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Mechanisms and molecular targets surrounding the potential therapeutic effects of psychedelics

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Abstract

Psychedelics, also known as classical hallucinogens, have been investigated for decades due to their potential therapeutic effects in the treatment of neuropsychiatric and substance use disorders. The results from clinical trials have shown promise for the use of psychedelics to alleviate symptoms of depression and anxiety, as well as to promote substantial decreases in the use of nicotine and alcohol. While these studies provide compelling evidence for the powerful subjective experience and prolonged therapeutic adaptations, the underlying molecular reasons for these robust and clinically meaningful improvements are still poorly understood. Preclinical studies assessing the targets and circuitry of the post-acute effects of classical psychedelics are ongoing. Current literature is split between a serotonin 5-HT_{2A} receptor (5-HT_{2A}R)-dependent or -independent signaling pathway, as researchers are attempting to harness the mechanisms behind the sustained post-acute therapeutically relevant effects. A combination of molecular, behavioral, and genetic techniques in neuropharmacology has begun to show promise for elucidating these mechanisms. As the field progresses, increasing evidence points towards the importance of the subjective experience induced by psychedelic-assisted therapy, but without further cross validation between clinical and preclinical research, the why behind the experience and its translational validity may be lost.

Keywords

Serotonin (5-hydroxytryptamine, 5-HT); 5-HT_{2A} receptor; G protein-coupled receptor (GPCR); classical psychedelics; hallucinogens; depression; substance use disorder

Introduction

Psychedelics are a class of pharmacologically active compounds that when taken by human subjects cause changes in perception, cognition, and sensory processing. The commonly reported subjective effects of psychedelics include visual hallucinations, euphoria, loss of sense of self, and some spiritual experiences. Ceremonial and medicinal use of psychedelics

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is dated back to Mesoamerica, Indo-European civilizations and ancient Greece, and has been reported throughout history on every continent[1–5]. Psychedelics became a topic of scientific research only following the synthesis and serendipitous discovery of the hallucinogenic effects of lysergic acid diethylamide (LSD) by Albert Hofmann while investigating the chemical and pharmacological properties of ergot derivatives at Sandoz Laboratories in Switzerland[6]. In the early to mid-20th century, these compounds were noted to produce effects similar to symptoms reported in schizophrenia patients [7–10] – the effects of mescaline and LSD aggravated the mental symptomatology in some schizophrenic patients[11] whereas relatives of schizophrenic patients were more susceptible to the psychotic responses induced by LSD[12]. More recent findings raised concerns about the translational validity of psychedelics as a preclinical model of psychosis, but this family of psychoactive compounds did provide key molecular insights into the pathology of schizophrenia mainly through the involvement of serotonin-related processes[13–15].

Interestingly, and in contrast to earlier studies where LSD was found to mimic psychotic symptoms, one study evaluated the effects of LSD when it was administered to twenty-nine schizophrenia patients or patients with related neuropsychiatric conditions, and found it to produce increased activity, sociability and emotional understanding in some patients[16]. Additionally, most of the patients made attempts to establish social connection with the personnel. In view of this, eight of the patients were chosen for psychotherapy and, interestingly, some were able to re-evaluate the emotional meaning of some of their symptoms, and improved – two of the patients were improved sufficiently to discontinue treatment. This was one of the first studies proposing that psychedelics may serve as a tool for shortening psychotherapy in schizophrenia patients.

With the increased use of LSD in experimental psychiatry, an upsurge of interest in brain chemistry and clinical evaluation on human subjects also increased. Beginning in the 1950s, LSD was prescribed as treatment to over 40,000 patients and was seen as a potential therapeutic agent for mental illnesses in combination with psychotherapy[16]. However, the growing interest in LSD by the scientific community trickled into the counterculture of the 1960s where it became a popular recreational drug, partially due to the testimonies of psychologists and philosophers such as Timothy Leary and Aldous Huxley[17–19]. This recreational use started to dismantle advances for mental health care based on psychedelic-assisted therapies, and reports of adverse and/or unwanted effects were brought to surface, therefore leading the U.S. Government to declare a War on Drugs. In 1970, the Controlled Substances Act was passed which classified drugs in five categories, of which classical psychedelics were put into Schedule I, meaning they have no medical use and high potential for abuse[20]. However, a preliminary report in 2015 indicating antidepressant effects of a single dose of ayahuasca[21] – followed by two key publications with psilocybin in 2016[22, 23] – provided strong evidence that psychedelic-assisted psychotherapy was highly effective in patients with depression. More recent years have brought great advances in our understanding of basic mechanisms related to the effects of classical psychedelics on both preclinical and clinical settings. Here we review these recent data related to the molecular target, cell signaling and neural circuit processes potentially involved in the still intriguing neurophysiological effects of classical psychedelics.

Common types of classical psychedelics

From a chemical perspective, serotonergic psychedelics including those naturally occurring such as psilocybin which comes from certain types of mushrooms and mescaline which is found in the peyote cactus, and synthetic such as LSD and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) can be grouped into three broad families: tryptamines, phenethylamines and ergolines. Simple tryptamines, such as psilocybin and its active metabolite psilocin or N,N-dimethyltryptamine (DMT), contain an indole ring and a chemotype that most closely resembles the neurotransmitter serotonin (or 5-hydroxytryptamine, 5-HT), while phenethylamines, such mescaline or DOI, contain a phenyl ring and have a chemotype similar to dopamine. Ergolines, such as LSD have a more complex tetracyclic structure and a chemotype closely related to ergotamine and are sometimes classified as complex tryptamines[2, 4, 5, 24].

While this review focuses on classical serotonergic psychedelics, there are also psychoactive compounds that produce some psychedelic-like effects, such as the dissociative ketamine and the entactogen 3,4-methylenedioxy-methamphetamine (MDMA)[25]. Although these compounds may produce some similar subjective effects, and are often also referred to as psychedelics[26], they work primarily through different molecular target mechanisms than classical psychedelics[27], which is another field of investigation out of the scope of this review article.

As expected, considering this diversity in their chemical composition (Fig. 1), psychedelics act on more than one target – meaning that their pharmacological profile is quite complex particularly with regard to synthetic psychedelics of the ergoline family including LSD. As a few examples[1, 2, 4] (Table 1), psilocin binds with moderate affinity to serotonin 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors (with K_i values ranging from 100 – 600 nM), as well as to the histamine H_1 receptor (K_i values of approximately 300 nM) and with higher affinities to 5-HT_{2B} and 5-HT_7 receptors (K_i < 10 nM)[28–30]. Psilocin, however, has negligible affinities targeting adrenergic (K_i > 1,000 nM) and dopamine (K_i > 1,000 nM) receptors. The prodrug, psilocybin, binds monoaminergic receptors including serotonin, adrenaline and dopamine with negligible affinity (K_i > 10,000 nM), and must be metabolized into psilocin by alkaline phosphatases to become pharmacologically active[28–30]. LSD, being a chiral compound, has four different enantiomers that could exist (*d*-LSD, *l*-LSD, *d*-iso-LSD and *l*-iso-LSD) of which only one (*d*-LSD) can be described as a hallucinogenic agent. It binds with high affinity to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors (K_i < 10 nM) and also with a slightly lower affinity to the 5-HT_{2B} receptor (K_i of approximately 30 nM)[28, 30, 31]. LSD has modest to high affinity for dopaminergic receptors as well, including D_1, D_2, D_3 and D_4 receptors (K_i values ranging from 30–200 nM). DOI has a high affinity for both 5-HT_{2A} and 5-HT_{2C} (K_i < 5 nM and < 10 nM, respectively), modest affinity for 5-HT_{2B} (K_i approximately 20 nM) and lower affinity for 5-HT_1 receptors[30, 32, 33], whereas mescaline binds almost exclusively to 5-HT_{2A} receptors[30, 33].

The active doses of these compounds as psychedelics can also vary greatly among different chemical forms. For example, in humans the average effective dose of mescaline is 200–400

mg with a duration of effects between 8 and 10 hours, whereas psilocybin effective doses are within the range of 1–5 g of dried mushrooms (20–40 mg psylocibin) and last 6–8 h. LSD is even more potent with an effective dose of 0.05– 0.2 mg that can last up to 12 h. Their route of administration is also an important factor for the duration and intensity of the subjective and physiological effects. Psychedelics such as mescaline and psilocybin are ingested orally and rapidly absorbed from the gastrointestinal tract, where the compounds are eliminated in the liver via first-pass metabolism. Psilocybin can be easily converted into psilocin, which then has increased lipid solubility due to the removal of the phosphate group and may more readily cross the blood-brain barrier compared to mescaline. Not all psychedelics are taken orally and may bypass the liver. LSD or DMT for example, are typically administered sublingually and through inhalation, respectively [1, 24].

Animal studies have also revealed insights into the differences in potency and efficacy of psychedelics as behaviorally active drugs. Drug discrimination studies assessing various psychedelics' ability to substitute for the phenethylamine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (0.1 – 1.0 mg/kg) showed that rats lever responded after LSD administration at reduced doses (0.05–0.1 mg/kg), and responded following DMT at increased doses (1.5 – 10 mg/kg)[34]. This suggests that psychedelics can have different efficacies and potencies in the same assay. In 1984, Richard Glennon and collaborators proposed that the affinities of psychedelics targeting 5-HT_2 receptors correlated with their hallucinogenic properties in human and animal behavioral models[35]. Further, as reviewed[36], head-twitch response (HTR) – a rapid side-to-side movement of the head – provides insights into relative efficacies for unconditioned behavior, and is currently used as a mouse behavioral proxy of human psychedelic potential.

Despite this heterogeneity in their pharmacological profiles and considering the potential role of alternative monoaminergic receptors such as the dopamine D_2 in the effects induced by ergoline-derived psychedelics, it is clearly established that 5-HT_{2A} receptors and particularly those in frontal cortical excitatory pyramidal neurons projecting to subcortical regions are the primary molecular target necessary to produce hallucinations in humans as well as HTR in rodents. This concept was first described by Franz Vollenweider and colleagues[8] as they demonstrated that the hallucinogenic action of psilocybin[8] and LSD[37] in healthy volunteers was prevented by administration of the serotonin receptor blockers ketanserin and risperidone, and was further corroborated in rodent models via genetic and pharmacological tools[36]. However, as reviewed below, several questions still remain unanswered with regard to the molecular target(s) responsible for the clinically relevant effects of classical psychedelics.

Serotonin and 5-HT receptors

Serotonin – a biogenic monoamine similar to dopamine and norepinephrine – is distributed throughout the entire physiological system while ~90% of the body's serotonin is produced in the gut. It was first isolated in the late 1930s from the gastrointestinal tract by Vittorio Erspamer, who described the compound as an indole amine and named it as enteramine. About a decade later, it was again isolated in blood serum by Maurice Rapport and collaborators where they identified it as a vasoconstrictor and subsequently named the

molecule serotonin. But it was not until another decade later in 1953, that Betty Twarog analyzed brain tissue using a serotonin-sensitive assay and reported for the first time the presence of serotonin in mammalian brain. Following this discovery, Rapport later found the structure to be that of serotonin. This chemical structure comes from the essential amino acid tryptophan, which is hydroxylated to 5-hydroxytryptophan before going through a decarboxylation to form serotonin (or 5-HT)[38–40].

Around this same time, Hofmann discovered the psychedelic effects of LSD. Following an accidental exposure to LSD, Hofmann returned to the compound and took it deliberately, recording his experiences[6]. He found the experience to be quite intense, describing "*kaleidoscopic, fantastic images*". Once the structure of serotonin was reported, it was put forth that this monoamine may be implicated in several neuropsychiatric diseases since LSD contains a tryptamine core scaffold like the endogenous ligand 5-HT[38, 41, 42]. From there, pharmacology research on serotonin began and the classification of serotonin receptors was soon to follow. The classification was initially divided into two groups with M- and D-type receptors that were designated based on their sensitivity to blockade by morphine and phenoxybenzamine in the ileum, respectively[41, 42]. In the 1970s, the development of radioligands for labeling receptors in animal tissue samples allowed researchers to target distinct serotonin receptor populations, two of which were named 5-HT_1 and 5-HT_2[38]. The affinity for these receptors was sensitive to guanine nucleotides which suggested they were likely G protein-coupled receptors (GPCRs). In the 1990s, serotonin receptors were reclassified into 7 main types and 14 different subtypes based on selective agonist/antagonist ligand affinities, sequence homology, and signaling pathways[42, 43].

GPCRs make up a large majority of signaling proteins in mammalian cells. The term GPCR comes from the association with and signaling through heterotrimeric (α, β and γ subunits) G proteins, which when the receptors are activated dissociate into Gα and Gβγ, and modulate the function of downstream effectors[44]. GPCRs are grouped into classes on the basis of conserved sequence fingerprints and endogenous ligand-binding sites: rhodopsin (A), secretin (B1), adhesion (B2), glutamate (C), Frizzled (F), and taste (T, reclassified as a separate family). Class A is the largest group implicated in several physiological functions such as vision, immune response, and hormonal and neurotransmitter signaling[45, 46]. Despite their diversity in terms of primary sequence and overall tertiary arrangement, they share the same general structural organization: GPCRs all have seven hydrophobic transmembrane (TM) α-helices connected by three extracellular loops and three intracellular loops, an extracellular N-terminal and an intracellular C-terminal. The N-terminal and extracellular loops are important for processes related to ligand recognition while the C-terminal and intracellular loops interact with G proteins, protein kinases and other downstream signaling proteins[45, 47]. With the rise of computational models in the service of X-ray and cryo-EM structural determination, it has been corroborated that despite primary sequence variability between GPCRs, there are several conserved motifs responsible for GPCR active and inactive conformations and ligand binding, particularly within each of the classes. For example, most GPCRs have a highly conserved cysteine disulfide bridge between extracellular tip of the third TM domain and the second extracellular loop, which

stabilizes the conformation of the extracellular domains. This stabilization allows for the structural conformation of the class A GPCRs to form the ligand-binding pocket [45].

The conformational shift from inactive to active states of GPCRs is thought to be a multi-state model[45, 48]. This suggests the receptor can have multiple distinct states, active and inactive, which can be stabilized specifically depending on the ligand and the given GPCR, allowing for existence of alternative signaling pathways or biased agonism[48, 49]. This is an important feature of GPCRs because it determines how different ligands, including endogenous and synthetic, bind to receptors allowing for differential signaling implicated in not only homeostasis, but also disease-related states. Once an orthosteric agonist binds to the GPCR, the induction of a conformational change will promote the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) within the Gα subunit of the heterotrimeric G protein, and the release of the Gβγ heterodimer[50, 51]. Deactivation of the G protein occurs when the GTPase activity hydrolyzes GTP to GDP, causing reassembly of the heterotrimeric G protein complex.

The signaling pathways following GPCR activation are dependent on the specific G protein associated with the receptor type. The four major classes of G proteins include G_s, $G_{i/o}$, $G_{q/11}$ and $G_{12/13}$[50, 51]. Receptors that interact mainly with G_s or $G_{i/o}$ typically stimulate or inhibit adenylate cyclase-related pathways, respectively, while those that interact with $G_{q/11}$ activate phospholipase C (PLC) which produces inositol phosphate 3 (IP3) and diacylglycerol (DAG), which ultimately cause an increase in intracellular calcium release from the endoplasmic reticulum[45]. The Gβγ dimer can recruit its own second messengers that are responsible for pathways associated with ion channels and protein kinases[52]. Ligand-bound GPCRs also activate non-canonical G protein-dependent pathways. For example, GPCR kinases (GRK) can phosphorylate GPCRs and recruit β-arrestin proteins, which are involved in processes related to desensitization, endocytosis, and downregulation, as well as alternative G protein-independent signaling processes[49].

Within the serotonin receptor family, there are seven receptor types, 5-$HT_{1–7}$, of which all receptors are class A GPCRs except for 5-HT_3, which is a ligand-gated cation channel. Within these seven receptor types, there are fourteen subtypes, which couple to different G proteins: $G_{i/o}$ protein-coupled receptors include the 5-HT_1 subfamily (5-$HT_{1A, 1B, 1D, 1E, 1F}$) and the 5-HT_5 subfamily (5-$HT_{5A, 5B}$)[39, 41, 42], whereas 5-HT_4, 5-HT_6, 5-HT_7 receptors are typically coupled to G_s proteins[53].

The 5-HT_{1A} receptor is expressed both presynaptically as an autoreceptor of serotonergic neurons in the dorsal raphe nuclei (DRN), and postsynaptically on pyramidal neurons in the frontal cortex[41, 54, 55]. The DRN is responsible for the release and regulation of serotonin[56, 57]. This receptor has been implicated in anxiety-like behaviors and has been investigated as a target for novel antidepressants and antipsychotics[58–60].

The $G_{q/11}$ protein-coupled receptors include the 5-HT_2 subfamily (5-$HT_{2A, 2B, 2C}$)[5, 35]. This group of receptors shares a 46–50% overall sequence identity, with 5-HT_{2A} receptor sharing an overall 45% homology with the 5-HT_{2B} receptor and 80% homology with 5-HT_{2C} receptor TM domains[5, 39, 61]. Despite their homology and overall activation

of PLC and stimulation of calcium release via $G_{q/11}$-signaling, these receptors can couple to second messenger pathways in a cell-specific manner[41, 42]. The 5-HT_{2B} receptor is implicated in cardiovascular physiology[62], whereas the 5-HT_{2C} receptor was first described as a "5-HT_{1C}" receptor in the choroid plexus[63] but more recent data have reported a more widespread expression in the CNS, mainly in the cortex and the ventral tegmental area[64, 65]. This 5-HT receptor subtype has been evidenced to be involved in appetite and control of dopamine release[66].

The 5-HT_{2A} receptor is widely distributed in the CNS, but is most notably densely spread across the cerebral cortex particularly in layers II/III and V on pyramidal cells and inhibitory interneurons[32, 67–69]. Intermediate levels of *5-HT_{2A} receptor* mRNA expression were also found in the limbic system in areas such as the caudate and nucleus accumbens in rodents[67]. Additionally, not only are 5-HT_{2A} receptors expressed in the CNS, they also play distinct roles in smooth muscle contraction specifically in the gastrointestinal tract and cardiovascular systems[41], and can be found in bronchial and uterine tissues and the ileum of the gut[39, 70].

There is also evidence suggesting that the 5-HT_{2A} receptor may be implicated in the etiology of schizophrenia and its treatment. Second generation, or atypical, antipsychotic drugs all share high-affinity for the 5-HT_{2A} receptor[71, 72]. The most effective antipsychotics such as clozapine have been found to act as 5-HT_{2A} receptor inverse agonists, competing for the orthosteric binding site on the receptor and stabilizing inactive conformation of the GPCR [73]. Alterations in 5-HT_{2A} receptor density and mRNA expression have been observed in postmortem frontal cortex samples from schizophrenia subjects[74–76]. Epigenetic changes associated with 5-HT_{2A} receptor-dependent mechanisms following repeated treatment with atypical antipsychotics have also been reported, including up-regulation of histone deacetylase 2 (HDAC2), which is involved in transcriptional repression of genes involved in synaptic plasticity and memory[77–79].

Structure of 5-HT_{2A} receptors

Several groups at the end of the 20th century used classical pharmacology and molecular biology techniques to map the binding site pocket of the 5-HT_{2A} receptor[80–82]. Although the first crystal structure of rhodopsin was published by Krzysztof Palczewski et al in 2000[83], the first crystal structures of serotonin GPCRs were revealed in 2013 for the 5-HT_{1B}[84] and 5-HT_{2B}[85] receptors bound to the antimigraine medication ergotamine. It was not until 2017 that the first psychedelic-bound serotonin receptor crystal structure was solved[86], followed by the first X-ray crystallography study reporting 5-HT_{2A} receptor conformation when bound to two second-generation antipsychotics: risperidone and zotepine[87].

Consistent across studies of active and inactive structures of class a GPCRS[44, 47], the orthosteric binding site was embedded in the TM core, encompassing several helices including TM3, TM5, TM6, and TM7. The extended binding site has been shown to occupy the extracellular portions of TM3, TM5, TM6 and TM7, as well as part of the extracellular loop 2. Previous reports suggested that $P^{5.50}$, $I^{3.40}$ and $F^{6.44}$ (superscripts in

this form indicate Ballesteros-Weinstein numbering for conserved GPCR residues[88]) – the “P-I-F” motif, form an interface between TM5, TM3 and TM6 near the base of the ligand binding pocket of the β_2-adrenergic receptor and many other GPCRs that is thought to be responsible for regulating receptor activation by allowing movement of helices toward the intracellular side[89]. Studies focusing on the serotonin receptors have also identified that the bottom hydrophobic cleft in the ligand-binding pocket is surrounded by conserved residues, including I163$^{3.40}$ and F332$^{6.44}$ in the PIF motif[84, 90]. The close proximity seen with the ligands and the PIF motif was hypothesized to contribute to stabilization of the structure in the inactive antagonist-bound state. Additionally, a side-extended cavity between TM4 and TM5 has also been reported and may allow for larger molecules to fit inside the binding pocket. This extended cavity connects the orthosteric binding site and the plasma membrane near a strictly conserved residue, D155$^{3.32}$, essential for ligand interactions[89, 90].

As mentioned above, first psychedelic-bound crystal structure of the serotonin GPCR was the 5-HT$_{2B}$ receptor[86], which was able to solve the LSD-bound X-ray structure as a model system for understanding how it might bind to the closely related 5-HT$_{2A}$ receptor. As an ergoline psychedelic, LSD exhibited a unique binding pose within the 5-HT$_{2B}$ receptor. It has similarities to other monoaminergic receptors, as it is anchored to the receptor via the highly conserved salt bridge through binding of the nitrogen of the ergoline moiety and the D135$^{3.32}$, which is analogous to the D155$^{3.32}$ in the 5-HT$_{2A}$ receptor. The ergoline moiety occupies the orthosteric binding pocket via hydrophobic side chains, whereas the diethylamide group binds between TM2, TM3 and TM7 with one ethyl group extending into what had been previously reported to be the extended binding pocket. The positioning of LSD in the structure is reported to be shallow – closer to the extracellular loop 2 compared to the non-psychedelic ergoline ergotamine. This positioning of LSD causes conformational changes in the receptor, which includes helical movements and re-positioning of several residues, suggesting changes in dynamic states of the receptor. This study also reported the LSD-bound conformation to have a substantial rotation of the diethylamide moiety of the molecule itself, suggesting that binding pose is necessary for the functionality of the bound-receptor, and more specifically β-arrestin recruitment. Perhaps the most interesting finding of the LSD-bound structure is the discovery of residues of extracellular loop 2 forming a “lid” over the docked molecule. In this bound structure, the side chain of the lid is stabilized via hydrophobic bonds with LSD and residues in TM3, TM4 and TM5, causing slow dissociation rates from the receptor – a structural mechanisms that was proposed as potentially involved in LSD’s long-lasting subjective effects[86].

Following the work with the 5-HT$_{2B}$ receptor, the 5-HT$_{2A}$ receptor structure was solved. Using cryo-EM to investigate the conformation of the 5-HT$_{2A}$ receptor complexed with different antipsychotics, risperidone and zotepine, it was found that both antagonists form a salt bridge between D155$^{3.32}$, which is also strictly conserved across serotonin receptors, as well as other aminergic receptors. They report that risperidone adopts an extended conformation in the ligand-binding pocket, whereas zotepine occupies the bottom of the pocket[87]. When evaluating docking poses of a more selective 5-HT$_{2A}$ antagonist, pimavanserin, they reported occupation of the side-extended cavity and when altered, the

affinity of the antagonist decreased[87]. This suggests that the side-extended cavity may contribute to high selectivity of some compounds.

It was not long after this investigation that a psychedelic-bound X-ray and cryo-EM structures of the 5-HT_{2A} receptor were found[91]. To stabilize the structural conformation of a psychedelic-activated G_q-coupled 5-HT_{2A} receptor, the authors utilized 4-(2-((2-hydroxybenzyl)amino)ethyl)-2,5-dimethoxybenzonitrile (25CN-NBOH), a psychedelic 5-HT_{2A} receptor agonist with a phenethylamine structure[91]. This was compared to the structure of the LSD-bound 5-HT_{2A} receptor to examine potential differences in binding configuration between two psychedelics. Similar to the 5-HT_{2A} receptor antagonists, when assessing the 25CN-NBOH-bound and LSD-bound structures, it was found that the highly conserved salt bridge between $D155^{3.32}$ and the basic nitrogen of the compounds produced a rotation of the toggle switch residue with a subsequent movement of the PIF motif consistent with ligand binding.

Considering the structural differences in the two compounds, it was proposed that 25CN-NBOH has a unique binding pose in that it seemed to bind in a previously undescribed pocket between TM3 and TM6, directly with a residue in the toggle switch and undergoing specific conformational changes. Further, the phenethylamine group of 25CN-NBOH binds in the orthosteric pocket, but unlike LSD, it does not have interactions with the conserved $S242^{5.46}$ residue typically required for orthosteric binding. In the LSD-bound 5-HT_{2A} structure, however, LSD forms a hydrogen bond between the indole-NH and side chain of $S242^{5.46}$. This serine residue was therefore an important and distinct residue for the 5-HT_{2A} receptor, as it was proposed to be involved in the different functional effects of two structurally distinct psychedelics[61]. Considering that this study focused on agonists bound to the G_q-coupled stabilized structure, they also reported interesting findings pertaining to the C-terminal helix, a major interface for the GPCR-$G\alpha_q$ complex. By mutating important residues in the intracellular loop 2, G_q-dependent signaling upon 25CN-NBOH administration was completely abolished, whereas β-arrestin recruitment was increased. This battery of mutations also decreased or abolished the ability of LSD and 5-HT to activate G_q-signaling[91], which corroborates previous findings with the 5-HT_{2A} I181D receptor construct[92].

Biased agonism refers to the ability of a ligand to activate a subset of receptor's signaling cascade, such a G protein coupling versus b-arrestin recruitment[93, 94]. Recent studies comparing the structure of different psychedelic and non-psychedelic serotonergic compounds bound to the 5-HT_{2A} receptor have provided insights into the mechanisms behind hallucinogenic action and ligand bias. In a particular study aimed at designing non-psychedelic ligands, the authors examined the 5-HT_{2A} receptor complexed with multiple compounds including the psychedelics psilocin and LSD, and the non-psychedelic 5-HT_{2A} receptor agonist, lisuride[95]. Consistent with previous studies[91], the ergoline moieties of LSD and lisuride were bound to the bottom of the orthosteric binding pocket. Considering that psilocin is a simple tryptamine psychedelic versus a complex ergoline structure, the binding of the indole core of psilocin was higher within the orthosteric binding pocket and closer to both the extracellular loop 2 and the extended binding pocket. Interestingly, this study also reported a secondary binding mode of both 5-HT and psilocin where the indole

core is positioned into a narrow cleft described by others as the extended binding pocket. Consistent with other complexed structures, both ligands form the salt bridge between D155$^{3.32}$ and the basic nitrogen, as well as an extra hydrogen bond with N352$^{6.55}$.

To assess whether the different binding poses affect receptor function, they mutated the key residues S239$^{5.43}$ and S242$^{5.46}$ in the orthosteric binding pocket, which diminished agonism of both compounds. However, substitution of L362$^{7.35}$ in the extended binding pocket showed no effect on agonist's potency on G_q coupling, whereas the agonist properties of psilocin and lisuride on β-arrestin recruitment were eliminated. This suggests the involvement of a key residue of the extended binding pocket for non-canonical signaling associated with some but not all 5-HT_{2A} receptor agonists. Further, the X-ray structures of LSD-bound and lisuride-bound 5-HT_{2A} receptor were compared to assess differences in the structural arrangement within the orthosteric binding pocket between psychedelic and non-psychedelic 5-HT_{2A} receptor agonists. Interestingly, the authors reported differences in contact with Y370$^{7.43}$ in TM7 in which the two ethyl groups of LSD interact versus only one ethyl group of lisuride (Fig. 2), and substitution of this residue strongly increased lisuride-induced 5-HT_{2A} receptor-dependent β-arrestin recruitment. This pivotal study suggests that despite similar occupancy to the orthosteric binding pocket, engagement with the extended binding pocket is crucial for biased agonism[95].

Psychedelic signaling and biased agonism

Determining the receptor target and signaling pathways of classical psychedelics is important for understanding their effects on the brain physiology and behavior. As mentioned above, it has been well-established that the principal molecular target of classical psychedelics to elicit hallucinations and subjective effects is the 5-HT_{2A} receptor [5, 91, 96]. This was first supported with the use of correlations between pharmacological parameters in vitro and in rodent models with hallucinogenic potencies in humans, as well as with antagonistic blockade with ketanserin and risperidone in healthy volunteers [8, 37, 97, 98]. However, it was not until the first 5-HT_{2A} knockout mouse was created and tested in preclinical models of psychedelic action that evidence became conclusive[96, 99].

To further explore the signaling processes involved in psychedelic action, and specifically the potential role of biased agonism *in vivo*, a transcriptome fingerprint approach was used following a single dose of psychedelic (DOI, DOM, 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane or DOB, LSD, psilocin, and mescaline) and non-psychedelic (*R*-lisuride, *S*-lisuride, and ergotamine) 5-HT_{2A} receptor agonists. Somatosensory frontal cortex samples were collected 1 h following drug or vehicle administration[96, 99]. Each agonist, psychedelic or non-psychedelic, tested produced unique responses in mouse somatosensory frontal cortex gene expression. Transcripts *c-Fos* and *I-κβα* were both induced at a similar level by psychedelics and non-psychedelics, but transcripts *Egr-1* and *Egr-2* were only consistently activated by psychedelic 5-HT_{2A} receptor agonists. This effect on gene expression upon psychedelic or non-psychedelic drug administration was mediated mostly via 5-HT_{2A} receptor since it was absent in the knockout animals. Further, it was found that both the non-psychedelic 5-HT_{2A} receptor agonist lisuride and the psychedelics 5-HT_{2A} receptor LSD induced expression of *c-Fos* via $G_{q/11}$ protein-dependent PLC-*β* signaling,

but only the psychedelic LSD augmented *Egr-1* and *Egr-2* transcription via pertussis toxin (PTX)-sensitive $G_{i/o}$ proteins. These findings have been validated by evaluating protein phosphorylation following administration of psychedelic and non-psychedelic 5-HT_{2A} receptor agonists [100]. Utilizing PTX, it was reported that ERK1/2 phosphorylation induced by the psychedelics DOI and LSD was abolished, whereas this blockade of $G_{i/o}$ protein coupling did not affect responses induced by lisuride.

Glutamatergic signaling is also heavily evidenced in the effects of psychedelics[101], considering the high level of expression of 5-HT_{2A} receptors on pyramidal neurons in the frontal cortex[102]. Interestingly, the effects of psychedelics on cellular, electrophysiological and behavioral responses are blunted upon administration of the metabotropic glutamate receptor type 2/3 receptor (mGlu2/3) agonist LY379268[74, 103], whereas psychedelic-induced HTR is augmented by the mGlu2/3 receptor antagonist LY341495[104]. Additionally, HTR induced by DOI and LSD is also absent in mGlu2 receptor knockout mice[105], a genotype effect that was not observed in mGlu3 receptor knockout animals [106]. This intriguing crosstalk between 5-HT_{2A} and mGlu2 receptors as well as its potential role in the acute and post-acute effects of classical psychedelics has been extensively reviewed elsewhere[107, 108].

It has also been proposed that psychedelics modulate differential signaling pathways as compared to the endogenous neurotransmitter 5-HT. Thus, treatment in mice with the 5-HT precursor, 5-hydroxytryptophan (5-HTP), produced β-arrestin-2-dependent HTR, whereas the effect of DOI on HTR was independent of β-arrestin-2[109]. Further, they also showed 5-HT-mediated activation of a signaling cascade via 5-HT_{2A} receptor and β-arrestin-2, as well elements important for neuronal maturation such as Akt and Src[109] – an effect that was not observed with DOI. More recent findings have also demonstrated the involvement of β-arrestins in the effects of psychedelics utilizing genetically modified mice. It was found that LSD-induced HTR was significantly reduced in β-arrestin-2, but not β-arrestin-1, knockout mice[110]. Harnessing the functional selectivity of a psychedelic ligand may prove useful for drug development, as it has been proposed that such biased agonists could activate pathways associated with therapeutic efficacy rather than cell signaling processes responsible for their unwanted side effects. For a summary schematic of signaling pathways and their potential outcomes see Fig. 3.

Another important consideration for 5-HT_{2A} receptor activation upon psychedelic administration and the various signaling cascades downstream is that related to single nucleotide polymorphisms (SNPs). These are random changes in gene sequence of an individual which may fall within coding or non-coding regions as well as in the intergenic regions. Those occurring within a coding sequence can by synonymous or nonsynonymous depending upon a change in the amino acid sequence of the protein that is finally translated. Nonsynonymous SNPs can have great impacts on pharmacokinetics and pharmacodynamics, and lead to alterations in the response to pharmacological therapeutics and interventions[111]. As one example, cytochrome P450 enzymes are responsible for the metabolism of most commonly used drugs through first-pass metabolism in the liver. SNPs at certain enzymes in this family of enzymes have been shown to alter the metabolism and pharmacokinetics of methadone, which is commonly prescribed as a pharmacotherapy

for opioid use disorder[112]. Just as it is important to understand the role of SNPs in metabolism, it is also necessary to focus on their role in terms of receptor dynamics, as it will alter downstream signaling outcomes.

Given the rising number of clinical trials focused on the potential therapeutic effects of psychedelics, it has been discovered that there are some important differences in response patterns to treatment with some study participants responding positively, negatively or in a neutral way to psychedelic administration. It has therefore become of interest to understand the mechanisms behind the non-responsive groups as it could shed light on how psychedelics may be useful or alternately unfavorable in future clinical settings. A potential explanation for these different responses to serotonergic compounds could be SNPs; as evidenced by a recent study by Schmitz and colleagues[113]. The seven SNPs in this study represent the most common 5-HT_{2A} receptor variants observed in the human population, with H452T being the most frequent (7.9%). Similar to the results from a study previous on antipsychotics and 5-HT_{2A} receptor agonists[114], they found that no compounds or mutations displayed a uniform pattern of increasing or decreasing signaling bias, and instead each had a unique pharmacological profile. Of note, psilocin was found to have increased propensity for G_q-dependent signaling versus both β-arrestin-1 and β-arrestin-2 recruitment cells expressing the H452T SNP, whereas 5-Meo-DMT had a decrease in G_q activity as compared to both β-arrestin-1 and β-arrestin-2 recruitment in the H452T SNP. Mescaline had a bias for G_q protein-dependent signaling in the A447V SNP only and more β-arrestin-1 bias in the S12N and T25N SNPs, whereas almost all SNPs caused a preference for β-arrestin-2 recruitment versus G_q coupling. Interestingly, LSD had no significant bias for G_q versus β-arrestin-1, but when comparing G_q and β-arrestin-2 the bias for G_q was increased in the A230T and H452Y SNP. These SNPs are not near the orthosteric binding site of the 5-HT_{2A} receptor, but still have significant effects on the *in vitro* pharmacology of psychedelics. Specifically, the H452Y and A230T polymorphisms may be of interest due to the distinct changes in G_q coupling and β-arrestin-2 recruitment, as well as alterations in efficacy for 5-HT, antipsychotics and psychedelics tested with the H452T polymorphism.

An additional interesting observation is related to the subcellular localization of the 5-HT_{2A} receptor and the potential role of location bias as an emerging paradigm in psychedelics research. Thus, although the majority of GPCRs are primarily located at the cell surface under basal state conditions, certain receptors including dopamine D_1[115], δ-opioid[116] and GPRC6A[117] present a notable intracellular presence. Accordingly, visualization of individual living cells indicates that, at steady stage, the bulk of 5-HT_{2A} receptors is present in punctate intracellular vesicles[92, 109, 118, 119]. Using electron microscopy techniques, it has also been reported that an important fraction of the 5-HT_{2A} receptor population is located intracellularly in brain regions such as the frontal cortex and the ventral tegmental area[77, 102, 120–123].

Agonist binding and activation of most GPCRs usually results in the rapid desensitization and endocytosis of the receptor[119, 124], and this trafficking effect has been described for the 5-HT_{2A} receptor with endogenous (5-HT)[119, 125–128] and synthetic agonists including quipazine[126, 129] and DOI[128, 130] using *in vitro* experimental systems such as HEK293 cells[131, 132]. This agonist-induced subcellular redistribution of the 5-HT_{2A}

receptor has been proposed to be mediated via dynamin-dependent yet arrestin-independent endocytosis[126]. Antagonists/inverse agonists, however, induce contradictory effects when targeting the 5-HT_{2A} receptor. As an example, ligands such as mianserin, volinanserin and altanserin enhance cell surface expression of the otherwise intracellularly located 5-HT_{2A} receptor[118, 130]. Treatment with clozapine – an antipsychotic medication that normally targets the 5-HT_{2A} receptor as an antagonist/inverse agonist yet can also activate certain signaling pathways such as Akt[133, 134]– retains the predominantly intracellular localization of the 5-HT_{2A} receptor in HEK293 cells[126, 128, 130, 133, 134]. Additionally, repeated administration of the antipsychotic clozapine as well as risperidone down-regulates 5-HT_{2A} receptor density in mouse frontal cortex samples[77, 135, 136]. It is clear that additional investigation is needed to unravel the role of these signaling pathways in the therapeutic response or alternatively unwanted side effects induced by clozapine and other atypical antipsychotics.

Alternative trafficking routes that retain this receptor intracellularly may also be related to the maturation pathway since it was reported that upon treatment with cycloheximide to inhibit protein synthesis, the 5-HT_{2A} receptor was observed only at the cell surface[127]. Different cell types as well as inter-experimental variability may also affect this pattern of subcellular localization since as two examples i) a truncation mutant affects agonist-induced 5-HT_{2A} receptor desensitization differently in HEK293 and C6 glioma cell lines[129], and ii) the elevated intracellular localization of the 5-HT_{2A} receptor at basal state has been validated by some[118, 125, 127, 130, 133, 137] but not all[126, 128, 129] studies testing GPCR trafficking in HEK293 cells[130]. More recent findings have suggested that psychedelics promote neuroplasticity effects via the activation of intracellular 5-HT_{2A} receptors[138]. Thus, studies in cortical neuronal primary cultures showed that only membrane-permeable but not membrane-impermeable 5-HT_{2A} receptor agonists promoted neuronal growth in cortical primary cultures. It remains unknown, however, whether this mechanism explains the psychedelic action of less lipophilic compounds such as mescaline, or the lack of psychedelic activity of 2-Br-LSD with more lipophilic properties than its psychedelic cousin LSD[139]. Additionally, it was suggested that, under these experimental conditions, serotonin was unable to elicit either an agonist response or structural plasticity effects because as a highly polar compound it lacked the ability to pass through the plasma membrane[138, 140]. These results raise intriguing questions about the endogenous ligand for 5-HT_{2A} receptors in the cortex, as well as the route of serotonin to the nucleus of cells across several brain structures than include frontal cortex, ventral tegmental area, dorsal raphe nucleus, caudate-putamen and cerebellum where serotonylation – the covalent linkage of serotonin to proteins including histone H3 tri-methylated lysine (H3K4me3) – alters chromatin accessibility and gene expression regulation[141].

It is important to recognize that classical psychedelics not only pharmacologically affect signaling cascades via activation of 5-HT_{2A} receptor in frontal cortex pyramidal neurons, since they also expressed in subcortical regions such as the ventral tegmental area[142], caudate putamen (or corpus striatum)[143–145] and amygdala[146]. Similarly, George Aghajanian in 1968 while testing the electrophysiological effects of LSD clearly suggested a reversible cessation of spontaneous activity in the rat dorsal raphe nucleus[140], one of the largest groups of serotonergic neurons in the mammalian brain. Additionally, a

more recent study using whole-brain two-photon microscopy to map immediate early gene expression following psilocybin and ketamine administration found that c-Fos expression was present in the frontal cortex, as well as central and basolateral amygdala, among other brain regions[147, 148]. A study focused on direct pharmacological manipulation of the nucleus accumbens with psychedelic DOI and 5-HT_2R antagonists have provided evidence that local DOI administration increases dopamine release, attenuated by co-administration of ketanserin[149]. This leaves an interesting field to be pursued since repeated administration of drugs of abuse such as cocaine increases the density of dendritic spines on nucleus accumbens medium spiny neurons[150]. Questions related to the effects of psychedelics on subcortical regions involved in processes such as reward, motivation and reinforcement also deserve additional investigation.

There is also a large body of research utilizing brain imaging techniques to understand global connectivity changes in the brain following psychedelic administration[151–154]. For example, the “relaxed beliefs under psychedelics” (REBUS) model, encompasses the idea that psychedelics reduce activity in the default mode network in order to promote activity in other brain regions such as the hippocampus[155]. According to this model, psychedelics would shift connections in higher-level brain regions to those in lower regions. Another neural network of interest is the cortico-striatal-thalamo-cortical loop (CSTC), which proposes that psychedelics modulate cortical and subcortical signaling through the striatum and thalamus, increasing the flow of sensory information and changes in cognition, perception and sensorimotor gating[156, 157]. Further research into neural networks is warranted before mechanisms behind psychedelic action on whole brain connectivity can be elucidated.

Acute effects of psychedelics in humans and rodents

The acute subjective effects of psychedelics in healthy subjects have been described in various ways, including visual hallucinations, changes in light/sound perception and touch sensations. Acute effects have also been related to increased feelings of oneness, joy, euphoria, ego dissolution, mysticism, and spiritual experiences. There are also changes in physiological measurements following psychedelic administration, such as alterations in blood pressure, heart rate, temperature, as well as gastrointestinal upset and in some cases nausea and vomiting[1]. One of the most accepted self-report measures to assess the subjective effects of psychedelics in through specific questionnaires in which clinical trial participants will rate their subjective feelings based on a Likert scale survey. Examples include the Mystical Experiences Questionnaire (MEQ)[158, 159], as well as the 5-dimensional Altered States of Consciousness Scale (ASC)[160]. The MEQ was developed by Walter Pahnke in 1963 to assess the major dimensions of classical psychedelic experiences[159], and includes measures of internal and external unity, transcendence of time and space, noetic quality, sacredness, positive mood, ineffability and paradoxicality. Several studies focused on understanding psychedelic-induced subjective effects and therapeutic outcomes are also based upon traditional questionnaires on anxiety[161] (The State-Trait Anxiety Inventory), depression[162, 163] (Beck Depression Inventory), and general moods and other responses[164] (Profile of Mood States and Visual Analog Scale).

Multiple studies in humans have demonstrated changes in brain dynamics that follow administration of classical psychedelics such as LSD and psilocybin using neurological techniques such as electroencephalography (EEG), positron emission tomography (PET) or functional magnetic resonance imaging (fMRI). When assessing the effects of psilocybin versus a placebo in EEG recordings, it was reported that the psychedelic decreased alpha-oscillations, which are involved in several top-down processes related to perception and working memory, as well as N170 visual-evoked potentials. Additionally, administration of the 5-HT_{2A} receptor antagonist ketanserin attenuated with changes in brain dynamics, evidencing the involvement of 5-HT_{2A} receptor activation in the perceptual changes associated with a classical psychedelic[98].

Measuring hallucinogenic activity in preclinical models including rodents is somewhat challenging due to the high subjectivity of the experience. While there are several behavioral paradigms that have provided insights into psychedelic pharmacology[36] such as drug discrimination[35], locomotion[133, 165] and pre-pulse inhibition (PPI) of startle[166, 167]. The most widely used behavioral method to test psychedelic drug action in rodent models is the HTR[168–171]. This unconditioned behavior is a rapid side-to-side rotational head movement that occurs in mice and rats after administration of a serotonergic psychedelic. A similar behavioral phenotype induced upon psychedelic administration and described as “wet dog shakes” has also been noted in other mammals such as rabbits, dogs and cats[169]. Since the first description of HTR induced by 5-HTP and LSD in the 1950s[172–174], consistent reports have demonstrated that this behavior increases in frequency following administration of serotonergic psychedelics, thus providing a mouse behavioral proxy of human psychedelic potential, as well as insights into molecular and neural circuit alterations potentially relevant to their mechanism of action.

It has been proposed that the mechanism behind psychedelic-induced HTR is via activation of the 5-HT_{2A} receptor. This effect was not induced by non-psychedelic 5-HT_{2A} receptor agonists such as lisuride or ergotamine, and undetected in mice with 5-HT_{2A} receptor knockout mice[96, 99, 123]. Since then, other lines of research have corroborated that the psychedelic-elicited HTR can be blocked using 5-HT_{2A} receptor antagonists such as ketanserin and volinanserin[166, 175–177]. Additionally, it was reported that Cre-mediated expression of 5-HT_{2A} receptor in cortical pyramidal neurons was sufficient to rescue the psychedelic-induced HTR behavior[96, 99], while direct injection of the psychedelic DOI into the frontal cortex also induced HTR in rats[178]. These findings suggest that frontal cortex 5-HT_{2A} receptor plays an important role in the behavioral effects of psychedelics including phenethylamines, tryptamines and ergolines, but does not exclude the involvement of other monoaminergic receptors since it was reported that the discriminative stimulus effects of LSD in rats occurs in two phases and these findings provided evidence that the later temporal phase is mediated by dopamine D_2 receptor stimulation[179].

While the acute subjective effects of psychedelics in humans have been consistently studied and reported, there has been little focus on differences across sexes and genders[180]. Many clinical trials report the use of both male and female subjects, but studies are typically insufficiently powered to extrapolate potential sex-driven effects. Recently, a case report regarding menstrual cycle and reproductive function in the context of psychedelics reported

anecdotal changes in menstrual cycle following acute classical psychedelic use[181]. Rodent behavior models using locomotion[182], HTR[171] and sensorimotor gating processes[166, 167] also suggest differences across sexes following acute administration of a psychedelic. As an example, DOI-elicited HTR was greater in female as compare to male C57BL/6J mice compared to male littermates, however the brain and plasma concentrations of DOI in females were lower at 30- and 60-minute time points[171]. This sex-related phenotype was not observed in 129S6/SvEv animals. Another study showed sex-specific effects of DOI and LSD on PPI – a model of sensorimotor gating – in 129S6/SvEv mice in which differences in male and female responses in %PPI was positively correlated with startle amplitude in males but negatively correlated in female mice[166].

As compared to opioids, alcohol and tobacco, psychedelics have low addictive potential and benign toxicity profiles[183, 184]. As an example, a lethal dose of LSD for humans could be around 100 mg, whereas the recreational dose is between 10–200 μg[185]. Case reports of LSD overdose, mostly nonlethal, have been published with most symptoms being extreme agitation, excited delirium and tachycardia[186, 187]. Although some case reports have attributed death to high doses of LSD, many of those include poly-substance use, or medical intervention including various types of restraints which may be involved in the deaths. Interestingly, some accidental nonlethal overdoses of LSD have been reported to produce positive outcomes like decreased physical pain and use of morphine[185]. The non-addictive nature of classical psychedelics is supported by their mostly sporadic use throughout life[188, 189]. Classical psychedelics produce subjective effects that last between 6–12 h, longer than other commonly used drugs. This long-period is thought to contribute to low-risk patterns of use of these compounds. An alternative although not mutually exclusive explanation for the non-addictive nature of psychedelics is rapid tolerance, or tachyphylaxis, observed upon repeated administration[132, 190]. Subjective and sought-after effects of psychedelics become less apparent after repeated exposure, which cannot typically be surmounted with a higher dose. In rodents, it has been reported that repeated administration of DOI induced a progressive decrease in HTR behavior. Pretreatment with the 5-HT_{2A} receptor antagonist volinanserin prevented the acute manifestation of DOI-induced HTR as well as the development of tolerance[132]. Arrestin proteins have been involved in processes related to agonist-induced GPCR desensitization and endocytosis, as well as tolerance to opioid-mediated analgesia[191]. However, this does not seem to be the case for psychedelics since development of tolerance to the effect of DOI on HTR remained unchanged in β-arrestin-2 knockout mice[132].

Studies assessing the reinforcing effects of psychedelics in nonhuman primates have reported low and unreliable rates of intravenous self-administration in a daily access procedure[192]. Similar results have been observed using a self-administration model of psychedelics in rodents. Interestingly, when mice self-administered ketamine, they took comparable numbers of infusions to that of cocaine[193]. However, in the two-lever drug discrimination paradigm, where animals are trained to recognize the internal state (discriminative stimulus) induced by a specific dose of a particular drug (training drug), it has been suggested that drug-appropriate responding in the test phase is generally observed when the training and the test drugs are both psychedelics, which was suggested as preclinical evidence for abuse liability[194, 195]. Substitution for LSD in drug

discrimination assays did not occur with other psychoactive drugs, such as phencyclidine, cocaine or amphetamine.

Intracranial self-stimulation (ICSS) is a commonly used rodent behavior model that evaluates the effect of psychoactive drugs on the brain reward system. In ICSS procedures, rats are equipped with intracranial microelectrodes implanted at the medial forebrain bundle and trained to engage in operant responding to earn pulses of electrical brain stimulation. Drugs with high abuse potential such as cocaine and heroin often induce increases in ICSS, whereas drug-induced decreases in ICSS provide a measure of behavioral disruption[196, 197]. Interestingly, recent findings show that the psychedelics DOI and LSD induced ICSS depression in rats. Volinanserin attenuated DOI-induced ICSS depression, but this antagonistic effect via the 5-HT_{2A} receptor was not observed with LSD-induced depression[175, 198]. This indicates that classical psychedelics have low abuse potential, but can produce behavioral disruption via pathways that are not mediated via the 5-HT_{2A} receptor depending upon their chemical identities.

Post-acute effects of psychedelics in humans and rodents

While the acute effects of psychedelics are a large topic of interest, specifically in understanding changes in perception and sensory processing, the post-acute effects of psychedelics have recently become a large research focus. These compounds are currently being tested as a treatment for several psychiatric conditions including major depression[199–201] and treatment-resistant depression[23, 202, 203], generalized[204] and end-of-life anxiety[22, 205], post-traumatic stress disorder[206], smoking cessation[207, 208], and alcohol use disorder[209, 210]. Even more recently, clinical trials assessing the safety and tolerability for classical psychedelics in patients with obsessive compulsive disorder and eating disorders have been registered. Regardless of these many indications, a largely debated question in the field is whether the acute subjective experience of psychedelics is clinically relevant, and if these long-lasting positive clinical effects upon psychedelic administration can happen without the acute hallucinations.

In human studies focused on depression and anxiety related disorders, there have been promising results with robust and sustained therapeutic effects. One of the first investigated indications in the 21st century was the use of psychedelics as a form of palliative care, in which a patient would undergo psychedelic-assisted therapy to ease existential distress and anxiety associated with life-threatening cancer. Several studies using crossover randomized controlled trials have shown overall safety and medium to large preliminary efficacy for psilocybin and LSD with psychological support for patients with cancer diagnosis or life-threatening illnesses[22, 205, 211]. In the past few years, long-term follow up studies have been completed to determine the sustained effects of this treatment, including a study which assessed patients at 4.5 years following psilocybin administration[212]. The results revealed 60–80% of participants showed significant anxiolytic and antidepressant responses, and 71–100% of participants attributed positive life changes to the psilocybin-assisted therapy.

Studies with major depression and treatment-resistant depression are ongoing, with over 25 clinical trials registered or completed across phases. Most of these studies focus on

psilocybin in conjunction with some form of psychotherapy and results have been replicated across research groups. In an open label study by a group at Imperial College London, it was found that psilocybin with psychological support showed marked reductions in depressive symptoms at week 5 and was sustained at the 3- and 5-month follow ups[153, 203]. Further research from this group has compared the effects of psilocybin with a commonly prescribed antidepressant, escitalopram, in a double-blind randomized control trial[199]. Results demonstrated that psilocybin had greater reductions in depressive symptom severity when compared to escitalopram, and was sustained at the 2-, 4- and 6-week follow-ups. Another research group at Johns Hopkins University has also been investigating psilocybin and depression. Recently, a prospective 12-month follow up study was published to examine the safety and efficacy of psilocybin in patients with moderate to severe major depression[201]. The parent study was a randomized waiting-list controlled study, of which participants received two doses of psilocybin and supportive therapy[200]. Participants had decreased depressive symptoms overall at 1-, 3-, 6-, and 12-months post-psilocybin. Further, 72% of participants showed a significant treatment response while 58% showed remission.

Moreover, other studies have focused on psychedelics as a treatment for substance use disorders, including tobacco smoking cessation and alcohol use disorder. In an open-label pilot study for smoking cessation, it was found that two to three moderate doses of psilocybin in combination with cognitive behavioral therapy produced higher 6-month abstinence rates than observed with other medications or therapy alone. At the 6-month follow-up, 67% of participants were biologically confirmed as smoking abstinent, suggesting that psilocybin-assisted therapy may produce sustained effects in smoking cessation[207]. Interestingly, similar results were found with alcohol abstinence in a double-blind randomized controlled trial of psilocybin-assisted therapy[210]. Percentage of heavy drinking days and mean alcohol consumption was decreased for the psilocybin group compared to placebo control, and was sustained up to 36-weeks. Taken together, these results suggest that psilocybin may be as efficacious, if not better, for treatment of depression and other psychiatric disorders as compared to currently approved medications.

Given the promising results of the clinical studies, many efforts have been directed towards a better understanding of the mechanisms related to the potential therapeutic-related outcomes of psychedelics in rodent models of psychiatric conditions, particularly depression and anxiety[176, 213–217]. These studies are typically coupled with molecular methods to provide fundamental insights into the effects of these compounds on functional and structural neuroplasticity. Studies across a variety of classical psychedelic compounds have demonstrated that these compounds produce robust and sustained effects on neuroplasticity and behavior[147, 148, 176, 214, 215, 217–220]. Using the phenethylamine psychedelic DOI, it was demonstrated that a single administration of the compound produces fast-acting effects on cortical dendritic spine structure and acceleration of fear extinction[217]. Further, this single administration had effects on chromatin organization, particularly at the enhancer region of genes involved in synaptic plasticity that lasted days after DOI administration. Other studies have shown that single doses of psilocybin promoted dendritic growth *in vivo* and *ex vivo* for up to one-month, increased excitatory postsynaptic potential in hippocampal neurons, and was associated with accompanying positive changes in hedonic behaviors that suggested antidepressant-like activity[176, 220]. Similar effects in

measures of structural and functional plasticity have been reported following LSD and DMT administration[215, 216, 218]. Most recently, it was evidenced that psychedelics may be able to induce neuronal plasticity through BDNF-related mechanisms. Psilocybin and LSD were found to allosterically bind the TrkB receptor, allowing for greater dimerization, and therefore greater activation via BDNF binding to TrkB[221]. This research suggests a potential 5-HT_{2A} receptor-independent mechanism for plasticity following psychedelic administration, although more research is necessary to understand whether this is due to potential synergistic effects from 5-HT_{2A} receptor downstream signaling and TrkB activation.

While the focus of rodent work is widening, only few preclinical studies have focused on the effects of classical psychedelics in addiction-relevant models[184, 193, 222–225]. Current preclinical literature has mainly assessed these compounds in the context of alcohol use with two-bottle choice models of drinking in rodents[226–229]. Across the existing studies, DOI, LSD and psilocybin are evidenced to produce decreases in alcohol consumption in similar models of rodent drinking. In the study administering DOI, it was also found that a high dose of the psychedelic acutely attenuated ethanol conditioned place preference, and these effects were blocked by the 5-HT_{2A} receptor antagonist volinanserin[229]. The group assessing the effects of LSD on ethanol drinking looked at post-acute effects of LSD 46-days following initial drug administration. It was found that both doses of LSD induced decreased overall ethanol consumption, and the higher dose also produced a decrease in ethanol preference, with no changes in overall fluid intake[227, 228]. Most recently, a study evaluating psilocybin on alcohol consumption in male and female mice found a sex-dependent effect, in which male mice had decreased consumption and preference of alcohol following a single dose of psilocybin and female mice displayed no changes in ethanol consumption or preference[226]. These changes in male ethanol drinking behavior were sustained up to day 3 at the different doses. This clearly underlines the importance of additional research on sex-related effects of psychedelics using rodent behavior models associated with drug-seeking and reward.

Targets and mechanisms of post-acute effects of psychedelics

The receptor target(s) of the potential therapeutic effects of psychedelics are not fully understood. Several groups are using preclinical models and molecular tools to understand how different receptor targets are involved in the post-acute effects of psychedelics. As mentioned, there is strong consensus that the acute subjective effects of psychedelics are mediated via the 5-HT_{2A} receptor, but the question remains whether 5-HT_{2A} receptor activation is also responsible for the potential therapeutic effects seen after the drug is eliminated from the system[230–234].

Most of the evidence in both support of and against the involvement of 5-HT_{2A} receptor-dependent signaling processes includes the use of antagonists such as ketanserin and volinanserin to test their effect on psychedelic-induced dendritic spine formation – a type of structural plasticity implicated in that pathology of depression[231]. The use of non-selective antagonists (particularly ketanserin and to some extent volinanserin) together with suboptimal dosing and pretreatment times, however, may alter receptor occupancy and

influence results contributing to discrepancies in the literature. Despite this, studies utilizing gene manipulation of the 5-HT_{2A} receptor have shown changes in dendritic spines following administration of DOI[217], psilocybin[215] and 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT)[138, 215] as compared to vehicle in wildtype but not 5-HT_{2A} receptor knockout mice, providing evidence for the role of the 5-HT_{2A} receptor, at least in structural plasticity in the cortex.

Many groups also have demonstrated that psychedelics promote escape-related behaviors in the forced swim test (shown as decreased immobility and increased swimming and/or climbing), and reduce fear extinction learning or fear expression through mechanisms dependent, at least in part, on 5-HT_{2A} receptor activation[215–217, 235]. On the other side of the coin, studies have tested similar behaviors but employed different models of stress, including chronic multi-model stress and/or electric shock, before assessing the effects of psychedelics on behavior. Interestingly, these findings corroborate that psychedelics promote antidepressant-like activity, but with the discrepancy that the mechanism is 5-HT_{2A} receptor-independent[176, 220, 236]. The use of stress models could at least in part account for differences in results on the involvement of 5-HT_{2A} receptor, as the neurocircuit responsible for stress-related behavior and the effects of psychedelics overlap[237–240]. Some literature has demonstrated that 5-HT_{2A} receptor antagonists volinanserin[241] and ketanserin[242] also produce some antidepressant-like effects *in vivo*. One possible explanation for this is the rapid down-regulation of 5-HT_{2A} upon single administration of a psychedelic[132]. This down-regulation would render the receptor unavailable for further activation and thus produce alternative results in sensitive behavioral outcomes. Other challenges associated with *in vivo* pharmacology include the non-specificity of psychedelics. Many of these classical psychedelics are characterized by their unique polypharmacology that extends across serotonin receptors and interact with other monoamine receptors involved in cognition, reward and learning, which makes it challenging to pinpoint a direct, and single, receptor-mediated effect.

An alternative, yet not mutually exclusive, explanation for the molecular target underlying the therapeutic-related effects of psychedelics may be related to their structural class. For example, it was reported that 5-HT_{2A} receptor antagonists only partially reduced the effects of the tryptamine psychedelic psilocybin on marble burying, whereas this pharmacological blockade of the 5-HT_{2A} receptor completely eliminated the effects of a phenethylamine psychedelic[243]. Additionally, volinanserin was able to reduce the repressive effect of DOI (but not LSD or psilocybin) on ICSS depression in rats, whereas comparable doses of this 5-HT_{2A} receptor antagonist completely abolished HTR induced by these three psychedelics in mice[175].

Molecular targets and mechanisms of the post-acute effects may also depend greatly on the outcome measures. The serotonergic system modulates other monoaminergic systems, including dopamine-related signaling in the reward system[55, 222, 244]. 5-HT_{2A} receptor and 5-HT_{2C} receptor are both expressed in the nucleus accumbens, and are thought to have opposing roles in models of drug-taking and impulsivity[145, 245–248]. Thus, it has been reported that blocking 5-HT_{2C} receptor in this neural circuit increased impulsive responding, whereas blocking the 5-HT_{2A} receptor decreased impulsive responding, suggesting that

the 5-HT_{2C} receptor may be an alternative potential target for psychedelics' therapeutic-like effects in rodent models of substance use disorder[249]. The inhibitory 5-HT_{1A} receptor is another potential target of psychedelic action[250–252]. Early experiments in the dorsal raphe nucleus found that direct stimulation with tryptamine psychedelics but not phenethylamine psychedelics suppressed cell firing, and this was due to selective stimulation of 5-HT_{1A} receptor autoreceptors[253, 254].

An important question is the relationship between the subjective psychedelic experience and 5-HT_{2A} receptor occupancy in the human brain. Interestingly, recent findings showed that the subjective intensity (using participant-rated global intensity scores) was correlated with both 5-HT_{2A} receptor occupancy and plasma psilocin levels. This work strongly supports the notion that 5-HT_{2A} receptor activation is necessary for the subjective effects of psilocybin[255]. A similar study was conducted with LSD, assessing global and thalamic connectivity, and the subjective effects of LSD-induced states. Using the ASC scale, it was reported that LSD produced increases in self-rated aspects of consciousness (*i.e.*, blissful state, changed meaning of percepts) and increases were not seen in the placebo group or those pretreated with ketanserin. They also demonstrate a positive correlation between mean connectivity change in the somatomotor network with ACS ratings[152, 256].

When it comes to correlating the sustained post-acute effects of psychedelics with activation of the 5-HT_{2A} receptor, research is still ongoing. Correlations between the MEQ and similar scales provide some evidence that subjective experiences predict long-term therapeutic outcomes. In a double-blind study containing a large number of psychedelic-naïve participants, it was reported that at the 2-month follow-up, participants attributed greater positive changes in life and behaviors to their experiences during psilocybin and not placebo sessions[257]. Further, when assessing the MEQ scores with therapeutic potential in smoking cessation, it was found that mean MEQ scores on session days was correlated with a change in smoking craving scores at the 6-month follow-up[258]. Most recently, a bottom-up approach with machine learning analytics was used to identify potential subtypes of the psychedelic experience and how these subtypes correlate with mental health outcomes[204]. Interestingly, this study showed that experiences characterized by higher mystical experiences and psychological insights are more likely to be associated with improvements in depression and anxiety symptoms. High scoring individuals on these measures also had higher scoring on the challenging experiences scale, but still showed significantly better outcomes on many measures compared to those with low scores. Additionally, this study also reported that that those using LSD with the high scoring subtype had an increased proportion of high doses suggesting that higher doses may be able to explain differences in subjective outcomes. Considering the importance of psychedelic-induced mystical-type experiences related to persisting positive effects, several questions remain open related to the translational validity of recent preclinical work suggesting therapeutic-related effects of non-psychedelic 5-HT_{2A} receptor agonists such as lisuride[259, 260], ariadne[261] and 2-Br-LSD[139] as well as newly designed non-hallucinogenic psychedelic analogs including TBG[214], IHCH-7086[95], or (*R*)-69[241].

Conclusions and future directions

The field of psychedelic research is growing at an exponential rate, but so are the questions and insights generated on the way to those conclusions (Fig 4). Several studies have attempted to reconcile the strong expectancy and unblinding in clinical trials by utilizing possible alternative methods of controlling for these confounds[262–266]. One example is using a psychotherapy component without a psychedelic as a control for psychedelic-assisted therapy, which has shown promising results in a small study[267]. Others have used low or inactive dose controls[268, 269], and alternative drug comparators[270]. An interesting approach is the administration of psychedelics in combination with an antagonist such as ketanserin or alternatively an anesthetic to examine whether the therapeutic effects are truly dependent on the subjective experience[265, 266, 271].

Another large focus for future clinical studies is the use of more diverse populations, including those from underrepresented demographics. Most clinical work has focused largely on white male populations, not allowing for statistical evaluation of potential differences in responses across demographics. It is also important to note that while classical psychedelics have pronounced therapeutic value for some, there are participants who do not respond, or respond negatively to treatments[272, 273]. This has been reported in multiple studies and by participants themselves. Some clinical work focused on treatment-resistant depression has reported increased suicidality in some patients[202]. It is therefore necessary to delve further into the negative-responders and study their psychedelic experiences the same way clinicians do for responding participants.

Preclinical research does not come without its own caveats. While it is not possible to infer whether an animal subject is having “hallucinations” during the acute administration of these compounds, most of the current efforts are focused on the evaluation the role of 5-HT_{2A} receptor in the post-acute effects of psychedelics, with some but not all evidencing a 5-HT_{2A} receptor-dependent mechanism. One of the greatest questions of this current renaissance of psychedelics research is: Are the molecular targets responsible for the subjective effects also involved in post-acute therapeutic effects? Additionally, most animal research has been performed in the context of plasticity and antidepressant-like or anxiolytic effects, but little is known about the effects of psychedelics using rodent models of drug seeking and reinstatement.

Overall, results during the past few years indicate that psychedelics promote changes in both structural and functional plasticity, cause long-lasting alterations in gene expression, alter global connectivity through increased and decreased activation of different brain regions, and could have the potential for alleviating suffering and enhancing quality of life. This growing body of preclinical and clinical results leads us to the following question: Are psychedelics contributing to an impossible panacea, or is there a distinct neural mechanism behind these therapeutic effects? Fostering greater multidisciplinary collaboration across disciplines including basic, preclinical and clinical mental health research could present one way to solve this open question.

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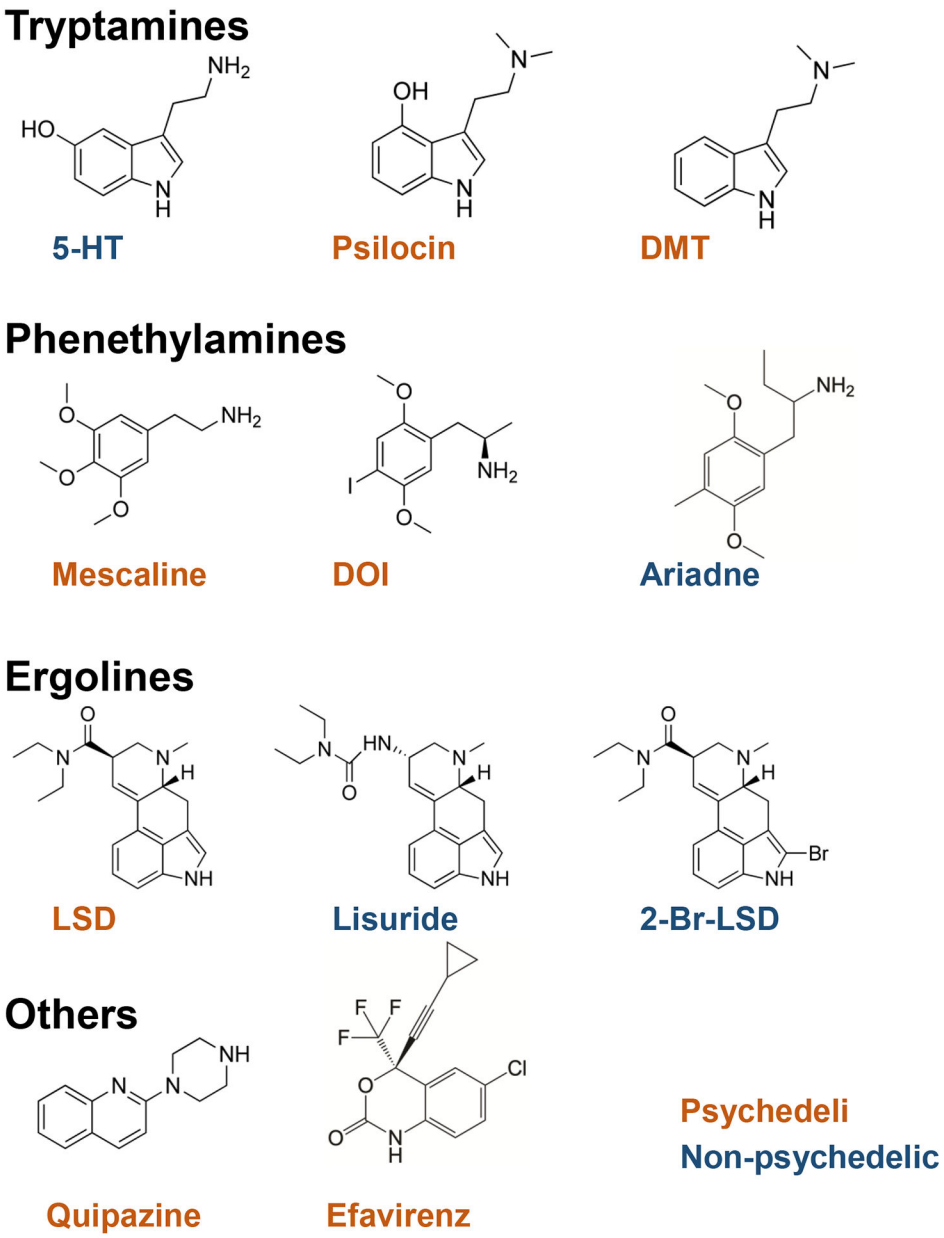

Figure 1.

Chemical structures of structurally different psychedelics and non-psychedelic 5-HT_{2A} receptor agonists.

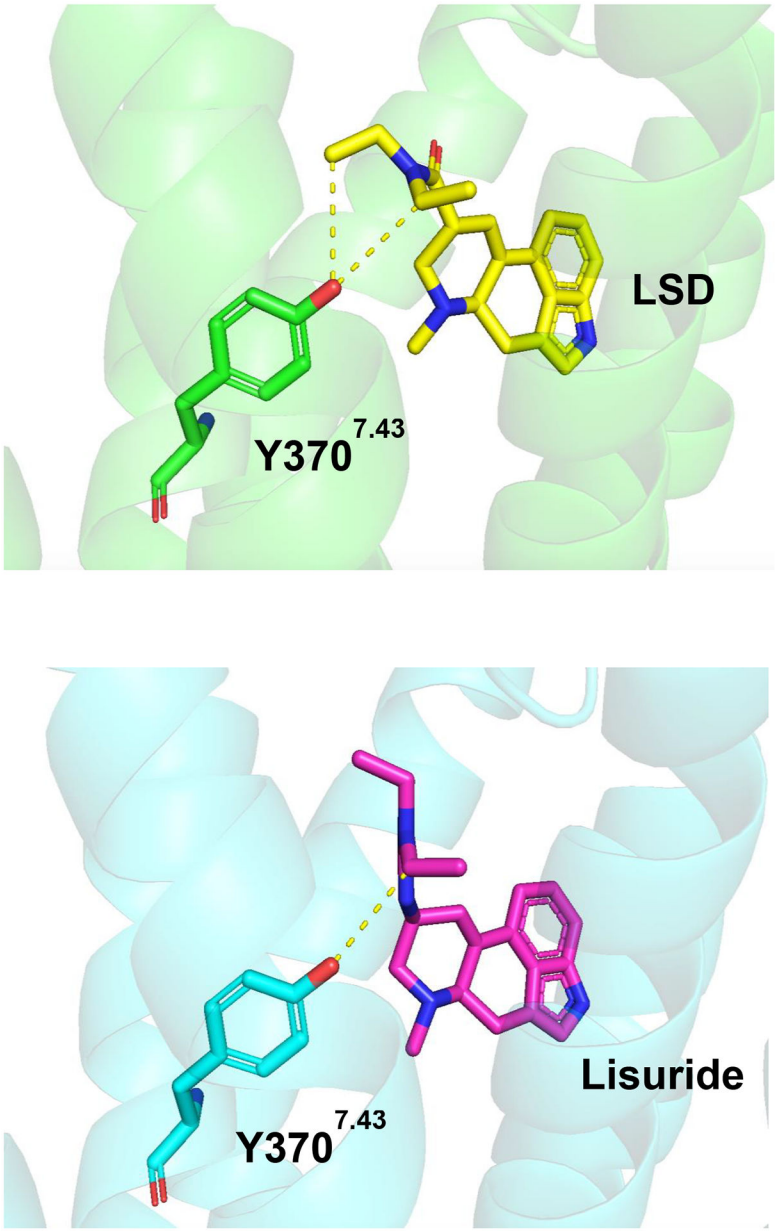

Figure 2.
Configuration of 5-HT_{2A} receptor with bound psychedelic and non-psychedelic 5-HT_{2A} receptor agonists.

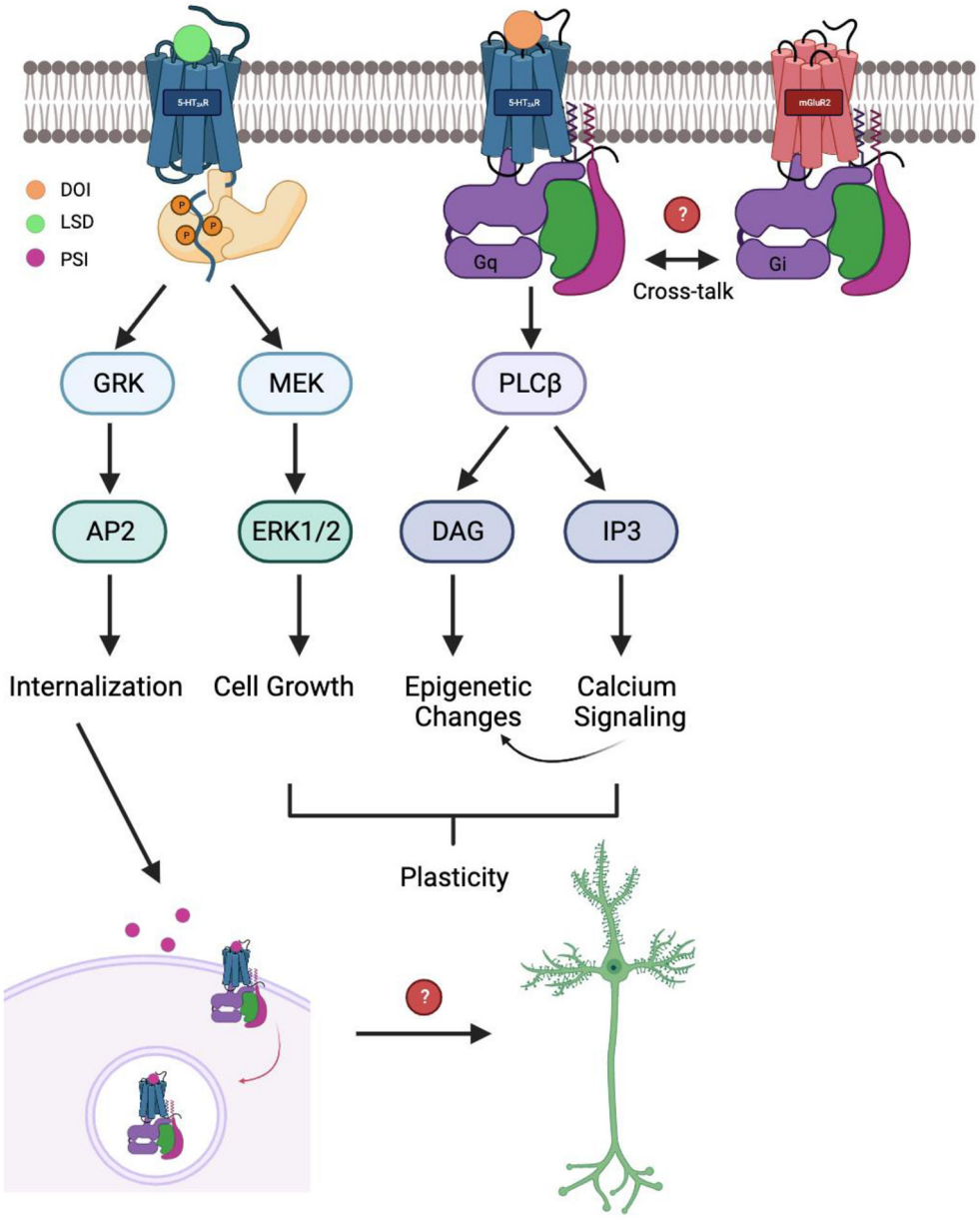

Fig 3.

5-HT_{2A} receptor signaling pathways and their downstream effects.

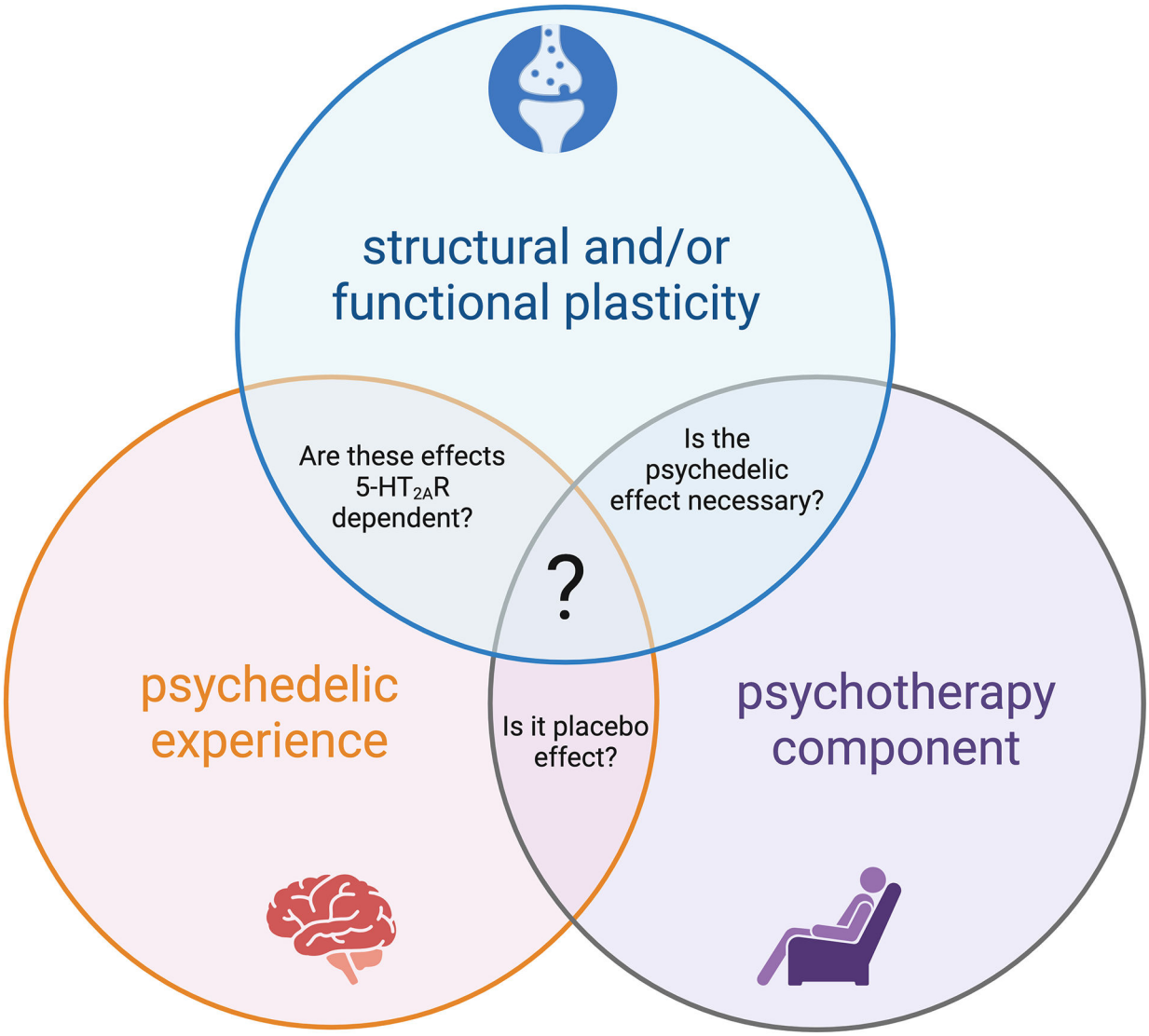

Fig 4.

Summary schematic of the mechanisms behind the potential therapeutic effects of psychedelic compounds.

Table 1.

Binding affinity of various classical psychedelics for selected serotonin and non-serotonin receptors. Values given as inhibition constant (K_i) via binding assays across studies.

	K_i values (nM)					
	Psilocin	**Psilocybin**	**LSD (racemic)**	**DOI**	**Mescaline**	**Data retrieved from:**
5-HT1A	567.4	>10,000	1.1	2,355	4,600	Halberstadt & Geyer, 2011; Chadeayne et al., 2020, Nichols et al., 2002; Roth et al., 2000; Sharp & Barnes, 2020; Barnes et al., 2021
5-HT1B	219.6	>10,000	3.9	1,261	---	Halberstadt & Geyer, 2011; Roth et al., 2000; Gonzalez-Maeso et al., 2007; Sharp & Barnes, 2020; Barnes et al., 2021
5-HT2A	107.2	>10,000	3.5	<5.0	150.0	Halberstadt & Geyer, 2011; Chadeayne et al., 2020; McKenna & Peroutka, 1989; Roth et al., 2000; Nichols et al., 2002; Sharp & Barnes, 2020; Barnes et al., 2021
5-HT2B	4.6	98.7	30.0	20.0	>10,000	Halberstadt & Geyer, 2011; Nichols et al., 2002; Chadeayne et al., 2020; McKenna & Peroutka, 1989; Roth et al., 2000; Sharp & Barnes, 2020; Barnes et al., 2021
5-HT2C	97.3	>10,000	5.5	<10.0	>10,000	Halberstadt & Geyer, 2011; Chadeayne et al., 2020; Roth et al., 2000; Nichols et al., 2002; Sharp & Barnes, 2020; Barnes et al., 2021
5-HT7	3.5	597.0	6.6	5,769	---	Nichols et al., 2002; Roth et al., 2000; Sharp & Barnes, 2020; Barnes et al., 2021
D1	>10,000	>10,000	180.0	---	---	Halberstadt & Geyer, 2011; Nichols et al., 2002; Sharp & Barnes, 2020; Barnes et al., 2021
D2	>10,000	>10,000	120.0	---	---	Halberstadt & Geyer, 2011; Nichols et al., 2002; Sharp & Barnes, 2020; Barnes et al., 2021
D3	2,645	>10,000	27.0	---	---	Halberstadt & Geyer, 2011; Nichols et al., 2002; Sharp & Barnes, 2020; Barnes et al., 2021
D4	>10,000	>10,000	56.0	---	---	Halberstadt & Geyer, 2011; Nichols et al., 2002; Sharp & Barnes, 2020; Barnes et al., 2021
H1	304.6	>10,000	1,540	---	---	Halberstadt & Geyer, 2011; Nichols et al., 2002; Sharp & Barnes, 2020; Barnes et al., 2021