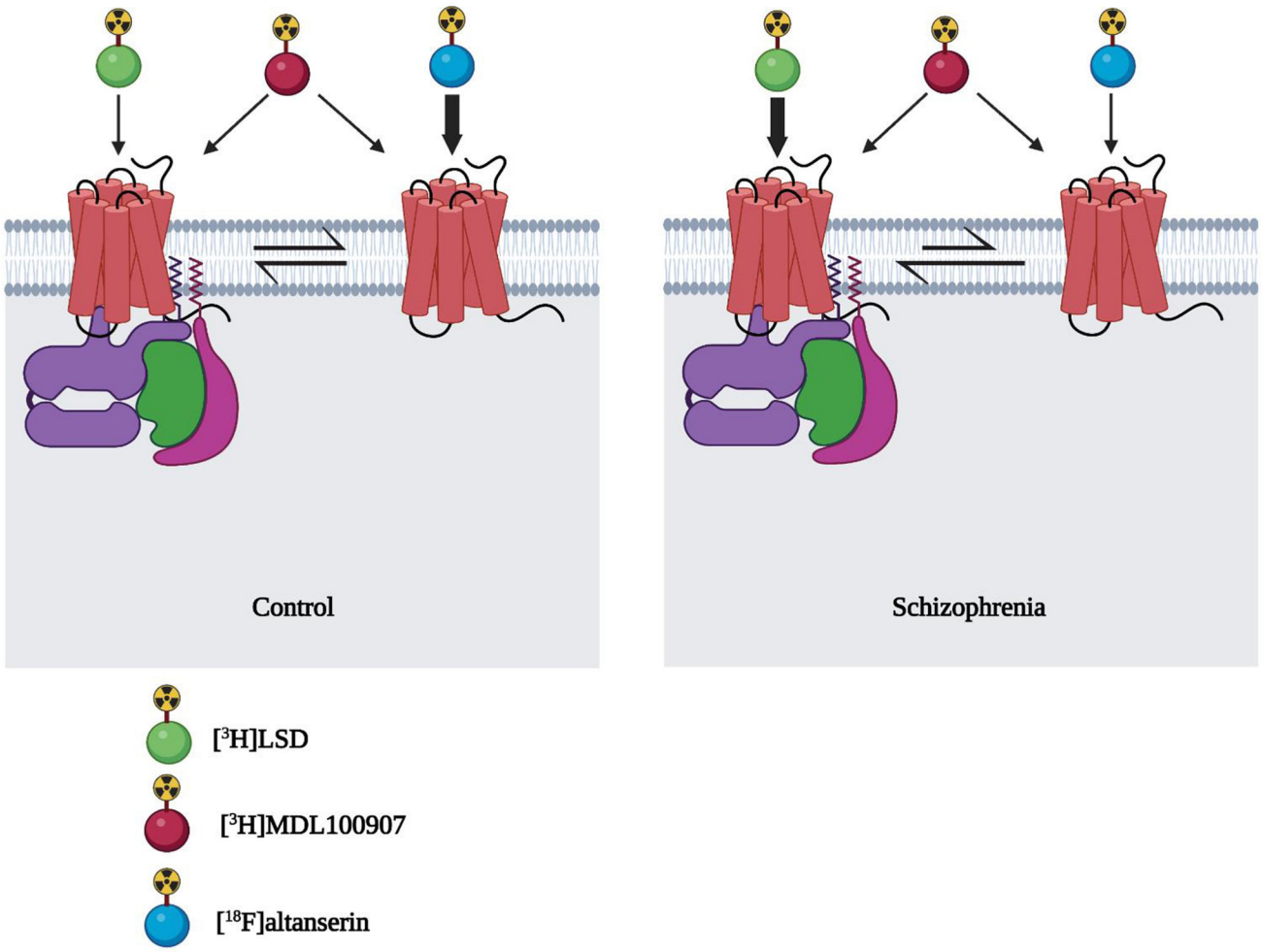

Fig. 2.

Binding assays with the 5-HT_{2A}R agonist [^{3}H]LSD, antagonist [^{3}H]M100907 and inverse agonist [^{18}F]altanserin show increased, unaffected and decreased 5-HT_{2A}R densities, respectively, in frontal cortex samples of schizophrenia subjects. These divergent receptor density values may be explained by their preferences in terms of affinity for the active vs inactive conformations of the 5-HT_{2A}R.

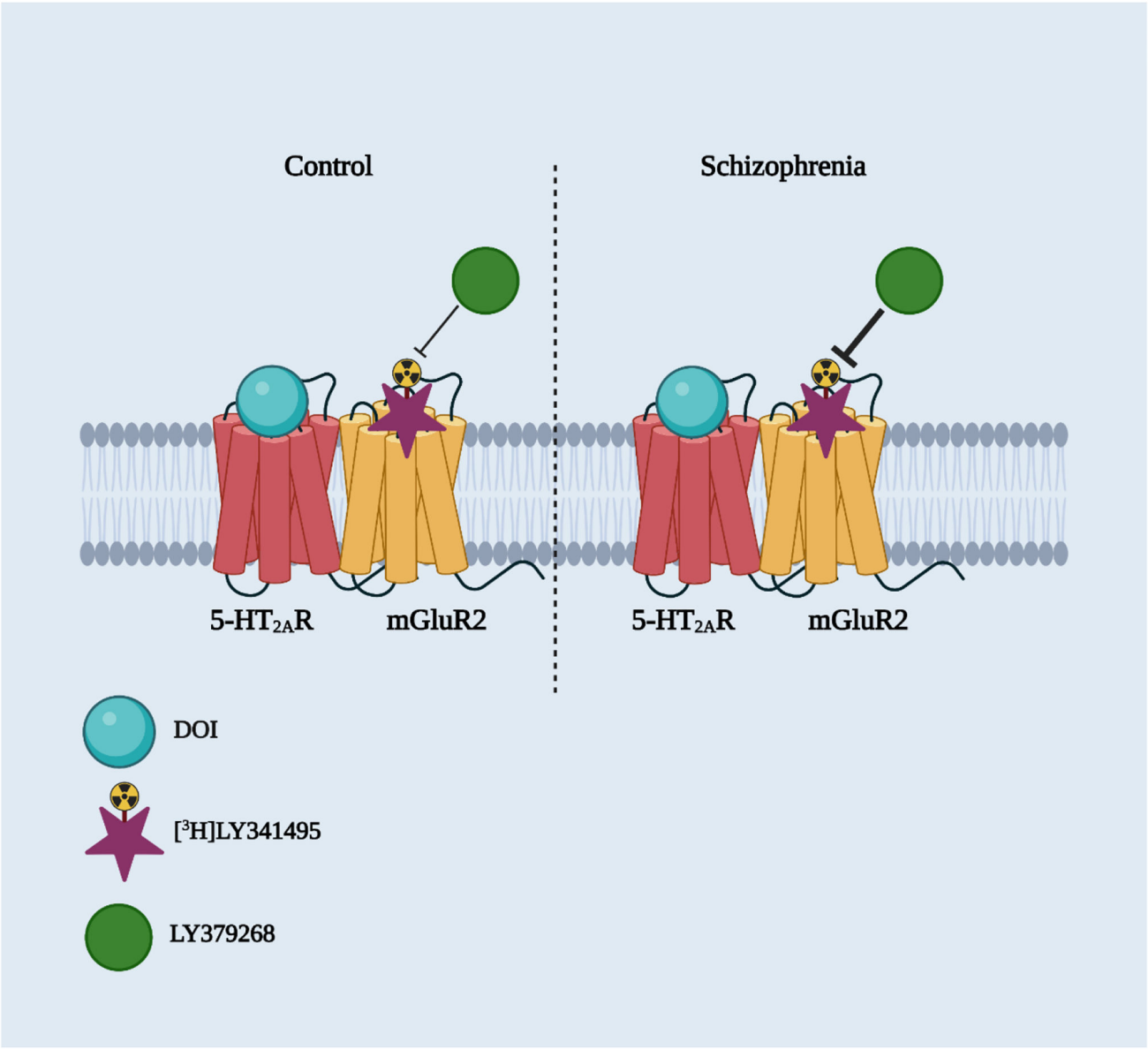

Fig. 3.

The allosteric effect of the 5-HT_{2A}R agonist DOI on the affinity of LY379268 displacing the mGluR2/3 antagonist [^{3}H]LY341495 is increased in frontal cortex samples of schizophrenia subjects. This suggests that the functional crosstalk between 5-HT_{2A}R and mGluR2 as a GPCR heterocomplex may be involved in the altered cortical processes in schizophrenia patients.

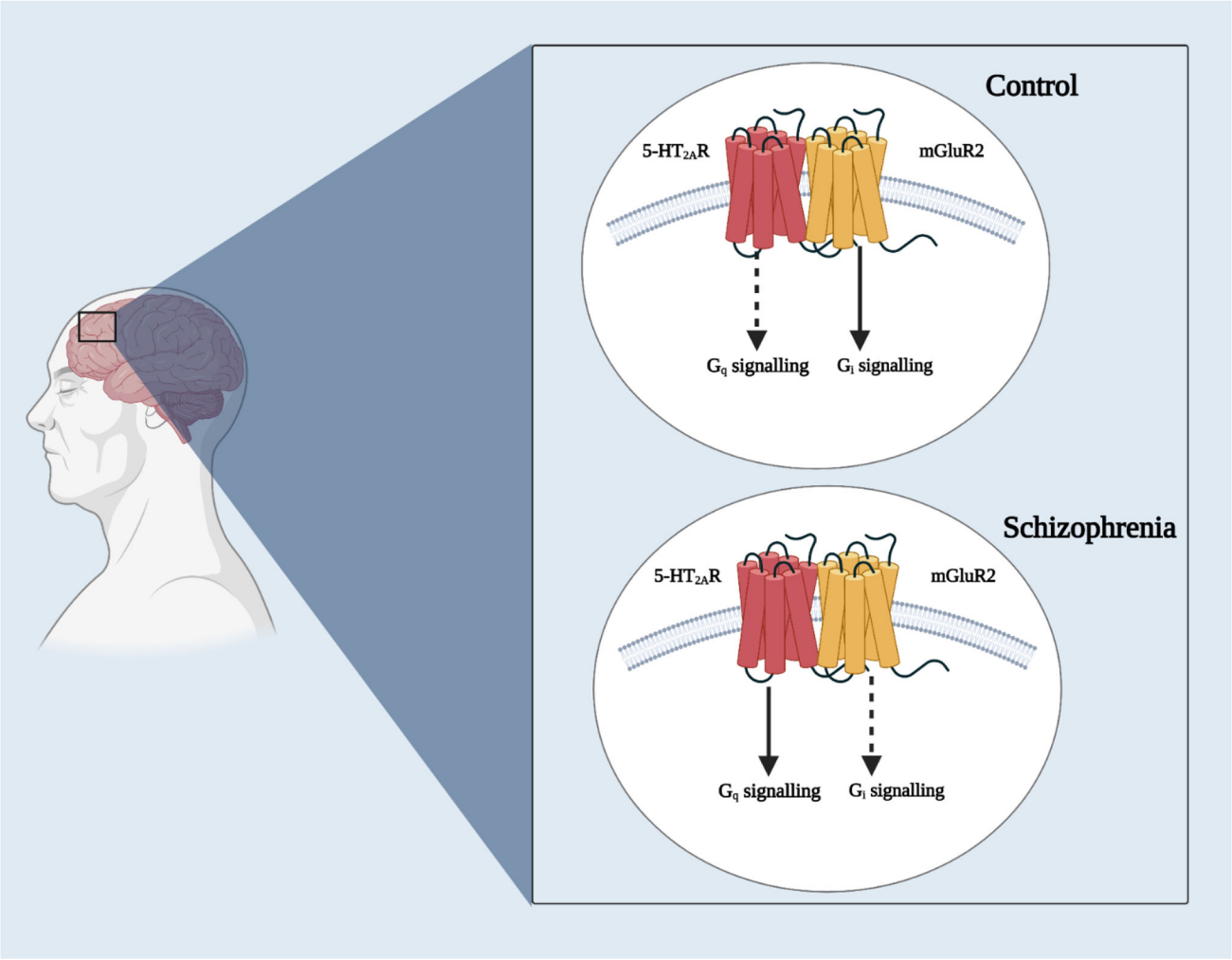

Fig. 4.

Alterations of G protein coupling to the 5-HT_{2A}R-mGluR2 complex in the frontal cortex of schizophrenia subjects. Activation of $G_{q/11}$ proteins by the mGluR2/3 agonist LY379268 potentially via 5-HT_{2A}R is reduced in postmortem frontal cortex membrane preparations from schizophrenia subjects, whereas the LY379268-dependent activation of $G_{i1,2,3}$ proteins is unaffected.

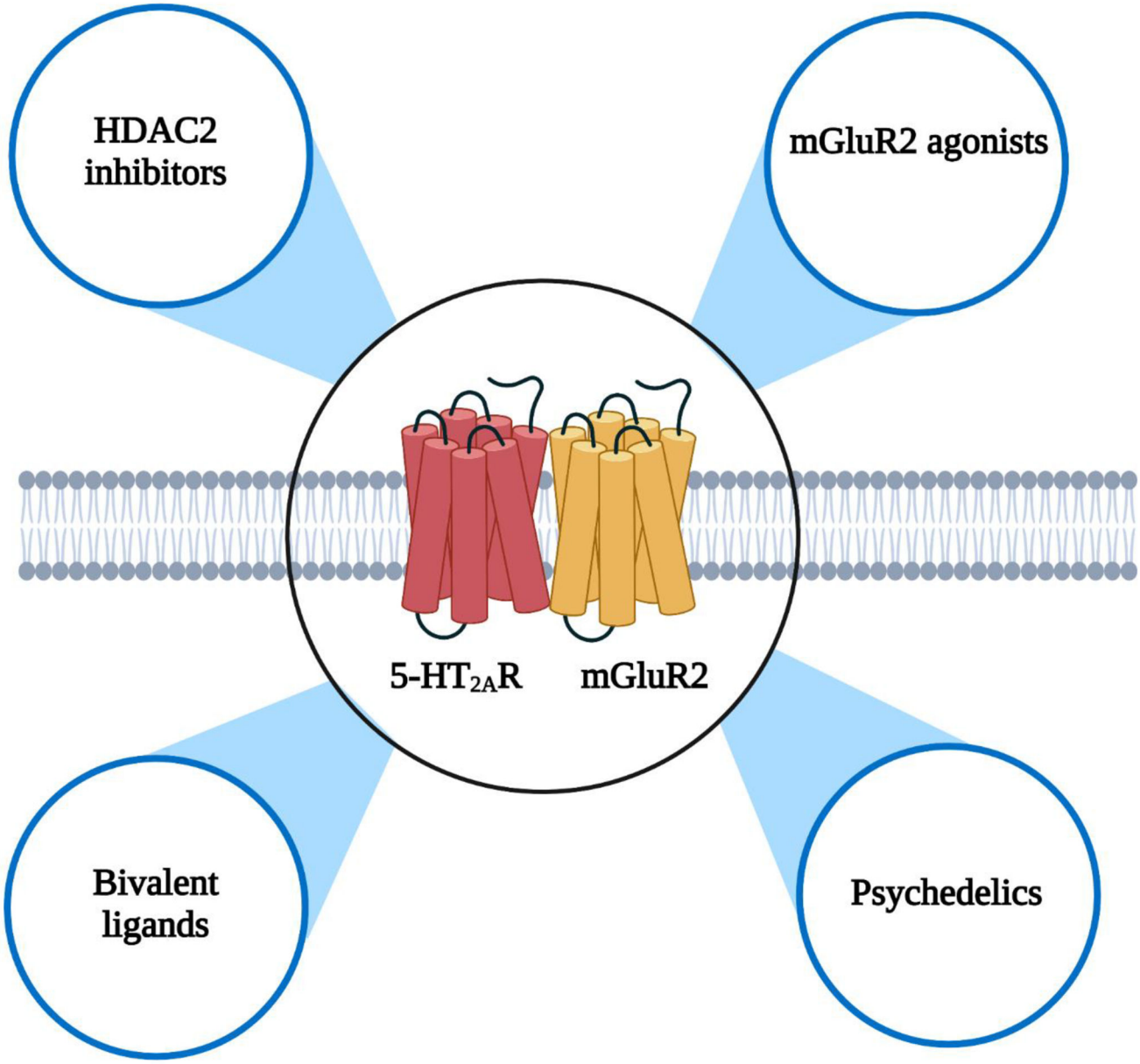

Fig. 5.

Pharmacological strategies to target the 5-HT_{2A}R-mGluR2 complex. Potential routes to affect the crosstalk between 5-HT_{2A}R and mGluR2 include i) HDAC2 inhibitors to prevent the 5-HT_{2A}R-dependent epigenetic modifications at the *mGluR2* promoter upon chronic antipsychotic treatment, ii) orthosteric and allosteric mGluR2 agonists as antipsychotics, iii) classical psychedelics as fast-acting antidepressants, and iv) bivalent ligands composed of 5-HT_{2A}R antagonists and mGluR2 PAMs as a pharmacological tool to treat schizophrenia.