Chapter 75

Molecular and Cellular Basis of Hallucinogen Action

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Abbreviations

2C-C 2,5-dimethoxy-4-chlorophenethylamine
2C-D 2,5-dimethoxy-4-methylphenethylamine
2C-I 2,5-dimethoxy-4-iodophenethylamine
5-HT 5-hydroxytryptamine (serotonin)
5-HTP 5-hydroxytryptophan
AA Arachidonic acid
CNS Central nervous system
DAG Diacylglycerol
DMT Dimethyltryptamine
DOB 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane
DOC 2,5-dimethoxy-4-chloroamphetamine
DOI (*R*)-(−)-DOI; (−)-2,5-dimethoxy-4-iodoamphetamine hydrochloride
DOM 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane
FISH Fluorescent in situ hybridization
GPCR G-protein-coupled receptor
HSV Herpes simplex virus
IP Inositol phosphate
LSD Lysergic acid diethylamide
LY379268 (1*R*,4*R*,5*S*,6*R*)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid
LY341495 (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid
M100907 MDL-100,907, volinanserin, (*R*)-(+)-*a*-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol
MAO Monoamine oxidase
MDMA 3,4-methylenedioxy-*N*-methylamphetamine
MK801 (+)-MK801; dizocilpine hydrogen maleate; (5*S*,10*R*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine hydrogen maleate
NMDA *N*-methyl-D-aspartic acid
OCD Obsessive–compulsive disorder
PIP_2 Phosphatidylinositol-4,5-biphosphate
PCP Phencyclidine
PLC Phospholipase C
SER-082 (+)-*cis*-4,5,7*a*,8,9,10,11,11*a*-octahydro-7*H*-10-methylindolo[1,7-*bc*][2,6]-naphthyridine
SSRI Selective serotonin reuptake inhibitor
TM Transmembrane domain

INTRODUCTION

Hallucinogens, such as psilocybin and mescaline, have been part of human culture for millennia, and since the 1960s, they have played a highly visible role in Western culture. Aside from their relevance to society, culture, and arts, hallucinogens and their mechanisms of action are important topics in basic and translational neuroscience research because of their dramatic effects on perception and mood. Comparing and contrasting these effects with schizophrenia has proven to be fruitful, as second- and third-generation antipsychotic drugs target signaling pathways that are also affected by hallucinogenic drugs. Here, we review current knowledge about the molecular mechanisms of hallucinogens, their use in research on neuropsychiatric disorders, and their potential use as therapeutic agents.

HISTORICAL POINT OF VIEW

Naturally occurring psychoactive substances have been used in numerous spiritual and magicoreligious practices since early stages of human history (Nichols, 2004). The ingestion of ergot alkaloids obtained from rye infected with the fungus *Claviceps purpurea* has been proposed as the focus of Eleusinian Mysteries in ancient Greece. Hallucinogenic ointments made from plants containing muscarinic compounds reportedly were used by medieval witches in Europe. *Datura* (angel's trumpet), *Belladonna*, and other plants with psychoactive compounds have been used by magicoreligious practitioners on every continent. Peyote's use appears to date back thousands of years, and its use by religious groups such as the Native American Church throughout the United States and Canada enjoys some measure of legal protection. Ayahuasca, a beverage brewed from the roots of *Banisteriopsis caapi*, which contains monoamine oxidase inhibitors, to which is often added extracts of plants with the hallucinogen dimethyltryptamine (DMT), has a long tradition of ceremonial use by indigenous groups in the Amazon region. More recently, in 1897, the German pharmacologist Arthur Heffter (1859–1925) identified mescaline as the centrally active compound in the peyote cactus. However, it was the serendipitous discovery in 1943 of the hallucinogenic properties of lysergic acid diethylamide (LSD) by the Swiss chemist Albert Hoffmann (1906–2008), which ushered in the modern era of psychedelics.

Hoffmann had synthesized LSD 5 years earlier while he was looking for respiratory stimulants at Sandoz Pharmaceuticals in Basel, Switzerland. While reevaluating its pharmacological properties, he suffered an accidental exposure to an unknown, but minute, amount of LSD that induced a sensation of "an uninterrupted stream of fantastic images of extraordinary plasticity and vividness and accompanied by an intense, kaleidoscope-like play of colors." His suspicion that these effects had been the consequence of the ergot derivative led him to ingest a solution containing 0.25 mg of LSD tartrate. A laboratory assistant accompanied Hofmann (2013) on his bicycle ride home, during which he experienced "visual disturbances with the faces of those around him appearing as grotesque colored masks." These psychopharmacological properties, as well as the extremely high potency of LSD, were confirmed by several other scientists at Sandoz Pharmaceuticals. With the introduction of LSD to Europe and to the United States in 1949, an era was begun in which these agents opened new lines of research related to mechanisms of neurotransmission, visual hallucinations, and schizophrenia. As they spread into popular culture, both recreational and spiritual uses were pursued by large numbers of people.

Serotonin (5-hydroxytryptamine, 5-HT), one of the oldest and most evolutionarily conserved neurotransmitters, was originally identified as the substance responsible for the vasoconstrictor effects of serum. In 1948, a few years after the discovery of LSD, serotonin was described in bovine blood serum and in the brain. In 1954, the psychedelic properties of LSD were proposed to be a consequence of its serotonergic properties in the central nervous system (CNS), a hypothesis that was further supported by findings of structural similarity between serotonin and hallucinogens such as psilocybin, which was isolated and synthesized in 1958 after Gordon Wasson brought back samples of mushrooms used by Mazatec healer Maria Sabina. In 1964 Kjell Fuxe at the Karolinska Institute in Stockholm demonstrated that the 5-HT cell bodies were located mainly in the raphe nuclei of the brain stem. In the mid-1970s, Solomon Snyder at Johns Hopkins University described 5-HT binding sites in membrane preparations of rat cortex. Importantly, his laboratory also suggested the existence of two different populations of 5-HT binding sites that were named 5-HT_1 and 5-HT_2.

Only a few years later, Richard Glennon, Milt Titeler, and their teams demonstrated a highly significant correlation between binding affinities of 22 hallucinogenic drugs for 5-HT_2 receptors and their psychedelic potential in humans (Glennon, Titeler, & McKenney, 1984; Glennon, Titeler, & Young, 1986). This achievement (see also the following), together with the effects of hallucinogenic drugs inviting comparison with schizophrenia symptoms, opened a new line of research regarding the role of serotonin and 5-HT_2 receptors in schizophrenia. The effects of LSD were one of the earliest models of this psychiatric disorder in healthy volunteers, and, since then, independent studies have validated the use of hallucinogens such as LSD, mescaline, psilocybin, and DMT as a model of some of the schizophrenia symptoms in clinical (Vollenweider, Vollenweider-Scherpenhuyzen, Babler, Vogel, & Hell, 1998) and preclinical (Hanks & Gonzalez-Maeso, 2013) studies.

CHEMICAL STRUCTURE

From a structural perspective, serotonergic hallucinogens are classified into two main groups: phenethylamines, such as mescaline, and tryptamines (Nichols, 2004). The latter group is divided into two subgroups: simple tryptamines, such as DMT and psilocin, which contain an indole ring structure and show substantial structural flexibility, and ergoline derivatives, including LSD and a handful of other relatively rigid closely related chemical compounds (Figure 1).

Mescaline, the principal active compound found in the peyote cactus (*Lophophora williamsii*), shows an extremely low potency, with effective doses in the range of 200–400 mg in human and 10–20 mg/kg in mice. Nevertheless, its hallucinogenic efficacy is well known, and this compound served as a backbone for the development of other phenethylamine hallucinogens such as (−)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI), 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), and 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB). There are more than 100 species of "magic" mushrooms, including *Psilocybe cyanescens* in Europe and the west coast of the United States and *Psilocybe cubensis* in South America and parts of Mexico (Figure 2). These fungi contain psilocybin, which, after ingestion, is processed by alkaline phosphatases of the gastrointestinal tract to generate the active simple tryptamine psilocin. Other simple tryptamines found in nature include DMT (contained in the admixture of plants included in Ayahuasca infusions; see previously mentioned) and bufotenin, which is found in the skin and glands of certain toads (Family Bufonidae). LSD is extremely potent, with effective doses as low as 0.025 mg. Although it has not been found in nature, other ergolines with similar psychoactive activity, but much lower potency, can be extracted from *C. purpurea* and from the seeds of some plants in the Convolvulaceae family. LSD is a chiral compound with two stereogenic centers at C5 and C8 with which four different enantiomers may exist (*d*-LSD, *l*-LSD, *d*-iso-LSD, and *l*-iso-LSD). Importantly, only one of these four enantiomers (*d*-LSD) is hallucinogenic (Figure 1), which suggests that this compound possesses unique pharmacological properties at the receptor it targets that lead to the cellular events responsible for its behavioral effects.

RECEPTOR TARGET

Two of the principal questions since the beginning of research related to the mechanism of action of hallucinogens were the receptor (or receptors) that they target and whether the same subtype of neurotransmitter receptor (or receptors) is responsible for the behavioral phenotypes induced by tryptamine and phenethylamine hallucinogens. Based on radioligand binding displacement experiments, which are interpreted in terms of simple competition of two ligands (one radiolabeled and one the unlabeled compound to be tested) for the occupancy of a single binding site of a receptor, significant correlation was found between the affinities (K_i values) of hallucinogens for the 5-HT_2 binding sites in brain frontal cortex membrane preparations of male Wistar rats (Glennon et al., 1984). The authors at Virginia Commonwealth University and Albany Medical College conduced binding displacement assays of [^{3}H]ketanserin and [^{3}H]5-HT with the following hallucinogenic ligands: (+)-LSD, (±)-DOI, (−)-DOI, (±)-DOB, (+)-DOB, (−)-DOB, (±)-DOPR, (±)-DOET, (±)-DOM, (−)-DOM, (±)-DOBU, (±)-DON, (−)-DON, (−)-*N*-Me-DOM, (±)-*N*-Me-DOM, 5-OMe-DMT, (±)-2-HMMP, (±)-DOF, (±)-2,4,5-TMA, (±)-MEM, (±)-2,5-DMA, and (±)-3,4,5-TMA (Glennon et al., 1984).

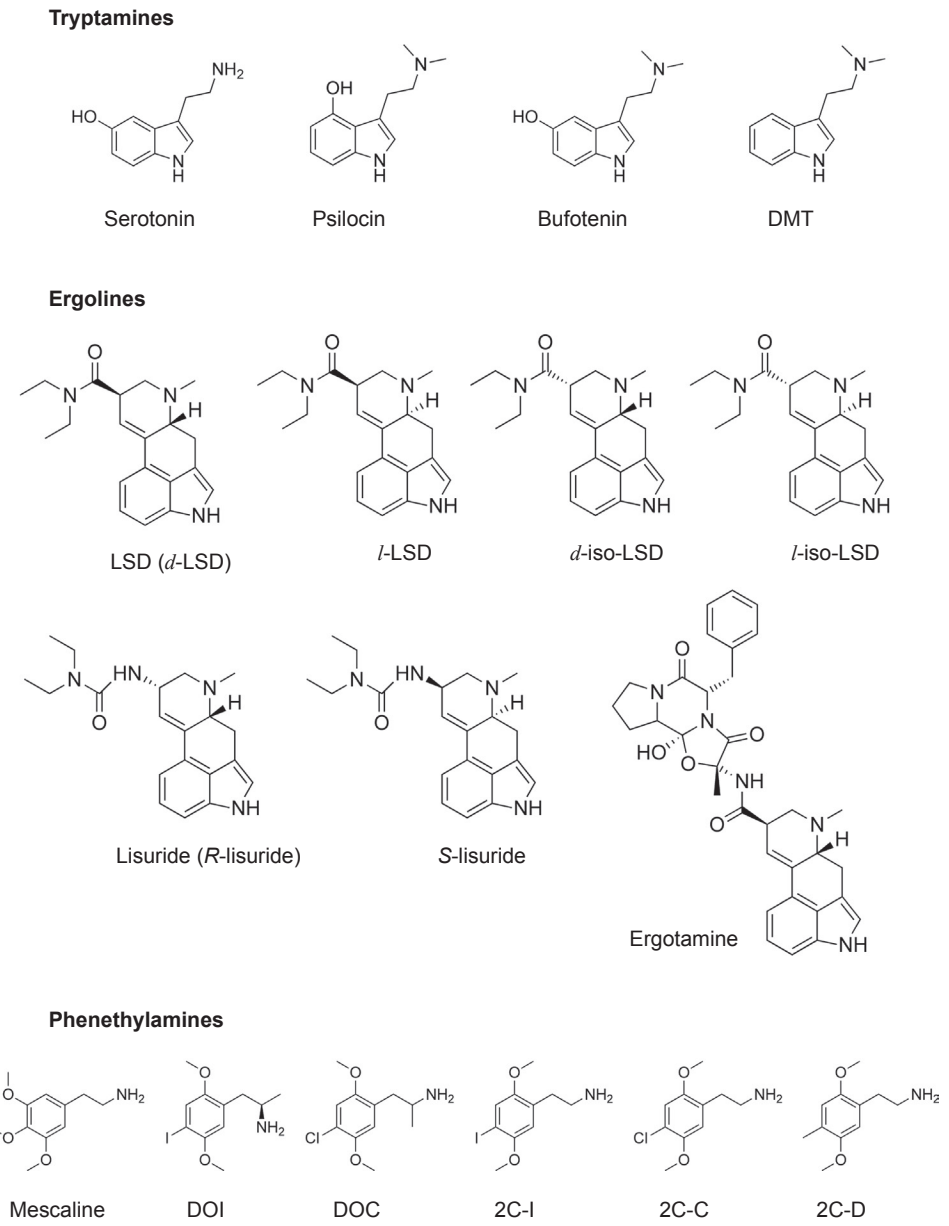

FIGURE 1 Chemical structures of hallucinogen and nonhallucinogen 5-HT_{2A} agonists. The hallucinogens are psilocin, bufotenin, dimethyltryptamine (DMT), lysergic acid diethylamide (LSD (*d*-LSD)), mescaline, (−)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI), 2,5-dimethoxy-4-chloroamphetamine (DOC), 2,5-dimethoxy-4-iodophenethylamine (2C-I), 2,5-dimethoxy-4-chlorophenethylamine (2C-C), and 2,5-dimethoxy-4-methylphenethylamine (2C-D). The nonhallucinogens are *l*-LSD, *d*-iso-LSD, *l*-iso-LSD, lisuride (*R*-lisuride), *S*-lisuride, and ergotamine.

Importantly, they found a highly significant correlation between the K_i values of these ligands for 5-HT_2 receptors and their potencies (EC_{50} values) obtained in previously examined drug discrimination paradigms using DOM as the training drug. There was also a significant correlation between human hallucinogenic potency and 5-HT_2 binding in rat frontal cortex. Similar findings were obtained using the radioligands [^{3}H]DOB, [^{3}H]8-OH-DPAT, [^{3}H]5-HT, and [^{3}H]mesulergine. Although previous findings using behavior and electrophysiology studies pointed toward the involvement of the serotonin system in the mechanism of action of both tryptamine and phenethylamine hallucinogens, the earlier mentioned findings represent the first evidence for the role of 5-HT_2 receptor binding in the molecular mechanism of action of hallucinogenic drugs. After the 5-HT_{2C} receptor (which was formerly called 5-HT_{1C}) was discovered by Ángel Pazos and his collaborators (Pazos, Hoyer, & Palacios, 1984), the next question was related to the serotonin receptor, specifically 5-HT_{2A} or 5-HT_{2C}, responsible for the behavioral effects of hallucinogens in rodents. To solve this, a very elegant study tested a series of serotonergic antagonists (risperidone, pirenpirone, metergoline, ketanserin, loxapine, LY53857, pizotyline, spiperone, cyproheptadine, mesulergine, promethazine, and thioridazine) on the stimulus effects of the phenethylamine DOM and the ergoline LSD (for a review regarding this behavior protocol, see Hanks & Gonzalez-Maeso, 2013). The authors carried out

FIGURE 2 Hallucinogenic mushrooms (*Psilocybe semilanceata*), whose principal active compound is psilocybin (see Persson, 2012).

dose–response studies and determined the IC_{50} values of each of these serotonergic antagonists on rats injected with DOM or LSD that had been previously trained to discriminate LSD from saline using an FR10 reinforcement schedule. The main conclusions of this article were that 5-HT_{2A} affinity correlated with IC_{50} values for the blockade of LSD and DOM and, most importantly, that the drug-stimulus effects of LSD and DOM were mediated by their agonist properties at 5-HT_{2A}, but not 5-HT_{2C}, receptors.

Following these findings, the development of the first 5-HT_{2A} receptor-selective antagonists such as (*R*)-(+)-*a*-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol (MDL-100,907, M100907, volinanserin) together with electrophysiological and behavioral assays led to the demonstration that 5-HT_{2A} is the main receptor responsible for the effects of both tryptamine and phenethylamine hallucinogens in rodents (Aghajanian, 2009). The role of this serotonin receptor subtype was further supported by Franz Vollenweider et al. (1998), who demonstrated that the psychosis-like effects of psilocybin in healthy volunteers were reversed by the 5-HT_{2A} receptor antagonist ketanserin. They also suggested that psilocybin intoxication in humans shows key similarities to the symptoms observed in first episodes of schizophrenia patients. This concept that hallucinogens induce psychotic states that are fundamentally similar to first-break schizophrenia had been previously suggested by Vardy and Kay (1983)—they compared patients hospitalized for LSD psychosis with drug-naïve first-episode schizophrenia patients (Vardy & Kay, 1983).

Although the use of batteries of ligands in rodent models provided evidence that 5-HT_{2A} receptors play an essential role in the psychoactive effects of hallucinogens, it was not until the development of 5-HT_{2A}-knockout mice in 2003 that this hypothesis was verified conclusively (Gonzalez-Maeso et al., 2003). Thus, it is now well accepted that the physiological and behavioral effects of tryptamine, ergoline, and phenethylamine hallucinogens are mediated primarily by 5-HT_{2A} receptors. These findings, however, do not exclude the possibility that other receptor subtypes to which they bind are also involved in their psychedelic properties.

One of these alternative candidates is the dopamine D_2 receptor. The effects of LSD in rats have been described as occurring in two temporal phases, with an initial locomotor suppression followed by increased exploratory behavior. David Nichols and collaborators showed in rats trained to discriminate LSD using a two-lever food-reinforced operant conditioning task that 5-HT_{2A} antagonists blocked the earlier effects of LSD and this effect lasted no longer than 60 min (Nichols, 2004). Importantly, they also demonstrated that the second phase of LSD was unaffected by 5-HT_{2A} antagonists but was inhibited by haloperidol. The role of the dopamine D_2 receptor in these distinct temporal phases of LSD was further suggested by the substitution of LSD obtained with the dopamine D_2 antagonists apomorphine, *N*-propyldihydrexidine, and quinelorane. Additional evidence that the delayed temporal effects of LSD were mediated through the dopamine system was provided by the demonstration of the effects of LSD with a 30-min preinjection (LSD-30) or a 90-min preinjection (LSD-90) using the same two-lever food-reinforced operant conditioning task. (LSD was injected 30 min (LSD-30) or 90 min (LSD-90) before testing.) The authors demonstrated that hallucinogens such as psilocin and mescaline substituted the effects of LSD 30 min after injection, but not 90 min after injection. The role of the dopamine system was supported by the use of the dopamine agonists ABT-724, aripiprazole, dihydrexidine, WAY-100635, and SKF-38393, which either fully or partially mimicked LSD-90, but not LSD-30. It was also demonstrated that the dopamine system is involved in the discriminative stimulus effects of the ergoline LSD, but not of the phenethylamine DOI. However, in humans, haloperidol did not attenuate most of the psychotomimetic effects of psilocybin (Vollenweider et al., 1998). Although further investigation is needed, these findings suggest that dopamine receptors may be involved in at least some of the psychoactive effects of ergoline hallucinogens.

Using an electrophysiological approach to measure single-cell functional properties from serotonergic neurons, George Aghajanian and his laboratory at Yale University demonstrated that LSD has a potent inhibitory effect on tonic activity of 5-HT neurons of the dorsal raphe nucleus (Aghajanian, Foote, & Sheard, 1968). Similarly, local administration of LSD suggested that these inhibitory effects were mediated through a direct action on the somatodendritic region of serotonergic neurons. The relevance of this to the action of hallucinogenic drugs was supported by further investigation, which provided direct evidence that additional hallucinogens, including simple tryptamines such as psilocin and DMT, also inhibit 5-HT neurons in the raphe nuclei. Importantly, although administration of phenethylamine hallucinogens such as mescaline also suppressed the firing of certain populations of 5-HT neurons, it was suggested that this effect of phenethylamine hallucinogens was mediated through an indirect pathway rather than through a direct effect on the somatodendritic region of serotonergic neurons in the dorsal raphe nucleus. The differences between phenethylamine hallucinogens and LSD on 5-HT neurons of the raphe nuclei were defined by the characterization of several 5-HT receptor subtypes (see earlier mentioned), after which it was found that LSD shows a high agonist potency at 5-HT_{1A} receptors, whereas mescaline and other phenethylamines show minimal affinity for 5-HT_{1A} receptors. As 5-HT_{1A} receptors are highly expressed on serotonergic raphe neurons, together these findings provide an explanation for the effects of LSD but not phenethylamine hallucinogens on direct inhibition of 5-HT neurons on the raphe nuclei. It has also been shown, however, that this effect on 5-HT_{1A} receptors does not correlate with the hallucinogenic properties of the tested ligand.

The 5-HT_{5A} and 5-HT_{2C} receptors have been reported to be involved in the locomotor activity induced by LSD. As discussed previously, LSD induces in rodents (mouse and rats) an initial decrease in exploratory behavior that is followed by an increase

in locomotor activity. After the generation of 5-HT_{5A}-knockout mice, René Hen and his laboratory explored the effects of LSD on locomotor activity (total distance) in 5-HT_{5A}-knockout mice and control littermates (Grailhe et al., 1999). The authors found that the effects of LSD on the initial decrease in locomotor activity remained unchanged in 5-HT_{5A}-knockout mice, whereas the resulting increase was attenuated. The 5-HT_{2C} receptor has also been involved in the locomotor activity induced by the phenethylamine hallucinogen DOI in mice (Halberstadt et al., 2009). Low doses (0.625–5.0 mg/kg) increased, whereas higher doses (≥10.0 mg/kg) decreased, locomotor activity. Based upon the use of 5-HT_{2A}-knockout mice and the 5-$HT_{2B/2C}$ receptor antagonist (+)-*cis*-4,5,7*a*,8,9,10,11,11*a*-octahydro-7*H*-10-methylindolo[1,7-*bc*][2,6]-naphthyridine (SER-082), the authors demonstrated that the increase in locomotor activity induced by DOI requires expression of the 5-HT_{2A} receptor and that the pretreatment with SER-082 attenuates the effects of DOI on locomotor reduction. Additional findings established that the serotonin 5-HT_{2C} receptor affects the behavioral effects of hallucinogens. As an example, Elaine Sanders-Bush and collaborators showed that the head-twitch response (a mouse behavioral proxy of a hallucinogenic response, see the following) induced by the hallucinogen DOI is significantly reduced in 5-HT_{2C}-knockout mice compared to wild-type littermates (Canal et al., 2010). Although these findings do not provide evidence that 5-HT_{2C} receptors are directly responsible for head-twitch behavior in mice, they suggest that head-twitch behavior is modulated by 5-HT_{2C} receptor function. The conclusion that hallucinogen 5-HT_{2A} receptor-dependent behaviors are modulated by other G-protein-coupled receptor (GPCR) subtypes is further supported by findings showing that the effect of the hallucinogen 5-HT_{2A} receptor agonist DOM on locomotor activity is increased in metabotropic glutamate receptor 5 (mGlu5)-knockout mice (Halberstadt, Lehmann-Masten, Geyer, & Powell, 2011) and that the head-twitch behavior induced by the hallucinogen 5-HT_{2A} receptor agonist DOI is reduced in cannabinoid CB_1 receptor-knockout mice (Hanks & Gonzalez-Maeso, 2013). Together, these findings suggest that direct activation of the 5-HT_{2A} receptor by hallucinogens initiates the molecular mechanisms and signaling cascades responsible for their unique psychoactive effects. However, GPCR subtypes such as dopamine D_2, serotonin 5-HT_{2C} and 5-HT_{5A}, cannabinoid CB_1, and metabotropic glutamate mGlu5 receptors also play a role in the modulation of the behavioral pharmacology of hallucinogens.

SIGNALING MECHANISMS

The serotonin 5-HT_{2A} receptor belongs to the superfamily of GPCRs (Pierce, Premont, & Lefkowitz, 2002), which owe their name to their functional interaction with heterotrimeric G proteins. These receptors can influence virtually every known cellular signaling event and are the targets of more than a quarter of the 100 top-selling therapeutic drugs. The first evidence that GPCRs have a central core domain composed of seven transmembrane α-helices, an extracellular N-terminus, and an intracellular C-terminus was based upon the hydrophobic and hydrophilic properties of the primary sequence of the β_2-adrenergic receptor obtained by Robert Lefkowitz at the beginning of the 1980s (Dixon et al., 1986). This was validated more recently with the first crystal structure of the photoreceptive GPCR rhodopsin

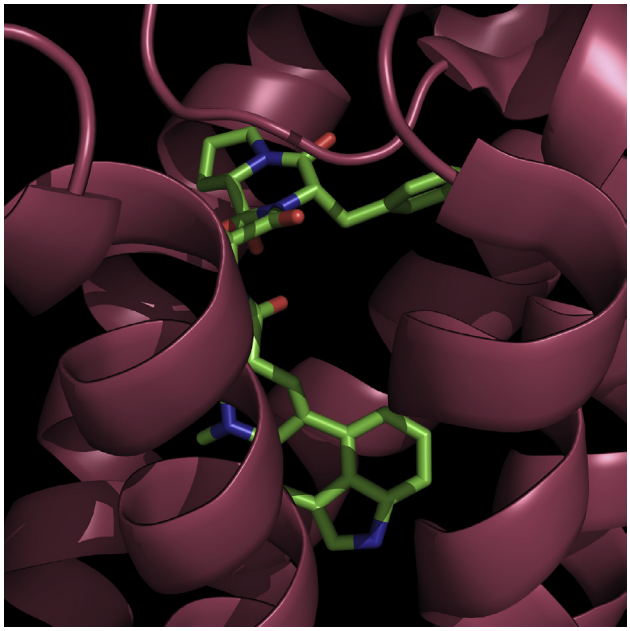

FIGURE 3 Docking of ergotamine into the binding pocket of the 5-HT_{2B} receptor subtype (PDB 4IB4) (see Wacker et al., 2013; Wang et al., 2013).

(Palczewski et al., 2000), after which the explosion of findings that have emerged from X-ray crystallography opened a new avenue for research on structural pharmacology and drug discovery (for review, see Audet & Bouvier, 2012).

Notwithstanding the remarkable advances in this field, the crystal structure of the 5-HT_{2A} receptor remains unresolved, mostly because of its unique functional properties and the stability of this intrinsically dynamic GPCR subtype. Nevertheless, findings showed that the crystal structure of the human 5-HT_{2B} receptor bound to the nonhallucinogen drug ergotamine (Wacker et al., 2013; Wang et al., 2013). The authors compared the structure of the 5-HT_{2B} receptor with that of the 5-HT_{1B} receptor, and their findings provided the structural basis for further investigation focused on understanding the differences at a molecular level between LSD and the closely related nonhallucinogenic compound ergotamine (Figure 3).

Paradoxically, although numerous biochemical, pharmacological, electrophysiological, and behavioral studies suggest that several effects of both tryptamine and phenethylamine hallucinogens involve agonist activity at the 5-HT_{2A} receptor, compounds such as lisuride and ergotamine, which have in vitro agonist activity on canonical 5-HT_{2A} receptor-mediated pathways that is indistinguishable from the profiles of structurally similar hallucinogens (Figure 1), are unable to induce psychedelic effects in humans. Findings based upon a pharmacological model termed "biased agonism" or "agonist trafficking of receptor signaling" provide a possible solution to this fundamental question about the mechanism of action of hallucinogens. The best-characterized signaling cascade of the 5-HT_{2A} receptor is its coupling to $G_{q/11}$ G proteins followed by activation of phospholipase C (PLC), which consequently leads to the formation of inositol phosphate (IP) and diacylglycerol (Figure 4). However, hallucinogenic and closely related nonhallucinogenic 5-HT_{2A} agonists show a similar pharmacological activity at this signaling pathway in vitro (Egan, Herrick-Davis, Miller, Glennon, & Teitler, 1998; Porter et al., 1999). Most importantly,

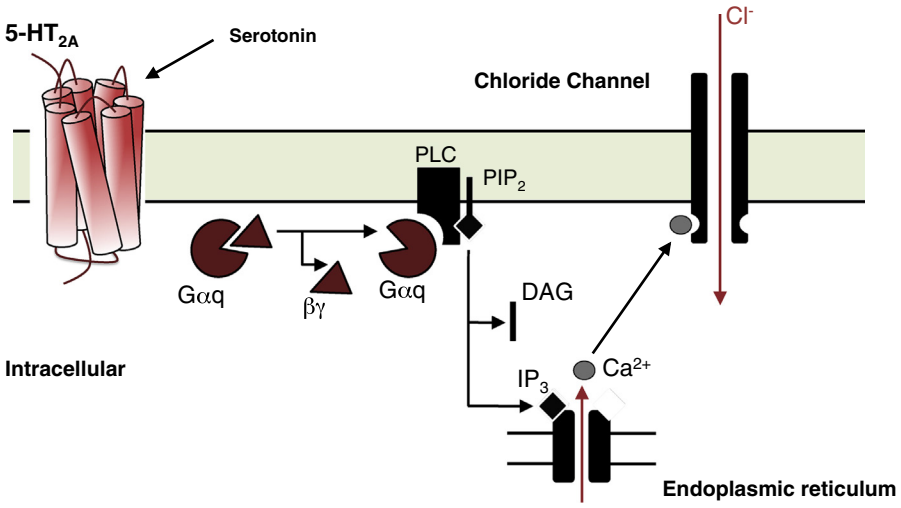

FIGURE 4 The intracellular Ca^{2+} signaling network. Binding of agonist to the 5-HT_{2A} receptor activates heterotrimeric $G_{q/11}$ proteins and releases Ca^{2+} from intracellular stores through inositol phosphate (IP_3) signaling pathways. Thus, upon $G_{q/11}$ activation, phospholipase C (PLC) hydrolyzes phosphatidylinositol-4,5-biphosphate (PIP_2) into diacylglycerol (DAG) and IP_3. IP_3 in turn mobilizes Ca^{2+} to the cytosol from the endoplasmic reticulum producing a characteristic peak current associated with calcium-activated chloride currents.

there is an absence of correlation between their hallucinogenic properties and their agonist properties activating G_q-dependent pathways such as IP stimulation (Egan et al., 1998) and Ca^{2+} release (Porter et al., 1999). Similarly, there is only a slight reduction in the head-twitch behavior induced by DOI in $G\alpha_q$-knockout mice compared to controls (Garcia, Smith, & Sanders-Bush, 2007), suggesting that although G_q-dependent signaling might be partially necessary for psychosis-like behavior induced in mice by hallucinogens, this G_q-dependent pathway is not fully required for the initiation of signaling events leading to hallucinogenic behavior induced by the phenethylamine DOI in mice. Studies in heterologous systems such as HEK-293 cells and NIH3T3 cells identified new 5-HT_{2A} receptor-dependent signal transduction pathways such as phospholipase A2 (PLA2) (Nichols, 2004), PLA2-mediated arachidonic acid release (Berg et al., 1998), and $G_{i/o}$-dependent $G\beta\gamma$-associated activation of ERK1/2 (Nichols, 2004), JAK/STAT (Singh, Dai, Staudinger, & Muma, 2009), ARF1 (Robertson et al., 2003), and Src family kinases (Xiang et al., 2012), some of which were shown to be differentially regulated by structurally distinct ligands. Using quantitative phosphoproteomics, it has also been suggested that hallucinogen, but not nonhallucinogen, 5-HT_{2A} receptor agonists induce phosphorylation of the 5-HT_{2A} receptor at serine-280 located in the third intracellular loop (Karaki et al., 2014). Importantly, the authors also demonstrated that pretreating cells with pertussis toxin (PTX) decreased PLC activation induced by the hallucinogens DOI and LSD, whereas PTX treatment did not affect lisuride and ergotamine responses (Karaki et al., 2014). Taken together, these observations suggest that hallucinogens selectively activate $G_{i/o}$-dependent signaling, whereas nonhallucinogen 5-HT_{2A} receptor agonists do not. Based on these findings, it was proposed that certain agonists have the capacity to selectively activate a subset of the numerous signaling transduction pathways that are coupled to a particular receptor subtype. This phenomenon, termed "agonist-directed trafficking of receptor stimulus" or "biased agonism," was originally proposed by Terry Kenakin (1995) and has been validated experimentally in numerous experimental systems, including tissue cultures expressing the 5-HT_{2A} receptor (Berg et al., 1998). This functional selectivity concept incorporates new concepts relevant to quantitative pharmacology suggesting that subtle changes in ligand structure may have profound effects on functional properties (Figure 5). However, with regard to the psychoactivity induced by hallucinogenic 5-HT_{2A} receptor agonists, the signaling pathways responsible for their unique effects on brain function remained unsolved.

Although heterotrimeric G proteins represent one of the main mechanisms involved in GPCR-dependent function, it has been demonstrated that G-protein-independent mechanisms related to β-arrestin and PSD-95/disc-large/zonula occludens-1 (PDZ) domains also play a key role in their signaling properties (Shenoy and Lefkowitz, 2011). Classically, upon receptor stimulation, β-arrestins have been shown to block G-protein-dependent signaling through a steric mechanism. Importantly, more recent evidence suggests that β-arrestins can also mediate G-protein-independent signaling events. This role of β-arrestins in GPCR function is further supported by the crystal structure of active β-arrestin-1 bound to a carboxyl-terminal peptide derived from a GPCR (Shukla et al., 2013). Using the serotonin precursor 5-hydroxytryptophan (5-HTP) or the hallucinogen DOI, Laura Bohn and her laboratory demonstrated that serotonin induced a head-twitch response in mice by a β-arrestin-2 mechanism, whereas the effect of DOI on this behavior is independent of β-arrestin-2 (Schmid & Bohn, 2010). They also showed that serotonin, but not DOI, activates a signaling cascade composed of β-arrestin-2, phosphoinositide 3-kinase, Src, and Akt that is responsible for head-twitch behavior. Interestingly, more recent findings by the same laboratory suggested that the atypical antipsychotic clozapine induces antipsychotic-like behavioral effects in mice acting as a 5-HT_{2A} receptor agonist through a β-arrestin-2-independent activation of Akt (Schmid, Streicher, Meltzer, & Bohn, 2014). Further work is needed to distinguish the role of Akt in the actions of 5-HTP and clozapine on psychosis-like and antipsychotic-like behaviors.

A large set of studies demonstrates that many GPCR functions implicate proteins that bind to their intracellular domains. Many of

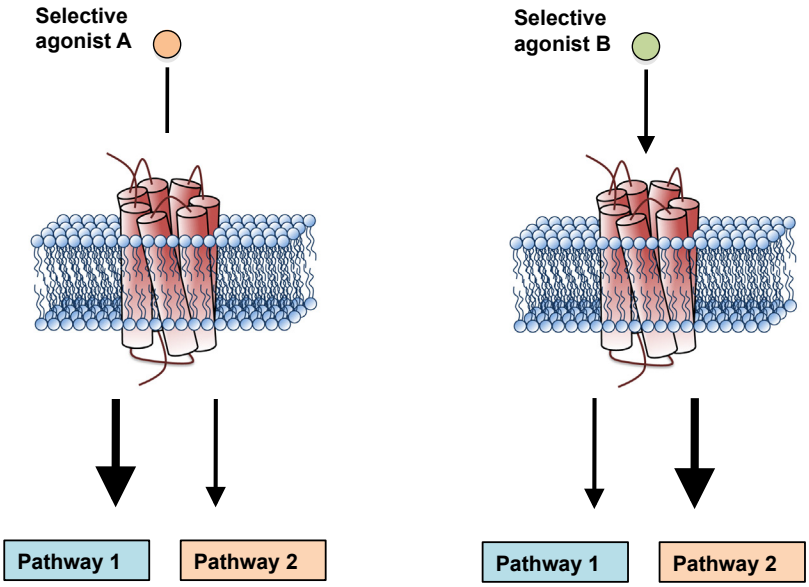

FIGURE 5 Cartoon depicting the pharmacological model of biased agonism through which selective agonists stabilize only a subset of receptor conformations that preferentially activate certain signaling pathways.

these proteins are PDZ-domain proteins, which recognize a PDZ recognition motif (PDZ ligand) located at their C-terminal end. The 5-HT_{2A} and 5-HT_{2C} receptors express a canonical type 1 PDZ ligand, (S/T)Xϕ, where ϕ is a hydrophobic residue, SCV and SSV, respectively. Philippe Marin and his group in Montpellier demonstrated that 5-HT_{2A} and 5-HT_{2C} receptors interact with specific sets of PDZ proteins (Becamel et al., 2002). Additional studies showed that PSD-95 is crucial in the regulation of functional activity and intracellular trafficking of 5-HT_{2A} receptors (Xia, Gray, Compton-Toth, & Roth, 2003). The potential role of PSD-95 in the behavioral effects of hallucinogens was demonstrated by a large and significant decrease in DOI-induced head-twitch behavior in PSD-95-knockout mice compared with wild-type mice (Abbas et al., 2009).

GPCRs have traditionally been assumed to function as monomeric structural units. This monomeric model of GPCR signaling is further supported by findings based on assays that measured G protein coupling of a single monomeric GPCR, such as β_2-adrenergic receptor or rhodopsin, reconstituted into a phospholipid bilayer (Whorton et al., 2007). These findings in reconstituted systems, together with the crystal structure of the active-state ternary complex composed of agonist-occupied β_2-adrenergic receptor and heterotrimeric G_s (Rasmussen et al., 2007), provide clear evidence that a single receptor is sufficient for G protein activation. Nevertheless, since 2005, multiple lines of evidence have provided support for the hypothesis that GPCRs may exist and function as dimeric or oligomeric complexes in vitro (Gonzalez-Maeso, 2011). With respect to family C GPCRs, it is well established that dimerization is required for receptor function. Findings have convincingly demonstrated a close functional interaction at the cellular, electrophysiological, and behavioral levels between the family A 5-HT_{2A} receptor and the family C mGlu2 receptor (Gonzalez-Maeso & Sealfon, 2009). Interestingly, the collaboration of several teams led to the demonstration that the $G_{q/11}$-coupled 5-HT_{2A} receptor and the $G_{i/o}$-coupled mGlu2 form a specific GPCR heteromeric complex in living cells (Gonzalez-Maeso et al., 2008). Coimmunoprecipitation of 5-HT_{2A} and mGlu2 receptors in mouse frontal cortex was also demonstrated (Fribourg et al., 2011), as well as labeling for both receptors in close subcellular proximity at or near cortical synaptic junctions by electron microscopy (Moreno et al., 2012). Additional work focused on the signaling properties of this receptor heterocomplex revealed that the 5-HT_{2A}–mGlu2 receptor complex establishes a balance between G_q and G_i signaling that predicts the antipsychotic (e.g., clozapine and risperidone) or propsychotic (e.g., LSD and DOI) properties of the ligand (Fribourg et al., 2011). Thus, hallucinogens decrease G_i signaling and increase G_q signaling, whereas antipsychotics increase G_i signaling and decrease G_q signaling (Figure 6). This assay might be used for screening new compounds with potential hallucinogenic or antipsychotic properties. When studying the behavioral properties of 5-HT_{2A} and mGlu2 as a GPCR heteromer in mouse models, the authors found that head-twitch behavior induced by LSD and the phenethylamine DOI was significantly decreased in mGlu2-knockout mice (Moreno, Holloway, Albizu, Sealfon, & Gonzalez-Maeso, 2011). Using an experimental approach based on intrafrontal cortical injections of bicistronic herpes simplex 2 viral particles expressing green fluorescent protein and either mGlu2 or mGlu2ΔTM4N (mGlu2/mGlu3 chimera that does not form the 5-HT_{2A}–mGlu2 receptor complex), further investigation demonstrated that the head-twitch response induced by DOI was rescued in mGlu2-knockout mice overexpressing mGlu2, but not mGlu2ΔTM4N, in frontal cortex compared to that observed in control mice (Moreno et al., 2012). These findings provide evidence that a cortical 5-HT_{2A}–mGlu2 receptor heterocomplex is necessary for at least part of the behavioral effects induced by hallucinogens in rodents.

APPLICATIONS TO OTHER ADDICTIONS AND SUBSTANCE MISUSE

One of the main conclusions of this chapter focused on the molecular mechanisms of action of hallucinogens such as LSD and mescaline is the findings based on pharmacological approaches demonstrating

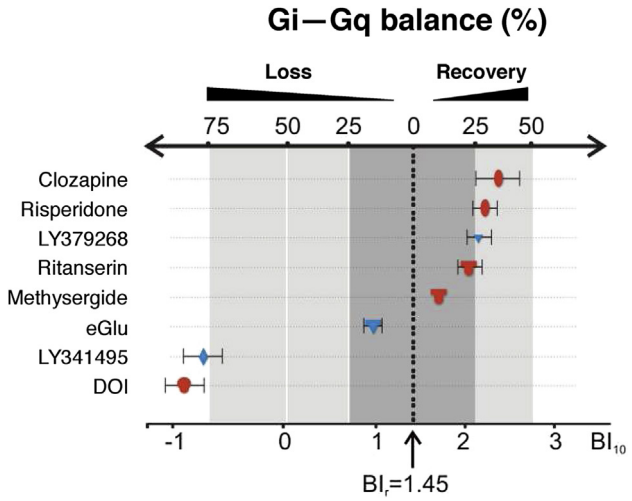

FIGURE 6 The 5-HT_{2A}–mGlu2 heteromeric receptor complex-dependent changes in $G_{i/o}$ and $G_{q/11}$ activity predict the psychoactive behavioral effects of serotonergic (red) and glutamatergic (blue) ligands. Clozapine, risperidone, and the mGlu2/3 receptor agonist LY379268 are antipsychotics. Ritanserin, methysergide, and eGlu are neutral antagonists. (−)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride (DOI) is a hallucinogen. The mGlu2/3 receptor antagonist/inverse agonist LY341495 is a propsychotic-like drug (see Fribourg et al., 2011).

that different drugs that target the same receptor may elicit distinct cellular signaling behavioral responses. This concept, described here with special emphasis on the serotonin 5-HT_{2A} receptor, has wider implications in other addictions as well as in the molecular basis of psychiatric disorders such as schizophrenia and dementia.

DEFINITION OF TERMS

G-protein-coupled receptor GPCRs are plasma membrane proteins that share a characteristic topology consisting of seven α-helical transmembrane spans. They respond to extracellular mediators such as hormones, neurotransmitters, small peptides, ions, and lipids, as well as sensory stimuli such as odorants, pheromones, and light.

Hallucinogens These are pharmacological compounds that affect cognition and perception. These drugs include dissociatives (e.g., ketamine and phencylidine), psychedelics (e.g., LSD and mescaline), and deliriants (e.g., scopolamine and atropine).

Heteromer or heterocomplex This is a macromolecular assembly formed by two noncovalently bound proteins.

Lysergic acid diethylamide LSD is the psychedelic compound discovered by Albert Hoffmann in 1943.

LY379268 This is an agonist of mGlu2/3 with antipsychotic properties in animal models of psychosis.

Psychosis This is a psychiatric term that refers to false beliefs (delusions) or hallucinations (false or distorted) perceptions.

KEY FACTS OF HALLUCINOGEN ACTION

- Hallucinogens and their mechanism of action are important topics in basic and translational neuroscience research because of their dramatic impact on perception, cognition, and mood.
- The serotonin 5-HT_{2A} receptor plays an essential role in the psychoactive effects of hallucinogens.
- All hallucinogens are 5-HT_{2A} receptor agonists, yet closely related 5-HT_{2A} receptor agonists such as lisuride and ergotamine lack comparable psychoactive properties.
- Recent findings have dramatically defined our understanding of the molecular mechanism of action of hallucinogens.
- The strategy to elucidate hallucinogen action should be applicable to CNS-active compounds with therapeutic potential in neuropsychiatric disorders.

SUMMARY POINTS

- This chapter focuses on the molecular and signaling mechanisms through which hallucinogens affect behavior in mice and humans.
- The psychoactive effects of naturally occurring hallucinogens have been recognized for millennia.
- The modern era of research on hallucinogens commenced in 1943, after the serendipitous discovery of the effects of LSD by Albert Hoffmann.
- Serotonin 5-HT_{2A} receptors are involved in the majority of signaling events triggered by hallucinogens, although hallucinogen-dependent responses are also affected by other neurotransmitter receptors, such as serotonin 5-HT_{1A} and 5-HT_{2C}, and dopamine D_2, either as direct targets or indirectly through separate signaling pathways.
- The signaling mechanisms responsible for the behavioral effects of hallucinogens in rodent models include G protein coupling ($G_{q/11}$ and $G_{i/o}$), receptor heteromeric formation, β-arrestins, and PDZ domains.

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