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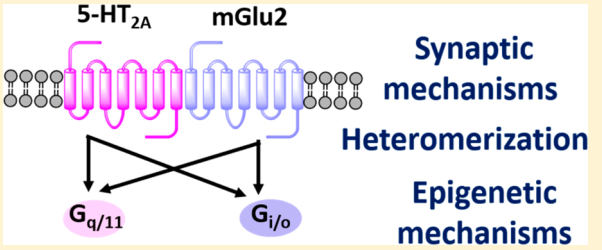
Serotonin and Glutamate Interactions in Preclinical Schizophrenia Models

Urjita H. Shah and Javier González-Maeso*

Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, Virginia 23298, United States

ABSTRACT: The serotonergic and glutamatergic neurotransmitter systems have both been implicated in the pathophysiology of schizophrenia, and there are multiple lines of evidence to demonstrate that they can interact in a functionally relevant manner. Particularly, it has been demonstrated that serotonin (5-hydroxytryptamine) 2A (5-HT_{2A}) receptors and metabotropic glutamate type 2 (mGlu2) receptors can assemble into a functional heteromeric complex and modulate each other's function. This heteromeric complex has been implicated in the mechanism of action of hallucinogens as well as antipsychotic agents, and its role has been demonstrated in both *in vitro* and *in vivo* systems. Additionally, the difference in the changes in $G_{i/o}$ and $G_{q/11}$ protein activity when a ligand binds to the heteromeric complex can be used as an index to predict the pro- or antipsychotic properties of an agent. Signaling via the heteromer is dysregulated in postmortem human brain samples of schizophrenia subjects, which may be linked to altered cortical functions. Alternative routes for the functional crosstalk between mGlu2 and 5-HT_{2A} receptors include synaptic and epigenetic mechanisms. This Review highlights the advances made over the past few years in elucidating the structural and functional mechanisms underlying crosstalk between 5-HT_{2A} and mGlu2 receptors in preclinical models of schizophrenia.



KEYWORDS: *5-HT_{2A} receptors, mGlu2 receptors, G protein-coupled receptor (GPCR) heteromerization, functional crosstalk, schizophrenia, psychosis, antipsychotics, hallucinogens, psychedelics, lysergic acid diethylamide (LSD)*

INTRODUCTION

Schizophrenia is a chronic psychotic illness that affects more than 1% of the global population.[1−5] The symptoms of schizophrenia surface during late adolescence and continue throughout adult life, and they can be divided into three categories: positive symptoms, characterized by hallucinations and delusions; negative symptoms, such as lack of affect, anhedonia, and avolition; and cognitive deficits, related to working memory and executive functions.[6−10] Currently available antipsychotic drugs ameliorate positive symptoms in certain groups of schizophrenia patients, yet they lack clinical effects on negative or cognitive symptoms.

Modeling psychiatric disorders in rodents is challenging, considering that most of the symptoms are evaluated on the basis of a subjective evaluation by the clinician, there is a lack of biomarkers for both diagnosis and prediction of clinical efficacy, such as those routinely used in cancer patients, and there are important limitations of behavioral models of certain psychiatric symptoms, particularly hallucinations and delusions.[11−15] However, several molecular mechanisms and neural circuits have been proposed to link altered brain function and schizophrenia,[7,16,17] with dopaminergic[18] and serotonergic hypothesis[16] receiving maximum attention. According to the dopamine hypothesis, dopaminergic dysfunction is at the presynaptic control level, and psychosis in schizophrenia is the result of dopaminergic dysregulation due to the interaction of "multiple hits" such as fronto-temporal dysfunction, stress, genes, and drugs.[18−20] The dopamine hypothesis of schizophrenia mainly explains the psychotic symptoms associated with schizophrenia, yet it does not address the negative and cognitive alterations of psychiatric disorder. According to the serotonin hypothesis of schizophrenia, a stress-induced serotonergic overdrive from the dorsal raphe nucleus can disrupt cortical neuronal function. This, along with the hyperactivity of 5-HT in the cerebral cortex, has been proposed as one of the upstream causes of schizophrenia.[16] This is further supported by the relatively recent approval of pimavanserin, a highly selective 5-HT_{2A} receptor antagonist, to treat hallucinations and delusions associated with psychosis in patients with Parkinson's disease.[21] Most currently available antipsychotic drugs act via the dopaminergic and serotonergic systems.[22−24] However, more recent data implicate the glutamatergic system as well.[25−29] The glutamate hypothesis suggests that the positive symptoms of schizophrenia can be linked to excessive release of DA from neurons in the mesolimbic pathway due to an abnormal functioning of the GLU-GABA-GLU-DA circuit. The negative and cognitive symptoms of schizophrenia can be attributed to an insufficient release of DA in the mesocortical region due to malfunctioning

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of the GLU-GABA-GLU-GABA-DA neuronal circuit.[30] Metabotropic glutamate receptor type 2/3 (mGlu2/3) orthosteric agonists as well as mGlu2 receptor positive allosteric modulators (PAMs) have been shown to possess antipsychotic-like character in rodent models and certain clinical studies.[31−34]

Psychoactive drugs such as hallucinogens (also known as psychedelics) lysergic acid diethylamide (LSD), psilocybin (and its active compound psilocin), and *N*,*N*-dimethyltryptamine are agonists/partial agonists at 5-HT_{2A} receptors and produce in healthy volunteers symptoms that resemble and share similarities with the core symptoms of schizophrenia.[35−39] Based on this, psychedelic-based models were some of the earliest ones used to model schizophrenia.[12,40] There is a good correlation between serotonin 2 (5-HT_2) receptor binding affinities (now known as 5-HT_{2A} receptors)[41] and hallucinogenic potencies of psychoactive drugs such as LSD and *N*,*N*-dimethyltryptamine in humans.[42] This coupled with the higher affinity of atypical antipsychotic agents for 5-HT_{2A} receptors as compared to D_2 receptors led to the serotonin hypothesis of schizophrenia with 5-HT_{2A} receptors being recognized as key players.[12,15,40] 5-HT_{2A} receptors are coupled to $G_{q/11}$ proteins, and activation of the receptor leads to hydrolysis of membrane phosphoinositides by the activation of phospholipase C (PLC) into diacylglycerol (DAG) and inositol phosphates. Inositol phosphates result in an increase in intracellular calcium levels and the subsequent activation of inwardly rectifying chloride channels, whereas DAG activates other downstream effectors such as protein kinase C.[41,43−45]

Glutamatergic neurotransmission is modulated by heterotrimeric G protein-coupled metabotropic glutamate receptors as well as ligand gated ion channels—kainate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and *N*-methyl-D-aspartate (NMDA) receptors.[46] Drugs that are noncompetitive NMDA receptor antagonists, such as the dissociative compounds phencyclidine (PCP) and ketamine, can resemble some of the negative, positive, and cognitive symptoms in healthy human subjects.[47−55] This suggests that there is a dysregulation of the glutamatergic system in schizophrenia, and consequently dissociative drugs are routinely used to model schizophrenia-related phenotypes in rodents.[15,56] Metabotropic glutamate 2/3 (mGlu2/3) receptor orthosteric agonists as well as mGlu2 receptor PAMs have antipsychotic-like properties and can reduce the behavioral effects such as hyperlocomotion and working-memory deficits induced by NMDA receptor antagonists.[15,31,34,57,58] mGlu2/3 receptors couple to $G_{i/o}$, and their activation leads to an inhibition of adenylyl cyclase.[32,59]

Recent studies have demonstrated that there is a close interplay between the serotonergic and glutamatergic systems that is relevant to schizophrenia and its treatment. Particularly, the 5-HT_{2A} and mGlu2 receptors can regulate each other in a functionally antagonistic manner. This crosstalk between 5-HT_{2A} and mGlu2 receptors occurs at various levels, and therefore it either could be due to synaptic mechanisms, regulation of downstream effectors, or modulation of epigenetic and genetic factors or might be a result of the assembly of 5-HT_{2A} and mGlu2 receptors into a heteromeric complex.[60−63] This Review focuses on recent advances in the field that demonstrate interactions between the serotonergic and glutamatergic systems, particularly 5-HT_{2A} and mGlu2 receptors, in preclinical models of schizophrenia and antipsychotic drug action.

FIRST EVIDENCE OF 5-HT_{2A} AND mGlu2 RECEPTOR CROSSTALK

Marek, Aghajanian, et al.[60,64,65] were the first to demonstrate that 5-HT_{2A} and mGlu2/3 receptors have an overlapping pattern of distribution in the medial prefrontal cortex and interact in a manner that is functionally antagonistic to one another. They demonstrated that LY293558, an AMPA receptor antagonist, 5-HT_{2A} receptor antagonists such as SR46349B and MDL100907 as well as the metabotropic glutamate receptor agonist (1*S*,3*S*)-aminocyclopentane-1,3-dicarboxylate can suppress the increase in amplitude and frequency of the spontaneous excitatory postsynaptic currents/potentials (EPSCs/EPSPs) induced by serotonin (5-HT) in layer V pyramidal cells (in neocortex and the transitional cortex) in rat brain slices.[64,65] This suggested that agonism at 5-HT_{2A} receptors results in a release of glutamate. Additionally, they have shown that activation of mGlu2/3 receptors by selective group II agonists, such as LY379268, LY354740, can suppress the EPSCs that occur as a result of activation of 5-HT_{2A} receptors by 5-HT in layer V pyramidal cells in the medial prefrontal cortex. This effect of LY354740 can be blocked by LY341495, an mGlu2/3 receptor antagonist/inverse agonist. LY341495 can also enhance the frequency and amplitude of the EPSCs induced by activation of 5-HT_{2A} receptors by 5-HT in layer V pyramidal cells in the medial prefrontal cortex.[60] Modulation of serotonin-dependent glutamate release in the frontal cortex was hypothesized to be negatively modulated via activation of mGlu2/3 receptors located presynaptically in the frontal cortex (Figure 1).[60] These electrophysiological studies[60,64,65] provided the first lines of evidence that 5-HT_{2A} and mGlu2 receptors can antagonize each other's effects in a physiological setting.

IDENTIFICATION OF THE 5-HT_{2A}-mGlu2 RECEPTOR HETEROCOMPLEX

The role of oligomerization in Class C GPCR function is well accepted with many Class C GPCRs such as the mGlu and $GABA_B$ receptors being obligatory homo-/heterodimers.[59,66]

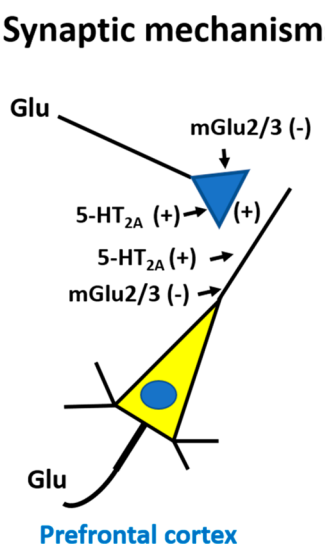

Figure 1. Synaptic mechanisms of allosteric crosstalk between 5-HT_{2A} and mGlu2 receptors. Activation of presynaptic mGlu2/3 receptors can negatively modulate serotonin-dependent glutamate release in the prefrontal cortex.

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However, the role of oligomerization remains controversial with regards to Class A GPCRs and does not seem to be necessary for the functional coupling of Class A GPCRs to their G proteins.[67] The existence of Class A GPCR dimers/oligomers has been questioned and is a subject of controversy even though physiologically/pharmacologically relevant non-covalent dimers/oligomers of Class A GPCRs have been detected.[68−70]

The 5-HT_{2A} and mGlu2 receptor crosstalk was initially attributed to synaptic mechanisms (Figure 1),[60] and the role of $G_{i/o}$ proteins in mediating hallucinogenic responses was unclear. However, co-localization of the *mGlu2* and *5-HT_{2A}* receptor mRNAs in cortical cultures of primary neurons led to the hypothesis that these receptors might interact and crosstalk by assembling into a heteromeric receptor complex (Figure 2).[61] The existence of this complex was demonstrated using a combination of approaches in cells as well as in mouse and post-mortem human brain samples. The 5-HT_{2A} and mGlu2 receptors co-immunoprecipitated in human post-mortem brain samples, as well as in HEK293 cells transfected with tagged 5-HT_{2A} and mGlu2 receptors, which suggested that the receptors were a part of the same protein complex. Biophysical approaches such as bioluminescence resonance energy transfer (BRET) and Förster resonance energy transfer (FRET) in HEK293 cells transfected with appropriate 5-HT_{2A} and mGlu2 receptor constructs also indicated that the receptors were in close molecular proximity.[61] The existence of the 5-HT_{2A}-mGlu2 receptor heteromeric complex has also been validated by other groups.[71−74] The 5-HT_{2A} and mGlu2 receptors have an overlapping pattern of distribution in the medial prefrontal cortex, and their ability to heteromerize provides an alternative, although not mutually exclusive, mechanism for the allosteric crosstalk that was previously hypothesized to occur via synaptic mechanisms (Figures 1 and 2).[60,61,64,65]

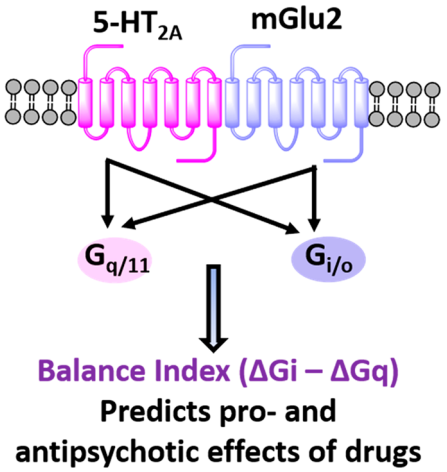

Figure 2. 5-HT_{2A} and mGlu2 receptors can assemble into a heteromeric complex. Binding of a ligand to the complex differentially modulates $G_{i/o}$ and $G_{q/11}$ coupling of these receptors. The difference in the changes in $G_{i/o}$ and $G_{q/11}$ coupling (balance index) can be used to predict the pro- or antipsychotic effects of a drug.

5-HT_{2A}-mGlu2 RECEPTOR HETEROMERIC COMPLEX IN THE MOUSE FRONTAL CORTEX

5-HT_{2A} and mGlu2 receptors co-immunoprecipitate in membrane preparations from human post-mortem brain samples[61] as well as from the mouse frontal cortex,[75] suggesting that they are a part of the same protein complex. As mentioned above, autoradiography studies suggest that 5-HT_{2A} and mGlu2/3 receptors have an overlapping pattern of distribution in the cortex.[60] *5-HT_{2A}* and *mGlu2* receptor mRNAs were found to co-localize in layer V mouse somatosensory cortex as well as in cortical cultures of primary neurons.[61] Additionally, 5HT_{2A} and mGlu2 receptor immunoreactivity co-localize in neuronal primary cultures from mice as well as in the mouse frontal cortex.[75] Ultrastructural localization of mGlu2 and 5-HT_{2A} receptors was studied using electron microscopy and labeled receptors were found to be located in close subcellular proximity at or near synaptic junctions in the mouse frontal cortex.[76] A subcellular fractionation approach was used to purify fractions of the mouse frontal cortex that were enriched in presynaptic active zone (PAZ) proteins and postsynaptic density (PSD) proteins. mGlu2 receptors were detected in both PSD and PAZ fractions, whereas 5-HT_{2A} receptors were detected only in the PSD fractions.[62] All of these data together collectively suggest that 5-HT_{2A} and mGlu2 receptors may assemble into a heteromeric complex at the PSD in the mouse frontal cortex.[61,62,75,76]

ALLOSTERIC CROSSTALK BETWEEN 5-HT_{2A} AND mGlu2 RECEPTORS

A measure known as the balance index (BI) reflects the difference between changes in $G_{i/o}$ and $G_{q/11}$ activity in *Xenopus* oocytes when a ligand binds to mGlu2/5-HT_{2A} receptors that are a part of the heteromeric complex.[75] The BI is defined as $\Delta G_{i/o}$ - $\Delta G_{q/11}$. In the absence of stimulation, the BI is 1.45, suggesting that under homeostatic conditions there is higher $G_{i/o}$ signaling and lower $G_{q/11}$ signaling. Fribourg et al.[75] have demonstrated that the BI can be used as an indicator to predict pro- and antipsychotic properties of a ligand. Pro-psychotic agents have a lower BI, whereas antipsychotic agents have a higher BI. Antipsychotic and antipsychotic-like agents such as clozapine, risperidone, and LY379268 have a higher BI and might exert their antipsychotic effects via restoring the $G_{i/o}$-$G_{q/11}$ balance back to baseline (Figure 2).[75]

To study the allosteric crosstalk at the 5-HT_{2A}-mGlu2 receptor heteromeric complex in mammalian cells, HEK293 cells were stably transfected with mGlu2 and 5-HT_{2A} receptors as well as GIRK1/GIRK4 (a reporter of $G_{i/o}$ and $G_{q/11}$ signaling).[77] Although many of the clones expressed the heteromeric complex, only 25−30% of the clones stably transfected with mGlu2 and 5-HT_{2A} receptors were positive for allosteric crosstalk in all the functional assays used. The clones that were positive for functional crosstalk showed certain common phenotypes that included higher levels of co-localization of 5-HT_{2A} and mGlu2 receptors at the cell surface, lower mGlu2 and higher 5-HT_{2A} receptor binding site densities in total membrane preparations as assayed by radioligand binding assays, and a higher ratio of mGlu2/5-HT_{2A} receptor normalized surface expression.[77] This suggests that an optimal ratio of expression of mGlu2 to 5-HT_{2A} receptors is needed to elicit functional crosstalk at the heteromeric complex.[62,77] This might be one of the contributing factors as to why under certain experimental conditions heteromeric complex assembly between mGlu2 and 5-HT_{2A} receptors was observed, yet their allosteric crosstalk remained undetectable.[71]

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In HEK293 cells transfected to co-express mGlu2 and 5-HT_{2A} receptors, mGlu2/3 receptor agonists such as LY379268, can cause a $G_{q/11}$ protein-mediated release of intracellular calcium. mGlu3 and mGlu2ΔTM4 (an mGlu2 receptor chimeric construct that contains the transmembrane domain [TMD] 4 of the mGlu3 receptor) do not co-immunoprecipitate or heteromerize with 5-HT_{2A} receptors.[62] Calcium signal induced by LY379268 was absent in cells co-expressing mGlu3 and 5-HT_{2A} receptors, as well as in cells co-expressing mGlu2ΔTM4 and 5-HT_{2A} receptors. Additionally, LY379268 induced $G_{q/11}$ protein-mediated intracellular calcium release in cells co-expressing 5-HT_{2A} receptors and mGlu3ΔTM4,5 (a chimeric construct that contains the TM4 and TM5 of the mGlu2 receptor, and hence heteromerizes with 5-HT_{2A} receptors). Collectively, this suggests that LY379268-induced intracellular calcium release may be mediated via the 5-HT_{2A}-mGlu2 heteromeric complex.[62]

Mutants of 5-HT_{2A}(5-HT_{2A}I181D) and mGlu2 (mGlu2F765S) receptors that are incapable of activating G-proteins do not cross signal when co-expressed with either mGlu2 or 5-HT_{2A} receptors, respectively.[62] Hence, this functional crosstalk induced by LY379268 requires the activation of both $G_{q/11}$ by 5-HT_{2A} receptors and activation of $G_{i/o}$ by mGlu2 receptors.[62] Furthermore, LY379268-induced intracellular calcium release is dependent on $G\alpha_{i/o}$ but not on $G_{\beta\gamma}$, since a blocker of the $G\alpha_{i/o}$-subunit abolished calcium signal whereas a $G_{\beta\gamma}$-subunit blocking peptide did not.[62] mGlu2 receptor mutants that are unable to bind orthosteric agonists or mGlu2 receptor mutants that do not couple to G proteins do not lead to a release of intracellular calcium in HEK293 cells co-expressing 5-HT_{2A} and mGlu2 constructs upon LY379268 stimulation.[62] However, co-expression of an mGlu2 mutant that does not bind orthosteric agonists with an mGlu2 mutant that is deficient in G protein coupling, along with 5-HT_{2A} receptors, can rescue $G_{q/11}$ protein-mediated calcium signaling upon activation of mGlu2 receptors by LY379268.[62] Thus, Moreno et al.[62] demonstrated that a functional mGlu2 receptor homodimer that couples to G proteins is essential for allosteric crosstalk at the heteromeric complex. Additionally, it has been shown that the distal mGlu2 protomer within an mGlu2 homodimer needs to couple to G proteins for this allosteric crosstalk to occur.[62] This allosteric crosstalk between 5-HT_{2A} and mGlu2/3 receptors has also been demonstrated *in vivo* in mice. mGlu2/3 receptor agonists such as LY379268 can reduce DOI-stimulated PI hydrolysis by DOI in the frontal cortex of living mice.[78]

Murat et al.[79] have identified that phosphorylation of the mGlu2 receptor residue Ser843 is a key event in the functional crosstalk between mGlu2 and 5-HT_{2A} receptors. They have demonstrated that phosphorylation of Ser843 in mGlu2 receptors upon stimulation by LY379268 or 5-HT is increased only in cells co-expressing both 5-HT_{2A} and mGlu2 receptors. These results were also observed *in vivo* wherein LY379268 promoted phosphorylation of Ser843 in the prefrontal cortex of wildtype mice but not 5-HT_{2A} knockout (KO) mice. Additionally, phosphorylation induced by LY379268 could be blocked by the 5-HT_{2A} antagonist M100907, whereas phosphorylation induced by 5-HT_{2A} receptor agonists was blocked by the mGlu2/3 receptor antagonist LY341495. Cells co-expressing Ser843Ala mutants with 5-HT_{2A} receptors showed reduced $G_{i/o}$ signaling on mGlu2/5-HT_{2A} receptor stimulation suggesting that phosphorylation of Ser843 is required for this allosteric crosstalk.[79]

Additionally, Olivero et al.[72] have demonstrated that 5-HT_{2A} heteroreceptors and mGlu2 autoreceptors assemble into a heteromeric complex in the rat spinal cord glutamatergic terminals and have also shown that they interact in a functionally antagonistic manner.

STRUCTURAL FEATURES

5-HT_{2A} and mGlu2 Receptors. Metabotropic glutamate and 5-HT_{2A} receptors are Class C and Class A GPCRs, respectively. mGlu2 receptors consist of a Venus flytrap domain (VFD; houses the orthosteric site) that is linked to the TMDs (houses the allosteric sites) via a cysteine-rich domain (CRD).[59,80] It has been shown that the CRDs need to be oriented in a particular manner to transmit signal from the VFT to the TMs.[81] The functional unit of mGlu2 receptors is an obligatory homodimer.[59,80,81] 5-HT_{2A} receptors consist of an N-terminal extracellular domain, a 7-transmembrane-spanning domain and an intracellular C-terminal domain.[41] Very recent findings have solved the crystal structure of the 5-HT_{2A} receptor in complex with the antipsychotics risperidone and zotepine.[82] Similarly, using a combination of X-ray crystallography and cryo-electron microscopy (cryo-EM), Brian Kobilka and his research team have proposed that the inactive conformation of the mGlu5 receptor homodimer involves a single physical interaction through the disulfide bond between the two VFT domains.[83] Interestingly, this recent work also showed that upon orthosteric agonist administration, there is a pattern of structural changes that lead to a TM6-TM6 interface that was proposed to be involved in mGlu5 homodimeric receptor activation.[83] Whether this agonist-induced transition by which the TM domains of the mGlu5 receptor homodimer come into close proximity represents a landmark of mGlu receptors' activation, including the mGlu2 receptor, remains to be investigated.

5-HT_{2A}-mGlu2 Heteromeric Complex. The mGlu2 and mGlu3 receptors have high sequence homology; however, mGlu2 receptors can assemble into a heteromeric complex with 5-HT_{2A} receptors, whereas mGlu3 receptors cannot.[61] This specificity of 5-HT_{2A}-mGlu2 complex formation and the similarity in the primary sequences of mGlu2 and mGlu3 guided the construction of mGlu2/mGlu3 chimeric receptors with the final goal of identifying the residues and domains that are necessary for 5-HT_{2A}-mGlu2 heteromeric complex assembly (summarized in Table 1). Molecular chimeras of these receptors were created wherein the TM domains 4 and 5 of the receptors (mGlu2ΔTM4,5 and mGlu3ΔTM4,5) were swapped. mGlu2ΔTM4,5 receptor chimeras were not able to co-immunoprecipitate with 5-HT_{2A} receptors, whereas mGlu3ΔTM4,5 receptor mutants were able to. This suggests that TM4 and 5 of the mGlu2 receptor mediate heterodimerization.[61] An mGlu2 receptor mutant that has the TM4 of mGlu3 was not able to co-immunoprecipitate, whereas an mGlu2 mutant that has the TM5 of mGlu3 retained the ability to co-immunoprecipitate with 5-HT_{2A} receptors. Hence, TM4 of mGlu2 is needed to mediate the interaction with 5-HT_{2A} receptors.[76] Additionally, three alanine ($677^{4.40}$, $681^{4.44}$, $685^{4.48}$) residues located at the intracellular end of TM4 were found to be important for mediating heteromerization.[76]

Mutations of conserved cysteines in the CRD to alanine abolished the response to agonist but did not affect the coupling of the TMs to G proteins.[81] The conformational changes in the TMDs have been shown to be responsible for constitutive activity of the mGlu2 receptor.[81] It has recently

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Table 1. Summary of Results When Various Chimeric Mutants Were Co-expressed with 5-HT$_{2A}$ or mGlu2 Receptors

chimeric construct	complex formation	allosteric crosstalk
Constructs co-expressed with 5-HT$_{2A}$ receptor		
mGlu2	Yes[61,62,71−76]	Yes[61,62,71−77,79]
mGlu3	No61,76	No[61,62,75,76]
ΔmGlu2	Yes[61]	Yes[61]
mGlu2ΔTM4,5	No[61]	No[61]
mGlu3ΔTM1−5	Yes[61]	Yes[61]
mGlu3ΔTM4,5	Yes[61]	Yes[61,62]
mGlu2ΔTM4	No[76]	Not tested
mGlu2ΔTM5	Yes[76]	Not tested
mGlu3ΔTM4	Yes[76]	Not tested
mGlu3ΔTM5	No[76]	Not tested
mGlu3ΔTM4N	Reduced/No[76]	No[61,62,76]
mGlu3ΔTM4C	Yes[76]	Yes[61,76]
mGlu2-A4.40S	Yes[76]	Not tested
mGlu2-A4.44F	Reduced[76]	Not tested
mGlu2-A4.48G	Yes[76]	Not tested
mGlu2-A4.40S-A4.44F	No[76]	No[76]
mGlu2-A4.40S-A4.48G	No[76]	No[76]
mGlu2-A4.44F-A4.48G	No[76]	No[76]
Constructs co-expressed with mGlu2 receptor		
5-HT$_{2A}$	Yes[61,62,71−76]	Yes[61,62,71−77,79]
5-HT$_{2C}$	No[61,62,71]	No[62,71]
5-HT$_{2A}$ΔTM1	Yes[62]	Not tested
5-HT$_{2A}$ΔTM4	No[62]	Not tested

been shown that the changes in the CRD and their relative orientations are more important than the relative orientation of the VFT (as previously proposed).[81] 3D models of the ECD suggest that the CRDs can associate in an active state with either one or both VFTs being closed.[81] This fits well with previous data suggesting that "homodimerization" between an mGlu2 mutant that cannot bind the orthosteric agonist with a second mGlu2 mutant that cannot activate $G_{i/o}$ proteins rescues the functional crosstalk between 5-HT$_{2A}$ and mGlu2 receptors.[62]

Using a 3-FRET experimental approach, previous data also suggest that 5-HT$_{2A}$ and mGlu2 receptors may assemble into higher order oligomeric complexes.[62] Although the role of oligomerization of Class C GPCRs is well established, the role of oligomerization with respect to Class A GPCRs is a subject of controversy with some recent studies suggesting that Class A GPCRs might predominantly exist as monomers.[67,70] However, this might be attributable to the transient nature of the oligomeric complexes formed. A fluorescence recovery after photobleaching based approach was used by Dorsch et al.[84] to demonstrate that β_1-adrenergic receptors interact transiently to form unstable oligomers, whereas β_2-adrenergic receptors were found to form more stable oligomeric complexes.[84] Micrometer-sized beads can be used to recruit affinity-tagged receptors on the cell surface, and by monitoring the ability of tagged receptors to co-recruit untagged protomers, Gavalas et al.[85] tested the physical stability of oligomerization in the membrane. The Class A GPCRs β_2-adrenergic and μ-opioid receptors were unable to recruit untagged protomers, whereas the tagged mGlu2 receptors were able to recruit untagged receptors suggesting the formation of a stable oligomeric complex.[85] Based on previous studies,[62,76] it is tempting to speculate that mGlu2 and 5-HT$_{2A}$ receptors might assemble into a stable heteromeric complex; however, further validation is needed. Additionally, we do not know where at a subcellular level the 5-HT$_{2A}$-mGlu2 receptors heteromeric complex is assembled. However, there is growing evidence for other GPCRs that suggests that homomerization may occur either during protein maturation in the Golgi complex or during the early stages of protein synthesis in the endoplasmic reticulum (ER).[70,86]

Behavioral Crosstalk between 5-HT$_{2A}$ and mGlu2 Receptors in Mouse Models of Psychosis. A long-lasting question in molecular pharmacology research is related to the behavioral phenotypes induced by 5-HT$_{2A}$ receptor agonists. Thus, even though agents such as LSD and lisuride both act as agonists at 5-HT$_{2A}$ receptors, only hallucinogenic 5-HT$_{2A}$ receptor agonists induce these unique behavioral pharmacological effects.[87−91] Interestingly, it was reported that hallucinogenic 5-HT$_{2A}$ receptor agonists such as LSD and DOI induce immediate early genes that non-hallucinogenic 5-HT$_{2A}$ receptor agonists such as lisuride do not.[90,91] Non-hallucinogenic 5-HT$_{2A}$ receptor agonists induce immediate early genes such as *c-fos*, whereas hallucinogenic agents that are 5-HT$_{2A}$ receptor agonists induce *c-fos* as well as additional genes such as *egr-1*, *egr-2*, and *period-1* in the somatosensory cortex of mice.[90] This effect was absent in 5-HT$_{2A}$-KO mice, suggesting that it is a 5-HT$_{2A}$ receptor-mediated effect.[90] Furthermore, it has been demonstrated that responses to hallucinogenic 5-HT$_{2A}$ receptor agonists are mediated via $G_{q/11}$ as well as $G_{i/o}$ and Src, whereas the response to non-hallucinogenic agents primarily utilizes $G_{q/11}$.[91] Hallucinogenic versus non-hallucinogenic agents have also been shown to differ in the receptor phosphorylation patterns that they trigger.[92] An mGlu2/3 receptor agonist did not block the induction of *c-fos* by hallucinogenic and non-hallucinogenic 5-HT$_{2A}$ receptor agonists; however, it blocked the induction of *egr-2* by hallucinogens both *in vivo* in the mouse frontal cortex and in cultured cortical neurons.[93] Additionally, chronic treatment of mice with an mGlu2/3 receptor antagonist (LY341495) led to a downregulation of 5-HT$_{2A}$ receptors in wild-type but not *mGlu2-KO* mice as well as a reduced induction of *c-fos*, *egr-1*, and *egr-2* in response to hallucinogenic 5-HT$_{2A}$ receptor agonists.[94] This suggests that the unique cellular effects of hallucinogens require the expression of mGlu2 receptors in addition to 5-HT$_{2A}$ receptors and might be due to their actions at the 5-HT$_{2A}$-mGlu2 heteromeric complex.

The head twitch response (HTR)[95,96]- a behavioral phenotype that is observed in rodents, is elicited by only hallucinogenic 5-HT$_{2A}$ receptor agonists such as LSD and DOI but not by non-hallucinogenic 5-HT$_{2A}$ receptor agonists such as lisuride. It is mediated by 5-HT$_{2A}$ receptors and is absent in *5-HT$_{2A}$-KO* mice.[90] Previous reports have also demonstrated that *mGlu2-KO* mice head twitch less as compared to wild-type mice,[93] and this behavioral phenotype has been validated recently.[97] This suggests that the HTR to hallucinogens such as DOI requires both 5-HT$_{2A}$ as well as mGlu2 receptors.[90,93]

LY379268, an mGlu2/3 receptor agonist, prevented the HTR induced by DOI and LSD, suggesting a role for the mGlu2 receptor in the hallucinogenic action of 5-HT$_{2A}$ receptor agonists.[61] However, chronic treatment with LY341495, an mGlu2/3 receptor antagonist led to a significant decrease in the HTR to hallucinogens as compared to control mice.[94] Notably, a similar decrease on HTR induced by the

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psychedelic 25CN-NBOH has been recently reported upon chronic treatment with LY379268.[98]

As discussed previously, TM4 of the mGlu2 receptor might play a role in mediating heteromerization with the 5-HT_{2A} receptor *in vitro*.[76] The relevance of the TM4 of the mGlu2 receptor in mediating heterodimerization with 5-HT_{2A} receptors has also been demonstrated *in vivo*. *mGlu2-KO* mice in which expression of mGlu2 receptors was rescued in the frontal cortex via a viral vector-mediated approach elicited HTR upon DOI administration. However, when mGlu2ΔTM4N was overexpressed in the mouse frontal cortex of *mGlu2-KO* mice, the HTR to hallucinogens was not rescued.[76,99] Although this approach does not unequivocally demonstrate 5-HT_{2A}-mGlu2 receptor complex formation, it suggests that the unique cellular and behavioral effects of hallucinogens may be due to their actions at the 5-HT_{2A}-mGlu2 heteromeric complex. Hyperlocomotor activity induced by NMDA receptor antagonists such as MK-801 and PCP serve as preclinical models of psychosis.[15] Recent findings suggest that antipsychotic as well as antipsychotic-like agents require both 5-HT_{2A} and mGlu2 receptors for antipsychotic-related behavior. Thus, the effect of clozapine and LY379268 on MK-801-induced hyperlocomotor activity was significantly decreased in *mGlu2-KO* and *5-HT_{2A}-KO* mice, respectively, as compared to wild-type controls.[75]

More recent findings also suggest that clozapine reduced hyperlocomotor activity induced by MK-801 and PCP, as well as that by amphetamine (which affects the dopaminergic system among others) and scopolamine (a muscarinic receptor antagonist) in wild-type mice. Importantly, clozapine reduced hyperlocomotor activity induced by amphetamine and scopolamine in *mGlu2-KO* mice, but not that induced by MK-801 and PCP. This suggests that mGlu2 receptors are needed for clozapine's 5-HT_{2A} receptor-dependent antipsychotic activity when glutamatergic signaling is disrupted by dissociative drugs such as PCP and MK-801. Clozapine's ability to reduce hyperlocomotor activity in *mGlu2-KO* mice was restored when mGlu2 receptors were overexpressed using a viral vector in the frontal cortex, but not when the mGlu2ΔTM4N construct was overexpressed. This suggests that clozapine's antipsychotic effects might be at least in part mediated via the 5-HT_{2A}-mGlu2 heteromeric complex.[100]

Epigenetic Mechanisms of Crosstalk between 5-HT_{2A} and mGlu2 Receptors. It has been shown that chronic treatment with atypical antipsychotic agents such as clozapine and risperidone can lead to a downregulation of mGlu2 receptors in the frontal cortex of mice.[63,101] Chronic clozapine treatment leads to 5-HT_{2A} receptor-dependent downregulation of IκBα (an NF-κB suppressor) expression mediated by hypophosphorylation of ERK1/2. This leads to an increase in the activity of NF-κB and a consequent increase of NF-κB binding to the *Hdac2* promoter, resulting in an increased transcription and upregulation of HDAC2.[63] HDAC2 binds to the *mGlu2* promoter and negatively regulates it, leading to downregulation of mGlu2 receptors in the frontal cortex and an increase in schizophrenia-related behaviors.[101] This downregulation of mGlu2 receptors might be responsible for the reduced therapeutic effects of atypical antipsychotics on chronic administration. Administration of HDAC inhibitors can help improve the effect of atypical antipsychotic agents by potentially preventing the repressive histone modifications at the *mGlu2* promoter, and hence reducing downregulation of mGlu2 receptors (Figure 3).[63,101]

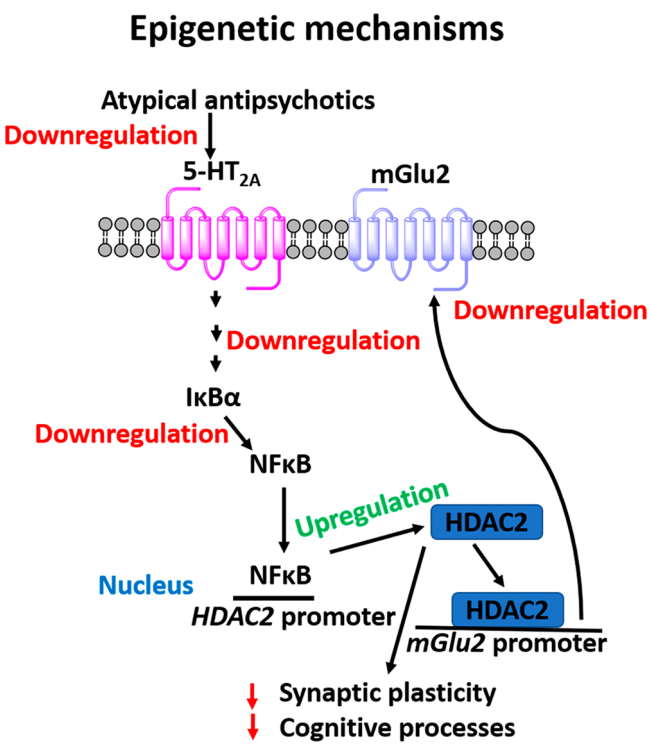

Figure 3. Epigenetic mechanisms for allosteric crosstalk. Antipsychotics such as clozapine lead to a 5-HT_{2A} receptor-dependent downregulation of *mGlu2* receptors.

Additionally, HDAC2 upregulation negatively affects genes that affect synaptic structure and behavioral plasticity. Chronic administration of clozapine leads to cognitive deficits and detrimentally affects short-term memory and latent learning in behavioral models in wild-type mice but not in *HDAC2-KO* mice.[63] *HDAC2-KO* mice show fewer behavioral phenotypes associated with psychosis as compared to wild-type control mice.[54] Chronic treatment with clozapine led to a decrease in the ability of mGlu2/3 agonists to activate G protein signaling in the frontal cortex of wild-type mice.[102] This effect was not observed in the frontal cortex of *HDAC2-KO* mice suggesting that this effect is mediated via upregulation of HDAC2. Chronic treatment with clozapine diminished the ability of mGlu2/3 receptor agonists such as LY379268 to reduce the hyperlocomotor activity induced by MK801 in wild-type but not in *HDAC2-KO* mice. Administration of SAHA, an HDAC inhibitor alongside chronically administered antipsychotic agents could rescue the diminished ability of mGlu2/3 agonists to reduce MK-801 induced hyperlocomotion.[102]

These findings are further validated by data from clinical trials.[103] It has been shown that schizophrenic patients that were previously treated chronically with atypical antipsychotic agents or by any medications that have a prominent 5-HT_{2A} receptor antagonist component do not respond to pomaglumetad methionil, an mGlu2/3 receptor orthosteric agonist. This could be a result of downregulation of mGlu2 receptors as a result of functional crosstalk with 5-HT_{2A} receptors. On the other hand patients previously treated with typical antipsychotics such as haloperidol showed a therapeutic response to pomaglumetad methionil as compared to placebo.[103]

This effect of chronic antipsychotic exposure on mGlu2/3 receptor density is further supported by findings in postmortem human brain samples. Thus, it was suggested

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that 5-HT_{2A} receptors are upregulated whereas mGlu2 receptors are downregulated in postmortem frontal cortex samples of untreated schizophrenic subjects as compared to controls.[61] 5-HT_{2A} receptor density was similar in postmortem frontal cortex samples of schizophrenia subjects treated with antipsychotic agents, whereas mGlu2/3 receptors remained downregulated.[61] Additionally, it was reported that the *mGlu2* receptor mRNA was down whereas the *mGlu3* receptor mRNA was unchanged in untreated schizophrenia subjects. This dysregulated pattern of distribution of 5-HT_{2A} and mGlu2 receptors might result in an altered pattern of signaling in the frontal cortex, leading to the psychosis associated with schizophrenia.[61]

The downregulation of mGlu2 receptors might be a consequence of antipsychotic treatment, as described in the previous section.[101] A 5-HT_{2A} receptor dependent-increase in expression of HDAC2 was also found in the frontal cortex of schizophrenic subjects that were treated with atypical antipsychotic agents as compared to untreated schizophrenics.[101] Additionally, decreased IκBα immunoreactivity was also observed in the frontal cortex of treated schizophrenic subjects as compared to controls.[63]

Many research groups have studied 5-HT_{2A} receptor density in postmortem human brain of schizophrenic subjects and controls.[104−109] However, the results of these experiments vary with some showing that 5-HT_{2A} receptors are upregulated or downregulated, whereas others reported that 5-HT_{2A} receptor density is unchanged. These discrepancies may be related to factors that include previous antipsychotic treatment of suicidal behavior in the schizophrenia cohort.[105] Muguruza et al.[110] studied 5-HT_{2A} receptor density in postmortem brain samples from treated and untreated schizophrenic patients as well as suicide victims that had other psychiatric disorders, as compared to controls that were individually matched by gender and age. The 5-HT_{2A} receptor density was unchanged in suicide victims with other disorders as compared to controls. As previously suggested,[61] findings in this separate cohort of samples also showed that 5-HT_{2A} receptor density was upregulated in postmortem frontal cortex samples of untreated schizophrenia subjects, but not in treated schizophrenia subjects, as compared to controls.[110] Additionally, the active conformation of 5-HT_{2A} receptors was increased in the frontal cortex of subjects with schizophrenia, suggesting that increase in 5-HT_{2A} receptor density is associated and linked with schizophrenia and not with suicide.[110] Consequently, it has been shown that there are no changes in the expression as well as the densities of 5-HT_{2A} and mGlu2 receptors in the postmortem prefrontal cortex brain samples of untreated subjects suffering from major depressive disorder as compared to control subjects.[111] In subjects that were treated with antidepressants there was a downregulation of 5-HT_{2A} receptors, whereas the density of the mGlu2 receptor was comparable to that of control subjects.[111]

The functional crosstalk between 5-HT_{2A} and mGlu2 receptors has also been shown to be dysregulated in frontal cortices of schizophrenic subjects.[76] Competition binding of LY379268 in the presence of [^{3}H]LY341495 to cortical membrane preparations was best fit by a two-site model, and the high-affinity binding of LY379268 was decreased in the presence of the psychedelic 5-HT_{2A} agonist DOI in schizophrenic brain samples as well as controls. However, the allosteric effect of DOI on LY379268 was significantly higher in schizophrenic subjects as compared to controls.[76] Activation of $G_{q/11}$ on mGlu2 receptor activation by LY379268 was reduced in the frontal cortices of schizophrenic individuals as compared to control subjects. The activation of $G_{i/o}$ proteins by LY379268 was unaffected in schizophrenic subjects as compared to controls.[62] Together, these findings suggest that altered signaling at the 5-HT_{2A}-mGlu2 receptor might be involved in pathophysiology of schizophrenia.

SUMMARY

Growing evidence suggests that the serotonergic and glutamatergic neurotransmission systems can interact with one another. Particularly 5-HT_{2A} and mGlu2 can assemble into a postsynaptic heteromeric complex in the frontal cortex. Additionally, these receptors can also interact at the epigenetic level as well as via synaptic mechanisms. Antipsychotic and hallucinogenic agents can mediate their effects via acting at this heteromeric complex. We have reviewed the importance of the interaction between the serotonergic and glutamatergic system and crosstalk in various preclinical models of schizophrenia. The epigenetic crosstalk between 5-HT_{2A} and mGlu2 receptors may have translational significance. The unresponsiveness of schizophrenic patients that were previously treated chronically with atypical antipsychotic agents/medications that have a prominent 5-HT_{2A} receptor antagonist component to mGlu2/3 receptor orthosteric agonists could be a result of downregulation of mGlu2 receptors as a result of functional crosstalk with 5-HT_{2A} receptors at the epigenetic level. Administration of HDAC inhibitors alongside chronically administered antipsychotic agents could potentially help improve the effect of atypical antipsychotic agents by potentially reducing downregulation of mGlu2 receptors. All of this suggests that alterations at the level of the 5-HT_{2A}-mGlu2 receptor heteromer might be linked to the altered cortical processes in schizophrenia and represents an attractive novel therapeutic target.

AUTHOR INFORMATION

Corresponding Author

*E-mail: javier.maeso@vcuhealth.org.

ORCID

Javier González-Maeso: 0000-0003-3105-3204

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ABBREVIATIONS

mGlu2 receptors, metabotropic glutamate 2 receptors; 5-HT_{2A} receptors, serotonin 2A receptors; PAMs, positive allosteric modulators; LSD, lysergic acid diethylamide; EPSCs/EPSPs, excitatory post-synaptic currents/potentials; PCP, phencyclidine; TMD, transmembrane domain; VFT, Venus fly trap; CRD, cysteine rich domain.

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