Interactions of Hallucinogens with the Glutamatergic System: Permissive Network Effects Mediated Through Cortical Layer V Pyramidal Neurons

Gerard J. Marek

Abstract Recordings made from layer V (L5) pyramidal cells of the prefrontal cortex (PFC) and neocortex in rodent slice preparations have shown that serotonin (5-hydroxytryptamine, 5-HT) and serotonergic hallucinogens induce an increase in the frequency of spontaneous excitatory postsynaptic currents (EPSCs) in the apical dendritic field by activating 5-HT_{2A} receptors. Serotonergic hallucinogens induce late EPSCs and increase recurrent network activity when subcortical or mid-cortical regions are stimulated at low frequencies (e.g., 0.1 Hz). A range of agonists or positive allosteric modulators (PAMs) for mostly $G_{i/o}$-coupled receptors, including metabotropic $glutamate_2$ ($mGlu_2$), adenosine A_1, or μ-opioid receptors, suppress these effects of 5-HT_{2A} receptor stimulation. Furthermore, a range of mostly $G_{q/11}$-coupled receptors (including $orexin_2$ [OX_2]; α_1-adrenergic, and $mGlu_5$ receptors) similarly induce glutamate (Glu) release onto L5 pyramidal cells. Evidence implicates a number of brain regions in mediating these effects of serotonergic hallucinogens and $G_{q/11}$-coupled receptors including the midline and intralaminar thalamic nuclei, claustrum, and neurons in deep PFC. These effects on 5-HT_{2A} receptors and related GPCRs appear to play a major role in the behavioral effects of serotonergic hallucinogens, such as head twitches in rodents and higher order behaviors such as rodent lever pressing on the differential-reinforcement-of-low rate 72-s (DRL 72-s) schedule. This implies that the effects of 5-HT_{2A} receptor activation on the activity of L5 pyramidal cells may be responsible for mediating a range of behaviors linked to limbic circuitry with connectivity between the PFC, striatum, thalamus, claustrum, striatum, amygdala, and the hippocampal formation.

Keywords In vitro electrophysiology · Layer V pyramidal neurons · DOI-induced head-twitch response · DRL 72-s behavior

Contents

The present chapter will detail the emergence over the last several decades of evidence demonstrating that hallucinogenic drugs share a common pharmacological action in stimulating cortical 5-hydroxytryptamine$_{2A}$ (5-HT_{2A}) receptors, which may provide an important clue to the treatment of psychotic disorders. While focusing on psychosis and potential links to lysergic acid diethylamide (LSD)-induced perceptual changes, we will also discuss hallucinogen-induced changes in mood and cognition as well as potential therapeutic applications. The genesis of this line of work began in many ways with the discovery of LSD by the Sandoz chemist Albert Hoffman in the early 1940s and the observation that this drug, like mescaline, appears to mimic certain symptoms exhibited by patients with schizophrenia. Woolley and Shaw first suggested that the serotonergic properties of LSD might explain its psychotomimetic effects (Shaw and Woolley 1956). By the late 1970s and early 1980s, convergent receptor binding, behavioral and electrophysiological studies suggested that LSD, mescaline, and psilocybin all share a common agonist action at 5-HT_{2A} receptors. While both stimulants like amphetamine and *N*-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine (PCP) and ketamine began to overtake serotonergic hallucinogens as models of a broader range of the symptoms of schizophrenia, others continued to use hallucinogen effects on 5-HT_{2A} receptors as a model psychosis with potential links to the family of schizophrenic disorders (Gouzoulis-Mayfrank et al. 2005; Vollenweider et al. 1998). More recently, evidence has accrued for pathophysiologic and therapeutic influences of 5-HT_{2A} receptors in neurodegenerative disease states ranging from Parkinson's disease (PD) psychosis to the psychosis-like symptoms associated with Alzheimer's disease (AD).

The initial clues indicating that serotonergic hallucinogens have effects on glutamate (Glu) release appeared around the time that it was recognized that selective 5-HT_{2A} receptor antagonists are not highly effective antipsychotic drugs in patients with schizophrenia. First, discoveries linking the glutamatergic system and hallucinogens will be discussed. The initial report showing that bath application of serotonin (5-HT) induces spontaneous (i.e., not evoked by electrical stimulation) excitatory postsynaptic potentials/currents (EPSPs/EPSCs) in prefrontal cortical and neocortical layer V (L5) pyramidal neurons in rat brain slice preparations was published in 1997 (Aghajanian and Marek 1997). The basic pharmacological observations were that these spontaneous 5-HT-induced EPSPs/EPSCs were potently blocked by 5-HT_{2A} receptor antagonists at concentrations that were consistent with their K_i values for the 5-HT_{2A} receptor, but were not affected by

5-HT_{1A} or 5-HT_3/5-HT_4 receptor antagonists. The other basic pharmacological finding was that the broad spectrum presynaptic metabotropic glutamate (mGlu) receptor agonist (*1S,3S*)-ACPD also suppressed the frequency of the 5-HT-induced EPSPs/EPSCs. Previously, inhibitory EPSPs were observed in piriform cortical and hippocampal neurons due to excitation of 5-HT_{2A} receptors on GABAergic interneurons. However, the 5-HT-induced synaptic currents in prefrontal and neocortical pyramidal cells were (1) potently and completely blocked by the AMPA receptor antagonist LY293558; (2) only minimally (<15%) suppressed by the $GABA_A$ receptor antagonist bicuculline; (3) completely suppressed by the fast sodium channel blocker tetrodotoxin (TTX); (4) completely suppressed by perfusing the slice with an artificial cerebrospinal fluid solution containing no added calcium ("0" calcium) and high magnesium; and (5) mimicked by iontophoretic application of 5-HT in layers I and Va in a distribution consistent with stimulation of the apical dendrite but not the basilar dendrites. Furthermore, the firing of pyramidal neurons in response to bath applied 5-HT could not be detected even after exhaustive searches, unlike the ready identification of firing interneurons in piriform cortical slices. Taken together, these observations suggested a model where 5-HT, via activation of 5-HT_{2A} receptors, releases Glu from glutamatergic afferents terminating in the layer I and Va dendritic field of L5 pyramidal cells. Subsequently, studies that will be described below suggested that the midline and intralaminar thalamic nuclei are at least one of the sources providing the glutamatergic afferents. Thus, these 5-HT-induced EPSCPs/EPSPs are a novel form of feedforward excitation of glutamatergic afferents to the principle output neurons of the prefrontal cortex (PFC) and neocortex.

Another model paradigm for observing Glu release induced by serotonergic hallucinogens via 5-HT_{2A} receptor activation in PFC slices was subsequently identified (Aghajanian 2009). After applying a serotonergic hallucinogen such as LSD (10 nM) or DOI (3 μM) to a prefrontal cortical slice for 10 min, focal electrical stimulation of the slice in the middle of the cortex at approximately 1 Hz results in an early fast EPSC followed by an UP state and recurrent network activity. More cumbersome protocols to induce what was originally described as "late EPSCs" involved identifying stimulation sites in the white matter below cortical layer VI where stimulation at approximately 0.1 Hz induced these late EPSCs/EPSPs or recurrent network activity during the washout of 5-HT. Subsequent application of a serotonergic hallucinogen for approximately 10 min would then induce a long-lasting (up to several hours) appearance of late EPSCs or recurrent network activity after low-frequency 0.1 Hz stimulation of the white matter. The fact that washout of 5-HT from the slice is required to observe this form of recurrent network activity highlights the likelihood that a primary but relatively brief effect of 5-HT in the PFC is to inhibit Glu release, probably via some combination of effects on 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1F} receptors (Fig. 1). By contrast, bath application of 5-HT appears to result in a more sustained activation of 5-HT_{2A} receptors. Bath applications of serotonergic hallucinogens such as LSD and DOI, which potently bind to and activate 5-HT_{2A} receptors, together with focal electrical stimulation, combine to produce late synaptic currents or Glu "spillover", which

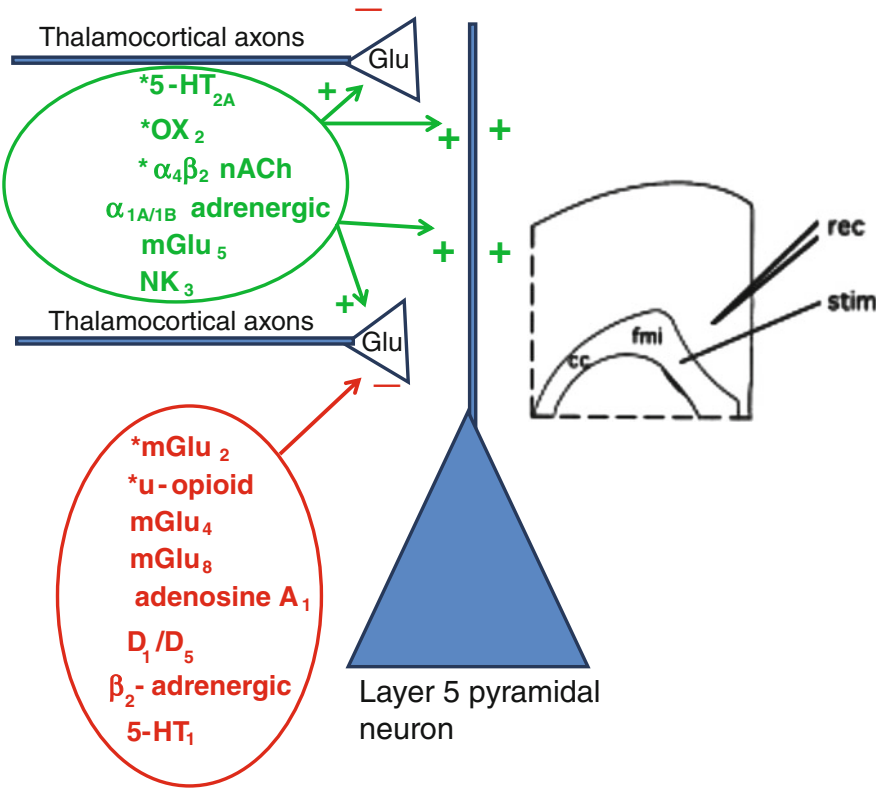

Fig. 1 Model of extrinsic (thalamus and/or claustrum) and intrinsic (medial prefrontal cortex (mPFC) and neocortex) afferents to the apical dendrites of layer 5 pyramidal cells that release glutamate (Glu) in response to activation of 5-HT_{2A}, $\alpha_{1A/1B}$ adrenergic, $mGlu_5$, OX_2, NK_3 or $\alpha_4\beta_2$ nicotinic acetylcholine (nACh) receptors (shown in *green*). A variety of presynaptic receptors suppress the 5-HT_{2A} receptor-induced Glu release including $mGlu_2$, $mGlu_4$, $mGlu_8$, μ-opioid, adenosine A_1, dopamine D_1 and D_5, β_2-adrenergic, and 5-HT_1 receptors. The Glu released in response to 5-HT_{2A} activation activates AMPA receptors on the layer 5 pyramidal neurons. To date, the most convincing evidence suggests that the midline and intralaminar thalamic nuclei are the primary sources of the afferent glutamatergic axons that terminate on layers I and Va of the PFC and neocortex. The inset diagram shows that these experiments were performed under in vitro conditions using slices of the rat brain containing the anterior cingulate and prelimbic regions of the mPFC

can otherwise be described as excitatory feedforward and feedback recurrent network activity.

The 5-HT-induced EPSCs and the recurrent network activity induced by the combination of DOI and focal low-frequency electrical stimulation are suppressed by a range of neurotransmitter receptors that are known to inhibit presynaptic Glu release (Table 1 and Fig. 1). The initial electrophysiological experiments suggested

Table 1 Neurotransmitter receptors suppressing glutamate release induced by 5-HT_{2A} receptor activation

Neurotransmitter	Receptor and Action	5-HT-induced EPSCs	DOI-induced recurrent network activity
Glutamate	$mGlu_{2/3}$ receptor agonists	Suppress	Suppress
Glutamate	$mGlu_2$ receptor PAMs	Suppress	*Not tested*
Glutamate	$mGlu_{4/8}$ receptor agonists	Suppress	*Not tested*
Adenosine	A_1 receptor agonists	Suppress	Suppress
Opioids	μ-opioid receptor agonists	Suppress	*Not tested*
Dopamine	D_1/D_5 receptor agonists	*Not tested*	Suppress
Norepinephrine	β_2 receptor agonists	*Not tested*	Suppress

that mGlu autoreceptors played an important role in modulating the Glu release induced by 5-HT_{2A} receptor activation (Aghajanian and Marek 1997). An autoreceptor role for the $mGlu_2$ receptor was initially suggested based on the fact that the $mGlu_{2/3}$ receptor orthosteric agonists LY354740 and LY379268 could potently suppress 5-HT-induced EPSCs and DOI-induced Glu spillover, whereas the $mGlu_{2/3}$ receptor antagonist LY341495 enhanced EPSC frequency (Marek et al. 2000). LY354740 was approximately threefold more potent in suppressing 5-HT-induced EPSCs and DOI-induced Glu spillover compared to the early evoked EPSP; importantly, the direct effects on LY354740 on membrane potential were minimal, and LY354740 failed to alter the inward currents induced by bath application of AMPA following perfusion of the slice with TTX to block fast sodium channels. Furthermore, $mGlu_{2/3}$ receptor binding sites in layers I and Va of the PFC appeared to overlap with the laminar distribution of 5-HT_{2A} receptor binding sites. Subsequent experiments demonstrated that midline thalamic lesions caused a ~20% reduction in the density of $mGlu_{2/3}$ receptors (measured with [^{3}H]LY354740) in PFC layers I and Va, likely a physiologically significant amount given that most [^{3}H]LY354740 binding sites were subsequently suggested to be $mGlu_3$ receptors (Marek et al. 2001; Wright et al. 2013). An even more definitive identification of a role for $mGlu_2$ receptors in mediating these effects was provided by experiments demonstrating that selective $mGlu_2$ receptor positive allosteric modulators (PAMs) suppressed 5-HT-induced EPSCs in the rat PFC (Benneyworth et al. 2007). These effects have also been replicated in other laboratories and with additional selective $mGlu_{2/3}$ receptor agonists (Wright et al. 2013; Klodzinska et al. 2002; Rorick-Kehn et al. 2007). Evidence for the behavioral salience of these functional 5-HT_{2A} and $mGlu_2$ receptor interactions will be described below. Thus, the $mGlu_2$ receptor appears to function as an autoreceptor on glutamatergic terminals releasing Glu (induced by 5-HT_{2A} receptor activation) from afferents reaching the layer I and Va dendritic field of L5 pyramidal cells in the PFC and neocortex.

Agonists of several other G-protein-coupled receptors (GPCRs) that generally had inhibitory effects on neurotransmitter release or hyperpolarizing electrophysiological effects ($G_{i/o}$-coupled receptors) were also found to suppress the spontaneous 5-HT- or DOI-induced EPSCs (Table 1 and Fig. 1). This included two other mGlu receptors ($mGlu_4$ and $mGlu_8$) that were also well known to decrease Glu release (Palucha-Poniewierg et al. 2008; Slawinska et al. 2013; Zhang and Marek 2007). The adenosine A_1 receptor, another well-known heteroreceptor that decreases Glu release, was also found to suppress spontaneous 5-HT-induced EPSCs and the feedforward and feedback Glu release induced by serotonergic hallucinogens and electrical stimulation (Stutzman et al. 2001). Finally, agonists for the μ-opioid receptor, a $G_{i/o}$-coupled GPCR with enrichment in PFC layers I and Va, were also found to suppress 5-HT-induced EPSCs (Marek and Aghajanian 1998). Thalamic lesions that decreased $mGlu_{2/3}$ receptor binding sites by ~20% also reduced μ-opioid receptor binding sites by ~40%, suggesting that the midline and intralaminar thalamic nuclei are a potential source for the 5-HT-induced EPSCs (Marek et al. 2001). These studies are all consistent with the known localization of $mGlu_2$, $mGlu_4$, adenosine A_1, and μ-opioid receptors in a potential thalamic source of afferents to cortical layers I and V, namely the midline and intralaminar thalamic nuclei (Kolaj et al. 2014). Thus, converging lines of evidence further suggest a model where 5-HT_{2A} receptor activation and a range of $G_{i/o}$-coupled GPCRs have countervailing effects Glu release from at least one pool of subcortical terminals reaching the apical dendritic field of L5 pyramidal cells throughout the PFC and neocortex (Table 1 and Fig. 1).

In addition to the suppression of hallucinogen-induced Glu overflow induced by a number of $G_{i/o}$-coupled GPCRs, several G_s-coupled receptors appear to have similar effects on hallucinogen-induced recurrent network activity (Table 1 and Fig. 1). This effect has been well characterized for the $D_{1\text{-like}}$ class of dopamine receptors (D_1/D_5), which are coupled to G_s. The ability of the D_1/D_5 partial agonist SKF38393 to suppress DOI-induced late EPSCs was blocked by the dopamine D_1/D_5 receptor antagonist SCH23390 but not the dopamine D_2 receptor antagonist raclopride (Lambe and Aghajanian 2007). In contrast, the dopamine D_2 receptor agonist quinpirole failed to suppress DOI-induced recurrent network activity. The critical role played by Glu spillover in the effect of SKF38393 is indicated by mechanistic experiments linking its suppressant effect to the known ability of the G_s pathway to upregulate the excitatory amino acid transporter 3 (EAAT3). Extending the effects observed to levels downstream from receptors, application of the adenylyl cyclase activator forskolin, or the phosphodiesterase-resistant cAMP analogue 8-Br-cAMP suppressed DOI-induced recurrent network activity. The modulatory effects of forskolin and 8-Br-cAMP on DOI-induced recurrent network activity occurred in the absence of effects on fast early EPSCs. A cortical versus thalamic site of action for the D_1/D_5 ligands is consistent with the known cortical versus thalamic localization of these receptors (Ciliax et al. 2000; Huang et al. 1992; Mansour et al. 1992). Both postsynaptic and presynaptic effects relative to L5 pyramidal cells are supported by a range of known mechanisms for dopamine D_1/D_5 receptors (Gao et al. 2001; Rotaru et al. 2007). Preliminary experiments have

also suggested that β_2-adrenergic receptor stimulation by epinephrine or clenbuterol can also selectively suppress DOI-induced recurrent network activity (GJ Marek and B Ramos, unpublished observations). The preferential localization of β_2-adrenergic receptors to the midline and intralaminar thalamic nuclei compared to the preferential localization of β_1-adrenergic receptors in the PFC and neocortex, as well as the known physiological effects of β_2-adrenergic receptor function in one of the midline thalamic nuclei, is also consistent with a role of thalamic inputs in DOI-induced Glu release in the mPFC (Nicholas et al. 1993; Rainbow et al. 1984; Zhang et al. 2009). Thus, suppression of DOI-induced Glu release by D_1/D_5 receptor agonists and β_2-adrenergic receptor agonists could support a model for intracortical circuitry or thalamocortical circuitry or a model combining both types of afferents onto cortical L5 pyramidal cells.

Similar to the effect of 5-HT via 5-HT_{2A} receptors, activation of a number of $G_{q/11}$-coupled GPCRs appears to induce feedforward Glu release onto L5 pyramidal cells in the PFC and/or neocortex (Table 2 and Fig. 1). Where tested, common features for spontaneous neurotransmitter-induced EPSCs/EPSPCs included dependence on fast sodium channels (i.e., suppression by TTX) and AMPA receptors (i.e., suppression by LY293558), and suppression by a μ-opioid receptor agonist (DAMGO). Thus, norepinephrine (NE), (*S*)-3,5-dihydroxyphenylglycine (DHPG), hypocretin-2, and senktide all induce EPSCs/EPSPs via activation of α_1 adrenergic, $mGlu_5$, $orexin_2$ (OX_2), and NK_3 receptors, respectively (Lambe et al. 2007; Lambe and Aghajanian 2003; Marek and Aghajanian 1999; Marek and Zhang 2008; Rekling 2004).

The NE-induced spontaneous EPSCs are especially interesting given the existence of significant similarities between α_1-adrenoceptors (especially the α_{1B} subtype) and 5-HT_{2A} receptors with respect to regional brain distribution, laminar cortical protein distribution, and laminar cortical mRNA distribution, in addition to a range of physiological and behavioral effects with salient medial PFC (mPFC) involvement as discussed elsewhere (Marek and Aghajanian 1999; Santana et al. 2012) and later in this chapter. Expression of α_1-adrenoceptors in glutamatergic axons and terminals in the mPFC, including colocalization with vesicular glutamate transporter 1 and 2 (VGluT1 and VGluT2), are consistent with cells of origin in the PFC and midline and intralaminar thalamic nuclei, respectively (Mitrano et al. 2012). When compared to the quite modest and more controversial localization of

Table 2 Neurotransmitters that induce EPSCs/EPSPs via a feedforward mechanism

Neurotransmitter	Receptor	TTX	LY293558	DAMGO
norepinephrine	α_1-noradrenergic	Suppress	Suppress	Suppress
Glutamate	$mGlu_5$	Suppress	Suppress	Suppress
Hypocretin-2	Orexin OX_2	Suppress	*Not tested*	Suppress
Neurokinin	NK_3	*Not tested*	*Not tested*	*Not tested*
acetylcholine	$\alpha_4\beta_2$	Suppress	Suppress	Suppress

5-HT_{2A} receptors to presynaptic neuronal compartments (primarily monoaminergic axons and varicosities) determined using well-known antibodies (Jakab and Goldman-Rakic 1998; Miner et al. 2003), the fact that the majority of α_1-adrenoceptors in the PFC, ventral tegmental area, and nucleus accumbens are expressed by unmyelinated axons and axon terminals rather than having a postsynaptic localization raises interesting questions about the reliability of monoamine antibody epitopes required to detect labeling in presynaptic terminals and axons.

In recordings from L5 pyramidal cells in rat brain slices, the type I mGlu receptor agonist DHPG induces an increase in spontaneous EPSCs that can be blocked by a selective negative allosteric modulator (NAM) of the $mGlu_5$ receptor (Marek and Zhang 2008). The pharmacology of DHPG-induced EPSCs is similar to 5-HT-induced EPSCs with respect to their suppression and enhancement by $mGlu_{2/3}$ receptor agonists and antagonists, respectively. In addition, a μ-opioid receptor agonist, as well as a AMPA/GluK5 receptor antagonist (LY293558), suppressed DHPG-induced EPSCs. DHPG was not observed to have consistent effects on membrane depolarization after blockade of sodium channels and impulse flow with TTX. Although $mGlu_5$ receptors are predominantly expressed at postsynaptic sites in the neocortex, according to light and electron microscopic studies, a minority of $mGlu_5$ receptors are localized in presynaptic axon terminals (Romano et al. 1995). The localization of $mGlu_5$ receptor mRNA and protein is consistent with a presynaptic role where the cells of origin might arise from either the thalamus, the PFC and neocortex, or both (Romano et al. 1995; Neto et al. 2000; Simonyi et al. 2005). Strong evidence for a presynaptic effect of orexin-B involving OX_2 receptors will be discussed shortly, including data indicating that the midline and intralaminar thalamic nuclei are a potential source for these afferents (Lambe et al. 2007; Lambe and Aghajanian 2003). The pharmacology of the senktide-induced increase in EPSC frequency has not been defined beyond blockade by NK_3 receptor antagonists. Immunohistochemical, receptor autoradiography, and mRNA studies in rats appear to suggest that NK_3 is largely a cortical receptor expressed in mid-cortical layers (layers IV and V) throughout the PFC and neocortex (Ding et al. 1996; Langlois et al. 2001; Saffroy et al. 2003; Shughrue et al. 1996). However, one study suggests that NK_3 receptors are present in the midline thalamic nuclei in primates (Rigby et al. 2005). Thus, agonists for a number of $G_{q/11}$-coupled receptors known to be expressed in the midline and intralaminar thalamic nuclei appear to induce an increase in the frequency of spontaneous EPSCs similar to the effect of 5-HT on L5 pyramidal neurons in the PFC and neocortex (Kolaj et al. 2014).

In addition to the neurotransmitters discussed above, acetylcholine (ACh) and nicotine were also found to induce EPSCs/EPSPs through activation of $\alpha_4\beta_2$ nicotinic receptors on thalamic afferents to L5 pyramidal cells (Lambe et al. 2003, 2005). Evidence also exists that ACh may increase the frequency of spontaneous EPSCs, although the pharmacology of this effect has not been defined beyond the involvement of muscarinic M_1 receptor activation. The potential for a $G_{i/o}$-coupled muscarinic receptor to suppress 5-HT-, NE-, orexin-B-, and DHPG-induced EPSCs should be explored as well.

Neurocircuitry: Thalamocortical versus cortical/claustrum projections to mPFC layer V neurons

At least a majority of the 5-HT-induced EPSCs, if not all, appear to originate from subcortical sources. The frequency of 5-HT-induced EPSCs in rat PFC slices was reduced 61–67% by fiber-sparing chemical or radiofrequency lesions of the midline and intralaminar thalamic nuclei that were made 12–18 or 6–14 days, respectively, before the intracellular recordings from L5 pyramidal cells (Marek et al. 2001). As described above, these thalamic lesions also reduced the expression of $mGlu_2$ and μ-opioid receptors, suggesting that about 40% of the $mGlu_2$ and μ-opioid receptors in the PFC are located on thalamic afferents presynaptic to the L5 pyramidal cells. The fact that these lesions greatly reduce the frequency of 5-HT-induced EPSCs suggests an important functional link to thalamic terminals. Postsynaptic $5\text{-}HT_{2A}$ receptors present in layers I and Va of the mPFC were significantly upregulated (∼114% of control levels) in response to the chemical fiber-sparing thalamic lesions. Because $5\text{-}HT_{2A}$ receptors are primarily localized postsynaptically on pyramidal cells and interneurons in the PFC and neocortex, an upregulation of postsynaptic $5\text{-}HT_{2A}$ receptors would likely conceal the fact that a relatively minor pool of presynaptic $5\text{-}HT_{2A}$ receptors was eliminated by the thalamic lesions. Less than 25% of the prefrontal cortical $5\text{-}HT_{2A}$ receptors are present on presynaptic sites on axons, and most of those $5\text{-}HT_{2A}$ receptors are thought to be present on the axons of monoaminergic neurons (Miner et al. 2003). This assessment, which was made using currently available antibodies, suggests that glutamatergic terminals and axons expressing $5\text{-}HT_{2A}$ receptors are relatively rare in the mPFC (Jakab and Goldman-Rakic 1998). A subsequent report confirmed that most of the 5-HT-induced EPSPs recorded in PFC slices appear to originate from axons and terminals of thalamic projections (Lambe and Aghajanian2001). Additional experiments suggested that 5-HT induces EPSCs by closing Kv1.2-containing potassium channels (Lambe and Aghajanian2001). Confirming previous reports that presynaptic $\alpha_4\beta_2$ nicotinic acetylcholine (nACh) receptors in mid-cortical layers originate from the thalamus, Lambe and colleagues reported that thalamic lesions reduced the frequency of nicotine- and ACh-induced spontaneous EPSCs by ∼80% (Lambe et al. 2003). Thus, agonists for both the $5\text{-}HT_{2A}$ receptor and the $\alpha_4\beta_2$ nAChR induced an increase in spontaneous currents in the PFC via activation of afferents from the midline thalamus.

Around this time, compelling converging evidence emerged suggesting that activation of $orexin_2$ (OX_2) receptors induced an increase in spontaneous EPSCs via activation of afferents from the midline and intralaminar thalamic nuclei (Lambe and Aghajanian 2003). First, in recordings from layer V pyramidal cells, the hypocretin-2 peptide was found to induce spontaneous EPSCs without directly depolarizing the pyramidal cells. Hypocretin-2, an OX_2 receptor agonist, induced calcium transients in a small minority of spines (<10%) in the layer I and Va apical dendritic fields of the pyramidal cells. Similar calcium transients were not induced in basilar dendritic spines. Furthermore, these calcium transients were induced in spines generally in apposition to anterogradely labeled terminals originating from

neurons in the midline and intralaminar thalamic nuclei. Midline thalamic lesions suppressed the hypocretin-induced EPSCs without affecting baseline EPSCs. TTX, μ-opioid agonists, and an AMPA receptor antagonist all suppressed the spontaneous EPSCs and the calcium transients, similar to the effects observed previously for 5-HT_{2A} receptor activation. These findings with hypocretin-2 are also consistent with the hypothesis that 5-HT_{2A} receptor activation induces Glu release onto the apical dendritic field of L5 pyramidal neurons via thalamocortical afferents.

The partial suppression (60–65%) of 5-HT-induced EPSCs in the mPFC by large thalamic lesions suggests that there is at least one additional source of afferents to the L5 pyramidal cells. The basolateral nucleus of the amygdala (BLA) projects to the deep portion of layer I, layer II, and the entire width of layer V, making this amygdaloid nucleus another potential subcortical source for 5-HT-induced EPSCs. However, complete destruction of the BLA bilaterally did not alter the frequency of 5-HT-induced EPSCs, ruling out the amygdala as a feedforward afferent to the L5 pyramidal cells (Marek et al. 2001).

Several cortical sources that could potentially mediate the EPSCs induced by 5-HT and serotonergic hallucinogens have also been proposed. Rodrigo Andrade and colleagues identified a subpopulation of neurons deep within the mPFC that are depolarized sufficiently by 5-HT_{2A} receptor activation to fire action potentials; these cells are a potential source of the 5-HT-induced EPSCs (Beique et al. 2007). This subpopulation of mPFC neurons needs to be defined in greater detail, especially with regard to the $G_{i/o}$-coupled GPCRs that suppress 5-HT-induced EPSCs and the other $G_{q/11}$-coupled GPCRs that produce similar increases of spontaneous EPSC frequency. To date, the μ-opioid receptor is the only GCPR known to modulate the activity of this population of deep cortical neurons. A more complete pharmacological characterization of these cells with respect to inhibitory effects of $mGlu_2$, $mGlu_4$, $mGlu_8$, adenosine A_1, dopamine $D_{1/5}$, β_2-adrenergic and 5-HT_1 receptors and the excitatory effects of OX_2, α_1-adrenergic, $mGlu_5$, $\alpha_4\beta_2$-nicotinic, and NK_3 receptors is necessary before concluding that these cells represent a cortical source of the afferents activated by 5-HT_{2A} receptors. Another cortical source for 5-HT-induced EPSCs may be L5 pyramidal neurons that send callosal and commissural projections to the contralateral cortex, which are excited by 5-HT (Avesar and Gulledge 2012). Unfortunately, the pharmacology of these cells with respect to inhibition and excitation by the range of GPCRs described above has not been explored.

One report, which analyzed 5-HT-induced EPSCs recorded from transgenic mice that were rescued from a constitutive knockout of 5-HT_{2A} receptors throughout the CNS, has suggested that 5-HT-induced EPSCs arise primarily from neocortical afferents intrinsic to the cerebral cortical mantle (Weisstaub et al. 2006). However, interpretation of the *htr2a* gene rescue experiment for the thalamus appears to have been compromised by the use of a promoter that is weakly expressed in the midline and intralaminar thalamic nuclei compared to a robust expression in the primary sensory thalamic nuclei (Lebrand et al. 1996; Narboux-Neme et al. 2008). Further, the *htr2a* gene rescue directed at the mPFC also included the claustrum as an area being rescued. Because the claustrum, which

sends afferents to layers I and V of the mPFC and neocortex, is also a potential source of the afferents mediating the 5-HT-induced EPSCs, it is premature to conclude that the spontaneous 5-HT-induced EPSCs are mediated by cortical afferents based solely on this experiment. Clearly, further work is necessary to understand whether circuitry between the mPFC and claustrum plays a role in mediating the effects of hallucinogenic drugs and 5-HT_{2A} receptor activation. Additional studies directed at understanding whether hippocampal afferents to the mPFC could be involved are also warranted. Further studies using different technologies to confirm or refute the presence of 5-HT_{2A} receptors in midline and intralaminar thalamic nuclei, such as optogenetic recordings with gene-based targeting strategies, could clarify models of interactions between hallucinogenic drugs and Glu in the PFC (Barre et al. 2012). Nevertheless, as indicated by both thalamic lesions and the combined two-photon imaging and anterograde labeling of thalamic projections arising from the intralaminar and thalamic nuclei, these "non-specific" thalamic nuclei clearly seem to serve as an important substrate for the spontaneous EPSCs induced by 5-HT and hypocretin-2. Additional experiments are required to understand the potential role of claustral and/or neocortical afferents to L5 pyramidal cells.

1 From 5-HT_{2A} Receptor-Induced EPSCs and Recurrent Neuronal Activity to Salient Behavioral Effects Induced by 5-HT_{2A} Receptor Activation

Head-Twitch Response

Given that a wide range of neurotransmitter systems appear to have modulatory effects on Glu released from subcortical terminals and/or intrinsic cortical afferents onto the apical dendrites of L5 pyramidal cells, salient behavioral effects would be expected to arise from these interactions since L5 pyramidal cells are the principal source of output from the neocortex to subcortical regions. A range of behaviors induced by systemic administration of hallucinogenic drugs, including a relatively simple behavioral response known as head twitches or head shakes, appear to be modulated by the same neurotransmitter relationships outlined previously for subcortical glutamatergic afferents to L5 pyramidal cells (Canal and Morgan 2012; Handley and Singh 1986).

According to a report published almost 20 years ago, local infusion of DOI into the prelimbic region of the mPFC induces a head-twitch response (HTR) that is blocked by systemic administration of 5-HT_{2A} receptor antagonists but not by a selective 5-HT_{2C} receptor antagonist, suggesting that hallucinogen-induced head twitches are mediated by activation of 5-HT_{2A} receptors in the mPFC (Willins and Meltzer 1997). Given that a range of antidepressant drugs, as well as typical and atypical antipsychotic drugs, suppress the HTR induced by DOI, 5-HTP, and quipazine (Czyrak et al. 1993; Eison et al. 1990; Friedman et al. 1983; Goodwin

et al. 1984; Moore et al. 1992; Nacca et al. 1998; Peroutka et al. 1981; Rojoz 2012; Sanchez and Arnt 2000; Wettstein et al. 1999), drugs acting on $mGlu_2$ receptors were tested for effects on the DOI-induced HTR. Several $mGlu_{2/3}$ receptor agonists (e.g., LY354740 and LY379268) and $mGlu_2$ receptor PAMs (e.g., BINA and CBiPES) were found to suppress DOI-induced head twitches, whereas the $mGlu_{2/3}$ receptor antagonist LY341495 potently enhances the frequency of DOI-induced head twitches (Fig. 2), consistent with an interaction between 5-HT_{2A} receptors and $mGlu_2$ autoreceptors on afferents to the mPFC (Benneyworth et al. 2007; Klodzinska et al. 2002; Benvenga et al. 2006; Gewirtz and Marek 2000; Gonzalez-Maeso et al. 2008; Moreno et al. 2011; Wieronska et al. 2013). These observations prompted efforts to determine whether a range of neurotransmitter receptor agonists, PAMs, or antagonists that modulate 5-HT-induced EPSCs can also alter the HTR induced by serotonergic hallucinogens. As expected, μ-opioid agonists (Marek 2003; Rojas-Corrales et al. 2007), adenosine A_1 receptor agonists (Marek 2009), AMPA receptor antagonists (Egashira et al. 2011; Zhang and Marek 2008), $mGlu_4$ receptor PAMs (Slawinska et al. 2013; Wieronska et al. 2012), $mGlu_5$ receptor negative allosteric modulators (NAMs; GJ Marek, unpublished observations), NK_3 receptor antagonists (GJ Marek, unpublished observations), α_1-adrenergic receptor antagonists (Dursun and Handley 1996; Schreiber et al. 1995), OX_2 receptor antagonists (GJ Marek, unpublished observations), and β_2-adrenergic receptor agonists (GJ Marek, unpublished observations) have been found to suppress head twitches induced by serotonergic hallucinogens (Fig. 2). Thus, impressive coherence exists between a range of targets modulating Glu release due to 5-HT_{2A} receptor activation and the ability of ligands acting at those targets to modulate the hallucinogen-induced HTR in rodents (Table 3).

In contrast to expectations from the molecular targets described above, dopamine $D_{1/5}$ receptor antagonists were found to suppress, rather than enhance, DOI-induced head twitches (Dursun and Handley 1996; Schreiber et al. 1995). However, the potency and efficacy of the dopamine D_1 receptor antagonists in suppressing DOI-induced head twitches was equally correlated with affinity for 5-HT_{2A} receptors and dopamine D_1 receptors, creating uncertainty regarding which mechanism was responsible for the behavioral action. Nicotine is another ligand that has been tested and found to have effects opposite to those expected based on its ability to increase spontaneous EPSCs. Nicotine suppresses DOI-induced head twitches (Gaynor and Handley 2001; Hayslett and Tizabi 2005; Tizabi et al. 2001). However, since hallucinogen-induced head twitches in rodents have been reported to have an inverted-U-shaped dose response relationship, it is not clear whether the apparent suppressant effect of nicotine on DOI-induced head twitches reflects a confounding effect of the position on the dose–response curve being studied.

Further pharmacological characterization with respect to Glu suggests that low doses of DOI (0.315–0.63 mg/kg) and the NMDA receptor antagonist MK-801 (0.2 mg) have synergistic effects on horizontal locomotor activity in rats when administered in combination (Zhang and Marek 2008). Taken together, these overall findings are consistent with the hypothesis that modulation of Glu spillover (arising from subcortical afferents and/or intrinsic cortical afferents and directed

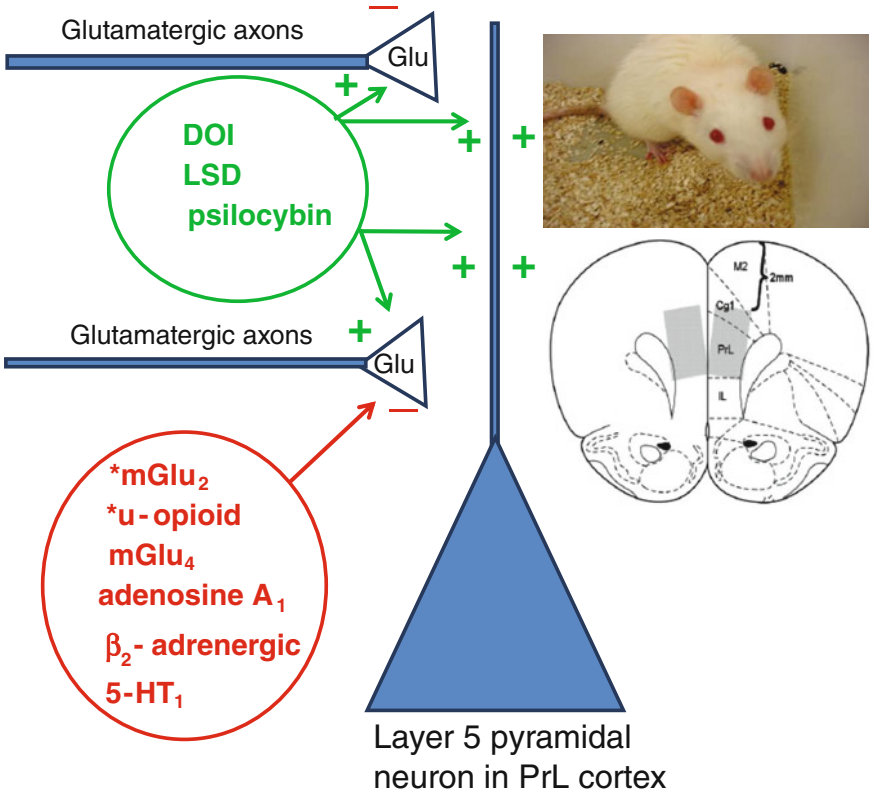

Fig. 2 Model of glutamatergic afferents to the apical dendrites of layer 5 pyramidal cells that release glutamate (Glu) in response to serotonergic hallucinogens such as LSD, DOI, and psilocybin (shown in *green*), which also induce head twitches in rats and mice. A variety of presynaptic receptors can suppress the hallucinogen-induced head twitches, including $mGlu_2$, $mGlu_4$, μ-opioid, adenosine A_1, β_2-adrenergic, and 5-HT_{1A} receptors. All of these receptors suppress recurrent network activity (late EPSPs) induced by stimulation of 5-HT_{2A} receptors on presumed thalamocortical afferents. Most of these agonists only partially suppress DOI-induced head shakes. The Glu released in response to 5-HT_{2A} receptor activation then stimulates AMPA receptors on the layer 5 pyramidal neurons. The inset picture on the top right shows a Sprague-Dawley rat. The inset illustration on the bottom right shows a coronal section through the brain with shading indicating the location of the prelimbic (PrL) region of the medial prefrontal cortex. As shown by David Willins and Herb Meltzer in 1997, infusion of DOI into the PrL region induces head-twitch behavior. *Abbreviations:* Cg1 cingulate area 1; IL infralimbic cortex; M2 premotor cortex

onto the apical dendritic field of L5 pyramidal cells in the mPFC) is a key substrate for modulating the HTR in mice and rats, a well-known behavioral effect of serotonergic hallucinogens.

Table 3 Corrrelation between PFC in vitro electrophysiology and in vivo screening for psychiatric drugs

Drug Type	Effect on feed forward neurotransmitter-induced EPSCs/EPSPs	Effect on feed forward and feedback recurrent network activity (late EPSPs)	Effect on fast EPSP/EPSC	Effect in the DRL 72-s behavioral paradigm	Effect on DOI-induced head shakes or NMDA receptor antagonist-induced hyperactivity
5-HT_{2A} receptor antagonist	Suppress	Suppress	No effect	Antidepressant-like	Suppress
Dextran	No effect	Suppress	No effect	Not tested	Not tested
$mGlu_{2/3}$ receptor agonist	Suppress	Suppress	Modest, less potent	No Effect	Suppress
$mGlu_2$ receptor PAM	Suppress	Not tested	No effect	Antidepressant-like	Suppress
Adenosine A_1 receptor agonist	Suppress	Suppress	No effect	Antidepressant-like	Suppress
$mGlu_{4/8}$ receptor agonist	Suppress	Not tested	No effect	Not tested	Suppress
μ-Opioid receptor agonist	Suppress	Not tested	No effect	No effect	Suppress
β_2-receptor agonist	Not tested	Suppress	No effect	Antidepressant-like	Suppress
Dopamine D_1 receptor agonist	Not tested	Suppress	No effect	Not tested	No effect
Orexin OX_2 receptor antagonist	Suppress	Not tested	No effect	AD-like	Suppress
$mGlu_5$ receptor NAM	Suppress	Not tested	No effect	No effect	Suppress
α_1-Adrenergic receptor antagonist	Suppress	Not tested	No effect	No effect	Suppress
NK_3 receptor antagonist	Suppress	Not tested	No effect	No effect	Suppress
Ketamine	Increase	Not tested	decrease	Antidepressant-like	Increased
Lithium (acute)	Increase	Not tested	Not tested	Not tested	Increased

Beyond their utility as an in vivo model for modulation of mPFC 5-HT_{2A} receptor activity, DOI-induced head twitches in rodents have been used as a relatively promiscuous screening model for potential antipsychotic and/or antidepressant drugs. The DOI-induced HTR has also been suggested to be a model for tic disorders, such as Tourette's syndrome. However, the actual physiological role for the HTR, observed to occur at a relatively low spontaneous rate in all vertebrate species outside of most primates and humans, remains to be delineated.

Motoric impulsivity studied using the 5-CSRTT

The five-choice serial reaction time test (5-CSRTT) is another intriguing behavioral paradigm that has been studied with respect to activation of 5-HT_{2A} receptors in the PFC. A particular type of impulsivity, motoric impulsivity or action impulsivity, is assessed using the 5-CSRTT. Important for our discussion, systemic administration of 5-HT_{2A} receptor antagonists such as ketanserin reduces motoric impulsivity (especially premature responses) in 5-CSRTT studies, but does not alter another form of impulsivity related to delayed reward presentation (Talpos et al. 2006). Anatomical validation of this behavior was demonstrated by the finding that infusion of selective 5-HT_{2A} receptor antagonists such as M100907 into the mPFC suppressed anticipatory responding induced by administration of a NMDA receptor

antagonist (Carli et al. 2006). As expected based on in vitro electrophysiological recordings from L5 pyramidal cells and studies of DOI-induced head twitches, pretreatment with the $mGlu_{2/3}$ receptor agonist LY379268 blocks impulsivity or over responding induced by DOI in rats performing the 5-CSRTT (Wischhof and Koch 2012). Furthermore, intra-mPFC infusions of DOI were shown to increase impulsive responding in the 5-CSRTT whereas the $mGlu_{2/3}$ receptor agonist LY379268 suppressed this effect of DOI (Wischhof et al. 2011). Also, as expected from electrophysiological and HTR studies, the α_1-adrenergic receptor antagonist prazosin suppressed DOI-induced premature responses on the 5-CSRTT, in contrast to the lack of effect for an α_2-adrenergic receptor antagonist (Koskinen et al. 2003).

Both consistent and contradictory behavioral evidence for a modulatory effect of dopamine $D_{1/5}$ receptors has been found. For example, the dopamine $D_{1/5}$ receptor agonist SKF38393 enhanced the accuracy of attentional performance under low baseline conditions; a dopamine D_1 receptor antagonist blocked the effect of SKF38393 (Granon et al. 2000). Furthermore, systemic administration of the dopamine $D_{1/5}$ receptor agonist SKF38393 improved choice accuracy in the 5-CSRTT, whereas the $5\text{-}HT_{2A}$ antagonist ketanserin suppressed premature responding when administered systemically or directly into the mPFC (Passetti et al. 2003). These findings were extended to the striatum where infusions of a dopamine D_1 receptor agonist improved accuracy in the 5-CSRTT; these effects were also blocked by a dopamine D_1 receptor antagonist. In contrast to the previous reports, other experiments suggested that the dopamine D_1 receptor antagonist SCH23390 suppressed the effect of DOI on premature responding (Koskinen and Sirvio 2001). Hence, although further pharmacological characterization with a range of other GPCR agonists and antagonists is necessary, initial experiments have shown that the 5-CSRTT has intriguing consistencies with the electrophysiology of L5 pyramidal cells and the DOI-induced HTR.

DRL 72-s operant behavior: A behavioral screen for antidepressant-like effects

Operant lever pressing by rats under a differential-reinforcement-of-low rate 72-sec (DRL-72 s) schedule of reinforcement is another behavioral paradigm involving PFC-striatal-thalamic-amygdala loops with a critical relationship to motoric impulsive behavior (Marek et al. 2016). Antidepressant drugs as a class appear to bias the responding of rats operating under DRL 72-s schedules away from impulsive responding (e.g., failing to wait 72-s from the last response to lever press in order to obtain water or food) and increase the probability that responding will occur at time durations matching reinforcement contingencies (Marek et al. 2016; O'Donnell et al. 2005). Not surprisingly, selective blockade of $5\text{-}HT_{2A}$ receptors induces cohesive antidepressant-like rightward shifts in the inter-response time (IRT) distribution (along with increases in the reinforcement rate and decreases in the response rate) alone or when added to the SSRI fluoxetine, the tricyclic antidepressant desipramine, and the monoamine oxidase inhibitor tranylcypromine (Ardayfio et al. 2008; Marek et al. 2005). Similar rightward shifts in IRT distributions were also induced by the selective $5\text{-}HT_{2A}$ receptor antagonist M100907

following administration of the channel blocking NMDA receptor antagonist MK-801 (Ardayfio et al. 2008; Higgins et al. 2003). As expected from the relationships between studies of L5 pyramidal cell electrophysiological responses and the DOI-induced HTR, suppressing Glu release with a $mGlu_2$ receptor PAM or an adenosine A_1 receptor agonist also resulted in antidepressant-like effects in rats responding under DRL 72-s schedules (Fell et al. 2011; Marek 2012; Nikiforuk et al. 2010). More recently, the blockade of OX_2 receptors was reported to result in antidepressant effects on DRL 72-s behavior (Fitch et al. 2014). Interestingly, several decades ago, β_2-adrenergic receptor agonists such as clenbuterol were demonstrated to produce antidepressant-like activity on DRL 72-s behavior in rats (Dunn et al. 1993; O'Donnell 1987, 1988, 1990, 1993; O'Donnell et al. 1994), effects that appear consistent with preliminary clinical observations and also match their profile of effects on rat PFC slice electrophysiology and the HTR induced by DOI. While some receptor systems discussed above have not yet been tested ($mGlu_4$ receptor agonists, dopamine $D_{1/5}$ receptor agonists, and NK_3 receptor antagonists), μ-opioid receptor agonists and α_1-adrenergic receptor antagonists do not appear to induce antidepressant-like effects on DRL 72-s behavior.

The lack of antidepressant-like effects on DRL behavior for some agents known to suppress Glu release onto L5 pyramidal cells is not surprising given the likelihood that actions on μ-opioid receptors or α_1-adrenergic receptors elsewhere in prefrontal cortical–striatal–thalamic–amygdaloid circuits may counter their modulatory effects on the thalamic and non-thalamic glutamatergic afferents to cortical L5 pyramidal cells. What is remarkable is that so many of the mechanisms that suppress 5-HT_{2A} receptor-induced Glu release onto L5 pyramidal cells possess either preclinical antidepressant-like (e.g., DRL 72-s behavior) or antipsychotic-like behavioral effects (e.g., blockade of DOI-induced HTR and/or NMDA receptor antagonist-induced hyperactivity; Table 3).

Heterocomplexes or functional interactions between 5-HT_{2A} and $mGlu_2$ receptors?

In vitro colocalization demonstrated using BRET and FRET technology and a wealth of both in vitro and in vivo functional interactions has led to the suggestion that a heterocomplex is formed between 5-HT_{2A} and $mGlu_2$ receptors in L5 pyramidal cells in the PFC and neocortex (Gonzalez-Maeso et al. 2008; Moreno et al. 2011; Fribourg et al. 2011). The in vitro localization of apparent complexes between 5-HT_{2A} and $mGlu_2$ receptors in transfected cell lines has been confirmed by others (Delille et al. 2012). However, functional evidence for heterocomplexes between 5-HT_{2A} and $mGlu_2$ receptors could not be confirmed in in vitro experiments (Delille et al. 2012). Thus, the presence of 5-HT_{2A} receptors did not appreciably appear to affect the binding of $mGlu_2$ radioligands and vice versa. Furthermore, it is not clear that heteromeric receptors are necessary for the occurrence of functional interactions such as those that exist between 5-HT_{2A} receptors and $mGlu_2$ receptors. The only other G_q-coupled GPCR that may be closely localized with $mGlu_2$ receptors in heteromeric receptor complexes is the $mGlu_5$ receptor (Delille et al. 2012), but such relationships are not known to exist for other receptors that are capable of inducing EPSCs, such as α_1-adrenergic, OX_2,

or NK_3 receptors (Delille et al. 2013). Similarly, heteromeric relationships have not been identified between 5-HT_{2A} receptors and a range of receptors that can suppress 5-HT-induced EPSCs (Table 1) (Delille et al. 2013). In contrast, Doumazane and colleagues did produce evidence for the existence of heterodimers between $mGlu_2$ and $mGlu_4$ receptors in overexpressing cell lines (Doumazane et al. 2011). The model for heterocomplexes between 5-HT_{2A} and $mGlu_2$ receptors places them both on L5 pyramidal cells and ignores the data suggesting that only a very minor subpopulation of 5-HT_{2A} receptors are expressed presynaptically, as well as the overwhelming evidence for a predominantly presynaptic function of the $mGlu_2$ receptor (Delille et al. 2013). Although it has been suggested that clozapine downregulates both $mGlu_2$ and 5-HT_{2A} receptors (Gonzalez-Maeso et al. 2008), this finding was not observed by others (Wright and Schoepp 2003). Furthermore, dissociations between $mGlu_2$ receptor distribution and 5-HT_{2A} receptor distribution appear to occur following sub-chronic antidepressant treatment in rodents or in depressed patients (Muguruza et al. 2014). Previous evidence for a dissociation between $mGlu_2$ and 5-HT_{2A} receptor distribution was derived from studies of midline thalamic lesions, where a $\sim$40% reduction of the density of presumed presynaptic $mGlu_2$ receptors occurred simultaneously with an increase in the density of postsynaptic 5-HT_{2A} receptors (Marek et al. 2001). In contrast to these previous lines of evidence, the in vitro and in vivo evidence that mutating three residues from the intracellular portion of the $mGlu_2$ receptor suppresses 5-HT_{2A} receptor activity strongly supports the existence of 5-HT_{2A}- and $mGlu_2$-containing heterocomplexes (Moreno et al. 2012). Thus, it remains to be completely understood which of the following three hypotheses explains the in vivo interactions between 5-HT_{2A} and $mGlu_2$ ligands: (1) only functional relationships occur between 5-HT_{2A} and $mGlu_2$ receptors; (2) only heterocomplexes containing 5-HT_{2A} and $mGlu_2$ receptors mediate the interactions; or (3) both independently acting 5-HT_{2A} and $mGlu_2$ receptors and 5-HT_{2A}–$mGlu_2$ heterocomplexes are involved.

As an aside with regard to receptor heteromers involving 5-HT_{2A} receptors, heterocomplexes between 5-HT_{2A} and cannabinoid CB_1 receptors appear to be involved in Δ^9-tetrahydrocannabinol (THC)-induced amnesia but not the locomotor, hypothermic, anxiogenic, or antinociceptive effects (Vinals et al. 2015). These 5-HT_{2A} and CB_1 heteromers appear to share some similarity to 5-HT_{2A}–$mGlu_2$ heterocomplexes since the 5-HT_{2A} receptor is primarily a postsynaptic receptor while $mGlu_2$ and CB_1 receptors are primarily presynaptic receptors.

2 Clinical Relevance of the Interactions Between Glutamate and 5-HT_{2A} Receptors in the mPFC

Based initially on insights regarding the interaction of hallucinogenic drugs with the 5-HT_{2A} receptor, several 5-HT_{2A} receptor antagonists were discovered, characterized, and tested in patients with schizophrenia. Clinical results with drugs lacking

selectivity at 5-HT_{2A} versus 5-HT_{2C} receptors (e.g., ritanserin) or with drugs having at least 30-fold selectivity for 5-HT_{2A} versus 5-HT_{2C} receptors (e.g., M100907, SR46349B, pimavanserin, and CYR-101) have been disappointing because none of the drugs exerted antipsychotic effects approaching those of either first- or second-generation antipsychotic drugs when tested as monotherapy for schizophrenia. At best, these drugs exert statistically significant but clinically disappointing intermediate efficacy between haloperidol and placebo (Marder 1999; Meltzer et al. 2004). Even when added to the atypical antipsychotic risperidone, the positive therapeutic effects of the 5-HT_{2A} inverse agonist pimavanserin were limited to those subjects on a very low (2 mg) daily dose of risperidone (Meltzer et al. 2012).

More recent results over the last 7 years with pimavanserin raise some hope that selective 5-HT_{2A} receptor antagonists or inverse agonists may provide clinically relevant therapeutic effects for psychosis in patients with neurodegenerative disease (Hacksell et al. 2014). Pimavanserin is only the second potent 5-HT_{2A} receptors antagonist or inverse agonist that has been shown to improve Parkinson's disease (PD) psychosis (Cummings et al. 2014; Meltzer et al. 2010). The other antipsychotic exerting both demonstrable antipsychotic efficacy and lacking clinically significant extrapyramidal side effects is clozapine (French Clozapine Parkinson Study Group 1999; Parkinson Study Group 1999). In April 2016, the FDA approved pimavanserin for psychosis associated with PD. With respect to the disease itself, multiple studies involving both postmortem data and in vivo PET imaging have reported increased levels of 5-HT_{2A} receptor expression in patients with Parkinson's disease (Ballanger et al. 2010; Chen et al. 1998; Huot et al. 2010; Rasmussen et al. 2016). Changes in brain $mGlu_2$ receptor expression in Parkinson's disease have yet to be examined in relationship to questions of functional interactions with 5-HT_{2A} receptors or the existence of an actual heterocomplex between these two receptors. Nevertheless, the effect of pimavanserin on Parkinson's disease does suggest that relatively selective 5-HT_{2A} blockade can suppress the delusions and hallucinations characteristic of this neurodegenerative disorder.

A phase 2 randomized, placebo-controlled trial addressing the effects of pimavanserin on psychosis associated with Alzheimer's disease (AD) was recently completed and reportedly showed positive results (www.ClinicalTrial.gov identifier NCT02035553). Thus, studies in psychosis associated with either PD or AD have brought to realization predictions made years ago by the late psychiatrist Dr. Daniel X. Freedman that the study of LSD would result in advances for the treatment of major mental illnesses.

The pimavanserin AD study is intriguing given that (1) down-regulation of 5-HT_{2A} receptors occurs in AD before other neurotransmitter receptors are altered (Blin et al. 1993; Cross et al. 1984, 1986; Hasselbalch et al. 2008; Lai et al. 2005; Lorke et al. 2006; Marner et al. 2011; Meltzer et al. 1999; Perry et al. 1984; Reynolds et al. 1984; Santhosh et al. 2009); (2) pharmacogenetic studies have revealed a potential association between certain 5-HT_{2A} receptor gene single nucleotide polymorphisms (SNPs) and psychosis in AD patients (Ramanathan and Glatt 2009); and (3) shared pathophysiology exists between AD and PD such as early degeneration of cholinergic and serotonergic neurons, amyloidosis, and the

presence of Lewy bodies (Bohnen and Albin 2011; Halliday et al. 2011; Petrou et al. 2012; Trillo et al. 2013).

The recent studies showing that a 5-HT_{2A} receptor inverse agonist can be used to treat psychosis associated with either PD or AD raises the provocative question of whether $mGlu_{2/3}$ receptor agonists might have a larger effect size in PD or AD psychosis compared with the apparent efficacy of the $mGlu_{2/3}$ agonist LY2140023 (pomaglumetad methionil) in only a modest subset of patients with schizophrenia. Given that clinical lore and experience in methadone maintenance clinics indicates that μ-opioid receptor agonists may possess clinical antipsychotic activity (Marek and Aghajanian 1998), the work with 5-HT_{2A} receptor antagonists in psychosis associated with neurodegenerative diseases suggests that testing compounds acting at novel molecular targets for antipsychotic activity should not be solely limited to patients with schizophrenia.

As described earlier, a number of drugs targeting receptor sites that modulate 5-HT-induced EPSCs in rat mPFC slice preparations also exert antidepressant-like effects in the DRL 72-s schedule. This includes selective 5-HT_{2A} receptor antagonists such as M100907, ketanserin, and pipamperone (Ardayfio et al. 2008; Marek et al. 1989, 2005; Marek and Seiden 1988). 5-HT_{2A} receptor antagonists also enhance the therapeutic effects of SSRIs, tricyclic antidepressants, and monoamine oxidase inhibitors (Ardayfio et al. 2008; Marek et al. 2005). These findings are consistent with the results of double-blind, placebo-controlled studies suggesting antidepressant efficacy for drugs such as trazodone, nefazodone, mirtazapine, and mianserin (Marek et al. 2003), which block 5-HT_{2A} receptors. Some, but not all, studies examining augmentation with mirtazapine or mianserin have demonstrated improved antidepressant efficacy (Carpenter et al. 2002; Ferreri et al. 2001; Maes et al. 1999). Furthermore, these preclinical data are also consistent with a meta-analysis confirming that adding atypical antipsychotic drugs, which block 5-HT_{2A} receptors among a range of pharmacological effects, onto ongoing treatment with SSRIs or SNRIs improves the clinical antidepressant action (Nelson and Papakostas 2009). Previous open-label and controlled studies suggested that the β_2-adrenergic receptor agonist salbutamol might have antidepressant effects in depressed patients (Lecrubier et al. 1980; Simon et al. 1984). A $mGlu_2$ receptor PAM is the only novel mechanism suppressing 5-HT-induced EPSCs that has also been tested in a clinical study. The results of a single trial investigating the effect of adjunctive treatment with a $mGlu_2$ receptor PAM in combination with SSRIs/SNRIs largely appeared negative with a numerical but not a significant advantage for the $mGlu_2$ receptor PAM over placebo (Kent et al. 2016). The primary limitation of this $mGlu_2$ PAM trial in major depressive disorder (MDD), which only showed a weak trend toward antidepressant efficacy, was uncertainty whether optimal doses and/or dosing schedules had been used. A number of open-label trials with buprenorphine, a μ-opioid agonist, and κ-opioid antagonist, in patients with MDD, including several involving patients with treatment-resistant depression, have suggested that this compound has antidepressant activity (Bodkin et al. 1995; Emrich 1982; Karp et al. 2014; Kosten et al. 1990). These effects of buprenorphine are consistent with the fact that opiates had a long history of use in depressed patients prior to the

development of other effective treatments (Carlson and Simpson 1963). A selective OX_2 receptor antagonist (Min-202 or JNJ-42847922) is currently being developed by Minerva Neurosciences and Janssen as a treatment for insomnia and MDD. Preliminary reports from phase 1b studies with this OX_2 receptor antagonist in patients with MDD indicate that there were improvements in both sleep and mood, but these results await confirmation in phase 2 proof-of-concept trials. Thus, a number of compounds that block 5-HT_{2A} receptors or suppress the effects of hallucinogens in either in vitro brain slice preparations or in vivo HTR studies in rodents appear to possess at least some potential antidepressant efficacy in patients.

A series of studies with the channel blocking NMDA receptor antagonist ketamine have raised the possibility that a unique pattern of results may have predictive utility for discovering drugs having positive effects in treatment refractory MDD or bipolar depression. In addition to having well-known pro-psychotic effect in subjects with schizophrenia, administration of subanesthetic doses of ketamine produces nearly immediate (90 min) but short-lived (up to approximately 2 weeks) antidepressant effects in subjects with treatment refractory MDD or bipolar depression (Berman et al. 2000; Preskorn et al. 2008; Zarate et al. 2006). When brain slices are prepared from rats treated 24 h earlier with ketamine, an increase in spontaneous EPSCs is observed in recordings from L5 pyramidal cells (Li et al. 2010). More remarkably, a single subanesthetic dose of ketamine (10 mg IP) induces the formation of new synaptic spines in the apical dendritic field of L5 pyramidal cells within 24 h. At the behavioral level, 24 h after ketamine administration antidepressant-like effects were observed in the forced swim test, the learned helplessness paradigm, and the novelty suppressed feeding test. At a mechanistic level, blockade of the mTOR pathway blocked these effects, which had been observed 24 h following an acute dose of ketamine. Deficits in synaptic electrophysiology, spine number, synaptic protein expression, and behavior induced by 21 days of chronic stress exposure are reversed by administration of a single dose of ketamine (Li et al. 2011). These effects of ketamine are also consistent with its antidepressant-like activity in a number of depression models and screens for antidepressant drugs (Table 3). Ketamine has even been found to exert antidepressant-like effects on DRL 72-s behavior (Hillhouse and Porter 2014), although this effect appears to be limited to a period ranging from minutes to 2 h after dosing and is not apparent 24 h after a single acute administration (GJ Marek, unpublished observations).

The evidence with ketamine is further strengthened by recent findings with lithium (Table 3). Lithium potentiates the electophysiological, synaptogenic, and antidepressant-like behavioral effects of ketamine in rats (Liu et al. 2013). These findings are of interest because lithium augmentation has long been a gold-standard treatment for treatment refractory MDD and bipolar disorder (Burgess et al. 2001). The fact that the muscarinic receptor antagonist scopolamine produces electrophysiological, synaptogenic, and antidepressant-like behavioral effects in preclinical rodent studies (Voleti et al. 2013) that parallel its clinical antidepressant effects observed for MDD and bipolar depression, including treatment refractory cases (Drevets and Furey 2010; Ellis et al. 2014; Furey and Drevets 2006;

Khajavi et al. 2012), provides additional support for the use of this paradigm to discover novel antidepressants for treatment refractory patients. From a theoretical standpoint, the effects of rapidly acting putative antidepressants such as ketamine and scopolamine on synaptogenesis may point to a fundamental link between depressive symptoms and the loss of the neuropil observed in MDD patients in postmortem histopathology studies and inferred from the decrease in cortical thickness seen in neuroimaging and postmortem neuropathology studies.

3 Conclusions

After the discovery of LSD's potent psychotropic effects by Albert Hofmann in Basel, Switzerland in April 1943, serotonergic hallucinogens have inspired research goals ranging from curing major neuropsychiatric diseases to understanding consciousness. Understanding the common shared effects of hallucinogens such as LSD, mescaline, and psilocybin has directed modern neuroscience toward the PFC and neocortex, where some of the highest densities of 5-HT_{2A} receptors are found. At the cellular level, a significant literature has described the effects of hallucinogens and 5-HT_{2A} receptor activation on L5 pyramidal cells, which are the principal output cells of the PFC and neocortex. A range of monoaminergic, glutamatergic, purinergic, and peptide neurotransmitter receptor ligands suppress Glu release from putative thalamic afferents and from other sources (potentially including the PFC, neocortex, and claustrum) where the release is induced by activation of 5-HT_{2A} receptors, as inferred from intracellular and whole cell recordings of L5 pyramidal cells in the PFC. Most of these transmitter systems also suppress the HTR induced by serotonergic hallucinogens. Administration of agonists or antagonists for most of these transmitter systems also results in antidepressant-like and/or antipsychotic-like effects for salient behaviors mediated by the PFC. Positive results have been observed for a number, though not all, of these targets when evidence for antidepressant or antipsychotic effects has been evaluated clinically in patients. One important recent lesson in CNS drug development regarding testing for clinical antipsychotic effects is that the recent positive results for pimavanserin in PD psychosis demonstrate that negative or disappointing results in schizophrenia do not always rule out a potential antipsychotic action in patients with neurodegenerative disease. Finally, recent studies with ketamine, lithium, and scopolamine raise the possibility that studying unique electrophysiological, synaptogenic, and behavioral profiles may help to uncover new medications for treatment refractory MDD and bipolar depression. However, there still remain a plethora of outstanding questions at multiple levels of inquiry ranging from receptor–receptor interactions, post-receptor transduction pathways, and both micro- and macro-circuit involvements in a range of behaviors, emotions, thoughts, and in consciousness itself.

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