Received: 2 September 2021 | Revised: 11 November 2021 | Accepted: 11 November 2021

DOI: 10.1111/jnc.15540

Journal of Neurochemistry JNC The Official Journal of the International Society for Neurochemistry WILEY

REVIEW ARTICLE

Molecular insights into psychedelic drug action

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Funding information
There are no funders to report for this Review article.

Abstract

A confluence of factors has renewed interest in the scientific understanding and translational potential of psychedelic drugs such as lysergic acid diethylamide (LSD), mescaline, and psilocybin: the desire for additional approaches to mental health care, incremental progress in basic and clinical research, and the reconsideration and relaxation of existing drug policies. With the United States Food and Drug Administration's designation of psilocybin as a "Breakthrough Therapy" for treatment-resistant depression, a new path has been forged for the conveyance of psychedelics to the clinic. Essential to the further development of such applications, however, is a clearer understanding of how these drugs exert their effects at the molecular level. Here we review the current knowledge regarding the molecular details of psychedelic drug actions and suggest that these discoveries can facilitate new insights into their hallucinogenic and therapeutic mechanisms.

KEYWORDS
psychedelics, G protein-coupled receptors (GPCR), serotonin, neurons, special issue

1 | INTRODUCTION

Psychedelic drugs comprise a group of psychoactive compounds that induce temporary physiological changes causing distortions in cognition, emotion, and perception (Nichols, 2016). While many psychedelic drugs interact with numerous receptors, their principal and defining activities are mediated by their activation of the serotonin 5-hydroxytryptamine 2A receptor (5-HT2AR) (Glennon et al., 1984; Gonzalez-Maeso et al., 2007; Keiser et al., 2009; Vollenweider et al., 1998) To date, hundreds of psychedelic compounds have been discovered and reported from which three distinct psychedelic chemotypes have classically been described: lysergamides, phenethylamines, and tryptamines, which are exemplified by LSD, mescaline, and psilocin, respectively (Figure 1) (Nichols, 2016). More recently, *N*-benzylated-*O*-methylphenethylamines (NBOMes) have been described, as typified by 2,5-dimethoxy-4-(2-((2-methoxybenzyl)amino)ethyl)benzonitrile (25CN-NBOMe) (Figure 1) (Hansen et al., 2014).

Psychedelics have historically remained restricted to traditional applications (e.g., religious ceremonies) and countercultural appreciation (Nichols, 2016). Precious few years separate the advent of their exposure to Western psychiatry in the 1950s and their wholesale ban and criminalization in the 1970s (Nichols, 2016). This included the exploration of their clinical promise for conditions such

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HT1AR, 5-hydroxytryptamine 1A receptor; 5-HT1BR, 5-hydroxytryptamine 1B receptor; 5-HT2AR, 5-hydroxytryptamine 2A receptor; 5-HT2BR, 5-hydroxytryptamine 2B receptor; 5-HT2CR, 5-hydroxytryptamine 2C receptor; 25CN-NBOH, 4-[2-[(2-hydroxyphenyl)methylamino]ethyl]-2,5-dimethoxybenzonitrile; 25CN-NBOMe, 2,5-dimethoxy-4-(2-((2-methoxybenzyl)amino)ethyl)benzonitrile; BDNF, brain-derived neurotrophic factor; CAV1, caveolin-1; ChIP-seq, chromatin immunoprecipitation sequencing; DMT, *N*,*N*-dimethyltryptamine; DOI, 2,5-Dimethoxy-4-iodoamphetamine; ECL, extracellular loop; Egr1, early growth response protein 1; Egr2, early growth response protein 2; ERK, extracellular signal-regulated kinase; GO, gene ontology; GPCR, G protein-coupled receptor; GSK-3β, glycogen synthase kinase 3β; HTR, head-twitch response; LSD, lysergic acid diethylamide; MAP1A, microtubule associated protein 1A; mRNA, messenger ribonucleic acid; mTOR, mechanistic target of rapamycin; MUPP-1, multi-PDZ domain protein 1; NBOMe, N-benzyl-O-methylphenethylamine; PAK, p21-activated kinase; PDZ, PSD-95/discs large/zonula-occludens 1; PKC, protein kinase C; PSD-95, postsynaptic density protein 95; RNA-seq, RNA-sequencing; RSK2, ribosomal S6 kinase; RT-qPCR, reverse transcription quantitative real time polymerase chain reaction; TM, transmembrane; TrkB, tropomyosin receptor kinase B; βarr1, β-arrestin1; βarr2, β-arrestin2.

This Review is part of the special issue "Psychedelics and Neurochemistry".

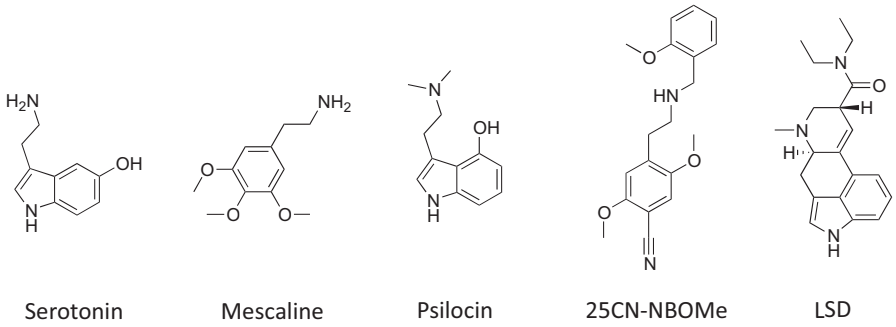

FIGURE 1 Structures of serotonin (5-hydroxytryptamine, 5-HT); the classical psychedelics mescaline, psilocin (4-hydroxydimethyltryptamine, 4-OH-DMT), and LSD (lysergic acid diethylamide); and the relatively recently described 2,5-dimethoxy-4-(2-((2-methoxybenzyl)amino)ethyl)benzonitrile (25CN-NBOMe)

as alcohol use disorder and anxiety and depressive disorders, which was essentially shelved for the better part of three decades (e.g., see (Abramson, 1967; Gasser, 2014; Gasser, 2015). The present renewal of interest in the clinical application of psychedelic drugs (Nutt et al., 2020) marks a potential shift in the tide of psychiatric practice, adding potentially novel molecules and mechanisms to the current treatments of psychiatric illnesses.

Historically, the study of psychedelic drug action is connected to the study of brain neurochemistry, and discoveries regarding the sites and effects of these drugs often have broad implications. For example, investigations of 5-HT2AR and related serotonin receptors have led to breakthroughs in the treatment of numerous neuropsychiatric conditions including depression, migraine headaches, and schizophrenia, among many other disorders (Roth et al., 1999). In the search for the mechanism of action of psychedelics, important neurological structures have been implicated. These include layer V pyramidal neurons in the prefrontal cortex (Jakab & Goldman-Rakic, 1998; Willins et al., 1997), a cell population with functional significance in affective, cognitive, and sensory processing.

The timing of increased interest in psychedelics is auspicious, as both basic scientific research into the mechanisms of psychedelics and technologies associated therewith have made tremendous advances over the past decade. Here we review what is known regarding the molecular mechanism(s) of action of psychedelics and highlight new findings and technologies. We also show how such insights could accelerate the discovery of novel psychedelic drug-like molecules with differential therapeutic actions.

2 | MOLECULAR PHARMACOLOGY

Psychedelic drugs such as LSD (Kroeze et al., 2015), *N*,*N*-dimethyltryptamine (DMT) (Keiser et al., 2009), and psilocin (the active metabolite of psilocybin) (Klein et al., 2021) have a robust pharmacology with, generally, high-affinity agonist actions at many serotonin and other biogenic amine receptors (Peng et al., 2018; Wacker et al., 2013, 2017; Wang et al., 2013) (Rickli et al., 2016). Despite their complex polypharmacologies, 5-HT2AR has emerged as the main molecular target for the hallucinogenic-like and hallucinogenic actions of psychedelics in animal models (Glennon et al., 1984; Gonzalez-Maeso et al., 2007; Keiser et al., 2009) and humans (Holze et al., 2021; Kometer et al., 2013; Preller et al., 2018; Vollenweider et al., 1998), respectively.

2.1 | Molecular biology of 5-HT2ARs

5-HT2ARs are endogenously activated by the monoamine neurotransmitter 5-hydroxytryptamine (5-HT), or serotonin, and are members of the large family of heptahelical proteins known as G protein-coupled receptors (GPCRs) (Kroeze & Roth, 1998). 5-HT2ARs were identified as integral membrane proteins in 1985 (Wouters et al., 1985) and cloned in 1988 by the late Dolan Pritchett and others (Pritchett et al., 1988), whereupon it became clear that 5-HT2ARs are GPCRs (Julius et al., 1990). Studies with cloned 5-HT2ARs expressed in heterologous systems showed equivalent pharmacological properties in terms of ligand binding profiles to receptors expressed in situ (Branchek et al., 1990; Julius et al., 1990; Teitler et al., 1990). Northern blot analysis showed high levels of 5-HT2AR messenger ribonucleic acid (mRNA) in cortex—similar to prior studies using radioligand binding experiments (see below) (Julius et al., 1990; Pritchett et al., 1988; Roth & Ciaranello, 1991; Roth et al., 1991).

The 5-HT2AR gene was characterized in 1992 (Chen et al., 1992) and demonstrated to have multiple introns and exons. Transcription start sites and promoters have also been identified for 5-HT2ARs (Shih et al., 1996) in vitro and in vivo (Toth et al., 1994). Although 5-HT2AR transcription is robustly developmentally regulated (Roth et al., 1991), there are conflicting results regarding transcriptional regulation by 5-HT2AR agonists and antagonists (Roth & Ciaranello, 1991; Roth et al., 1990; Toth, 1996; Toth & Shenk, 1994).

Sequence analysis of 5-HT2AR coding regions disclosed a high overall conservation across species (Kroeze et al., 2002; Roth et al., 1998), with the exception of non-conserved changes in transmembrane helix 5 (TM5) (Johnson, 1994; Johnson et al., 1993), most notably at residue 242, which is a serine in humans and an alanine in rodents. Several groups (Johnson et al., 1993, 1995; Johnson, 1994; Kim et al., 2020) have demonstrated that this single amino acid difference has a substantial effect on the affinity and efficacy of a variety of psychedelic and non-psychedelic 5-HT2AR agonists. Thus, the affinities and potencies of N1-substituted tryptamines and various ergolines, including LSD, may have attenuated actions at the

rodent receptor compared to the human receptor (Johnson, 1994; Johnson et al., 1993, 1995; Kim et al., 2020).

2.2 | 5-HT2AR signaling and relevance to psychedelic drug actions

5-HT2ARs have long been known to couple via the Gαq family of heterotrimeric G proteins to activate phospholipase C and induce phosphatidylinositol-4,5-bisphosphate production (Conn & Sanders-Bush, 1984; De Clerck et al., 1984; Roth et al., 1984). This activation leads to production of inositol 1,4,5-triphosphate, which causes subsequent mobilization of intracellular calcium, and diacylglycerol, which in turn activates protein kinase C (PKC) (Roth et al., 1986). Activated PKC then induces desensitization of 5-HT2ARs (Roth et al., 1986), as well as activation of a number of downstream effector pathways (Bhatnagar et al., 2004; Nakaki et al., 1985; Roth & Chuang, 1987) (Figure 2). To date, all tested psychedelic drugs are 5-HT2AR agonists and activate this pathway (Newton et al., 1996; Rabin et al., 2002). Intriguingly, many non-psychedelic drugs are also 5-HT2AR agonists, including 6-fluoro-*N*,*N*-diethyltryptamine, ergotamine, and lisuride (Newton et al., 1996; Rabin et al., 2002), while the non-psychedelic LSD analog 2-bromo-LSD does not appreciably activate rodent 5-HT2ARs (Rabin et al., 2002). in vivo genetic deletion of one copy of Gαq attenuates the psychedelic drug-induced head-twitch response (HTR) and abolishes psychedelic drug-induced c-Fos expression (Garcia et al., 2007).

Psychedelic and non-psychedelic 5-HT2AR agonists can also activate other downstream signaling pathways, including arachidonic acid release (Berg et al., 1998; Berg, Maayani, Goldfarb, Scaramellini, et al., 1998; Felder et al., 1990; Kurrasch-Orbaugh et al., 2003; Parrish & Nichols, 2006) simultaneously with phosphatidylinositol hydrolysis (Berg, Maayani, Goldfarb, & Clarke, 1998; Berg, Maayani, Goldfarb, Scaramellini, et al., 1998; Felder et al., 1990), albeit with differential abilities of psychedelic and non-psychedelic agonists to activate each pathway. Additional downstream effector pathways include the activation of extracellular signal-regulated kinases (ERKs) via phosphorylation (Florian & Watts, 1998), transforming growth factor beta release (Grewal et al., 1999), ribosomal S6 kinase (RSK2) activation (Strachan et al., 2008), arrestin translocation (Gray et al., 2003; Schmid & Bohn, 2010; Schmid et al., 2008; Wacker et al., 2013), calmodulin binding (Turner & Raymond, 2005), and p21-activated kinase activation (Jones et al., 2009). Quantitative phosphoproteomic studies have identified a large number of proteins downstream of 5-HT2ARs that are phosphorylated after 5-HT2AR activation (Karaki et al., 2014). Significantly, differential phosphorylation signatures were observed comparing psychedelic and non-psychedelic 5-HT2AR agonists (Banerjee & Vaidya, 2020), along with differential 5-HT2AR phosphorylation patterns (Karaki et al., 2014).

Although most studies have indicated that 5-HT2ARs mainly couple to pertussis-toxin insensitive Gαq-like transducers (Garcia et al., 2007; Garnovskaya et al., 1995; Goppelt-Struebe & Stroebel, 1998; Kim et al., 2020; Saucier & Albert, 1997) (Pottie et al., 2020), there have been reports that psychedelic compounds differentially couple 5-HT2ARs to Gαi/o signaling in brain (Garcia-Bea et al., 2019; Gonzalez-Maeso et al., 2007). However, a thorough evaluation of 5-HT2AR coupling in human embryonic kidney 293T cells using a G protein dissociation-based bioluminescence resonance energy transfer-based platform (Olsen et al., 2020) found that this receptor couples nearly exclusively to members of the Gαq family, with some activity at the pertussis toxin-insensitive Gαi/o family member, Gαz (Kim et al., 2020).

5-HT2ARs interact with a number of scaffolding proteins including postsynaptic density protein 95 (PSD-95) and other PSD-95/discs large/zonula-occludens 1 (PDZ)-domain-containing proteins (Becamel et al., 2004; Xia, Gray, et al., 2003; Xia, Hufeisen, et al., 2003), caveolin (Bhatnagar et al., 2004), microtubule-associated

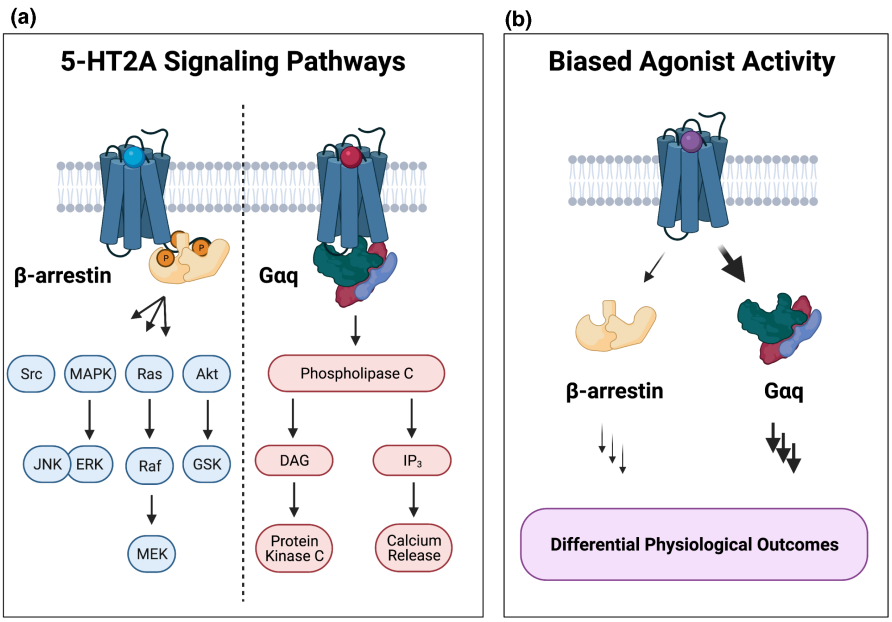

FIGURE 2 (a) Schematic of intracellular signaling pathways coupled to 5-hydroxytryptamine 2A receptor (5-HT2AR) activation and their downstream effectors. (b) Schematic of activity of a Gαq-biased agonist of 5-HT2AR

protein 1A (MAP1A) (Cornea-Hebert et al., 2002; Gelber et al., 1999), RSK2 (Sheffler et al., 2006), viral proteins (Elphick et al., 2004) and other miscellaneous synaptic proteins (Jones et al., 2009). The interactions of 5-HT2ARs with PSD-95 (Abbas et al., 2009), caveolin-1 (CAV1) (Allen et al., 2011; Bhatnagar et al., 2004), RSK2 (Strachan et al., 2010) and β-arrestin2 (Rodriguez et al., 2021) are essential for a variety of in vitro and in vivo actions of prototypical psychedelics like 2,5-dimethoxy-4-iodoamphetamine (DOI) and LSD. Taken together these studies provide a potential molecular model of psychedelic drug actions at 5-HT2ARs whereby the interactions of a variety of scaffolding proteins tether 5-HT2ARs to distinct neuronal subdomains, at least some of which are essential for the expression of psychedelic drug actions in vivo (Figure 3).

2.3 | 5-HT2AR expression and localization

Initial studies on 5-HT2ARs based on radioligand binding studies with non-selective 5-HT2AR antagonist radioligands (e.g., ^{3}H-ketanserin, ^{3}H-spiperone) revealed enrichment in both rodent (Leysen et al., 1978) and human cortex. Subcellular fractionation studies showed that putative 5-HT2 receptors (now known as 5-HT2ARs; see (Berger et al., 2009)) are enriched in synaptic membranes as well as intracellular microsomal-like compartments (Laduron et al., 1983). With the advent of receptor autoradiography, many studies showed that 5-HT2ARs are enriched in cortical layer V in many species (Pazos et al., 1985, 1987; Roth et al., 1987). Intriguingly, ^{3}H-ketanserin labels both 5-HT2ARs in cortex as well as tetrabenazine-sensitive sites in brain regions enriched in the vesicular monoamine transporter 2 (Leysen et al., 1987; Roth et al., 1987). In situ hybridization studies showed similar distributions of 5-HT2AR, as assessed by receptor autoradiography and mRNA distribution (Mengood et al., 1990).

Immunofluorescent and immunohistochemical studies with 5-HT2AR-specific antibodies have demonstrated that 5-HT2ARs are highly localized to cortical layer V pyramidal neurons in rodents (Cornea-Hebert et al., 1999; Willins et al., 1997) and non-human primates (Jakab & Goldman-Rakic, 1998). Targeting of 5-HT2ARs to apical dendrites and various postsynaptic regions is dependent upon interactions with the scaffolding proteins PSD-95 (Xia, Hufeisen, et al., 2003) (Abbas et al., 2009), multi-PDZ domain protein 1 (MUPP-1) (Jones et al., 2009), and MAP1A (Cornea-Hebert et al., 2002). Surprisingly, a large proportion of 5-HT2AR-like immunoreactivity is intracellularly localized in apical dendrites (Cornea-Hebert et al., 1999, 2002), which appears to depend at least to some extent upon interactions with MAP1A.

2.4 | Biochemical actions of psychedelics in vivo

Not surprisingly, psychedelic drugs via actions at 5-HT2ARs induce a variety of biochemical and cellular sequelae. In terms of electrophysiological effects, 5-HT2ARs in cortical layer V pyramidal neurons induce depolarization and enhanced excitability (Aghajanian & Marek, 1997), presumably via PKC activation. In interneurons of the rat piriform cortex, PKC activation was shown to blunt 5-HT-induced excitation, perhaps because of differences in how this kinase is utilized between different cell types (Marek & Aghajanian, 1995). Psychedelic drugs also induce expression of many RNA transcripts including the immediate early genes c-Fos (Abbas et al., 2009; Nichols & Sanders-Bush, 2002), early growth response protein 1 and 2 (Egr1/2) (Abbas et al., 2009; Gonzalez-Maeso et al., 2007), brain-derived neurotrophic factor (BDNF) (Vaidya et al., 1997), and several others (Martin & Nichols, 2016; Nichols et al., 2003). Studies by Gonzalez-Maeso and colleagues suggested that the Egr1/2 responses are specific to psychedelic drugs while both psychedelics and non-psychedelics can induce c-Fos (Gonzalez-Maeso et al., 2007).

More recently, two studies using RNA-sequencing (RNA-seq), which enables an unbiased assessment of the whole transcriptome, were reported. In a study in mice treated with a single administration of DOI, de la Fuente et al. (2021) used RNA-seq and chromatin immunoprecipitation-sequencing (ChIP-seq) to examine the effects of this drug 24, 48 h, or 7 days post-administration. The authors report a number of genes that were regulated by DOI and used weighted correlation network analysis to detect co-expression modules of genes. Gene ontology (GO) analysis found modules involved in synapse organization, synaptic plasticity, and *N*-methyl-D-aspartate receptor activity regulation, among other areas. Additionally, these authors (de la Fuente et al., 2021) used ChIP-seq to probe acetylation of histone H3 lysine 27 residues. The authors utilized data from these experiments for K-clustering of enhancers, including clusters showing enhancers with increased and decreased acetylation signals. GO analysis conducted on these enhancers showed overlap with RNA-seq terms, including in areas of synaptic alterations. The authors (de la Fuente et al., 2021) concluded, therefore, that the persisting effects of DOI, and perhaps those of other psychedelics, may be because of these epigenetic phenomena.

In another paper, (Raval et al., 2021) administered a single dose of psilocybin to pigs, a higher species with a comparatively larger frontal cortex than rodents. The authors examined the transcriptional effects of drug administration on animals either one day or three weeks post-treatment. In contrast to (de la Fuente et al., 2021) the authors found that only a modest number of genes—19 after 1 day, and 3 after 1 week—were transcriptionally regulated by psilocybin treatment, and that a considerable subset of these genes are involved in the immune response. However, these authors were unable to confirm their RNA-seq for the immune-related genes using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). The authors mention several considerations (e.g., the chosen timepoints) as potential caveats to their study, as mouse experiments with shorter timepoints showed that many transcripts peaked in their increased levels around 90 min post-treatment. Additional considerations, such as species differences, particularly in frontal cortical areas, cannot be ruled out.

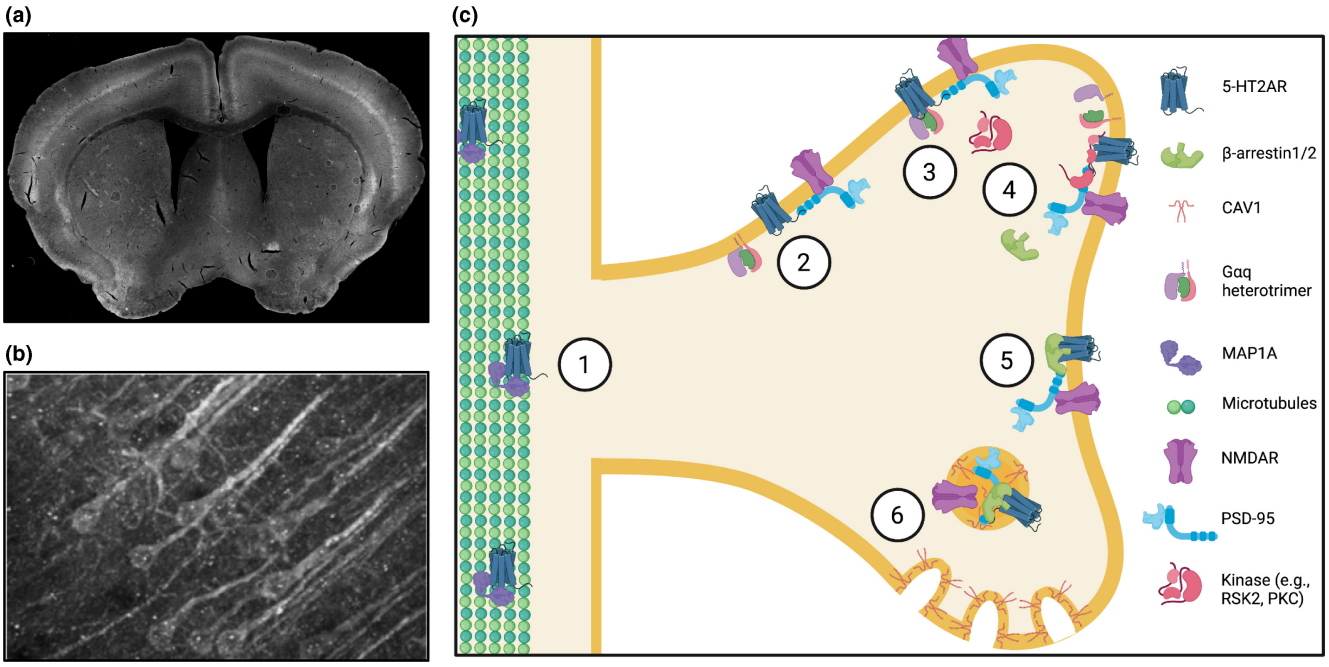

FIGURE 3 Immunohistochemical staining of 5-HT2ARs shows dense receptor expression in pyramidal cells of cortical layer V (a), having a somatodendritic localization pattern leading to receptor expression both in this and the more superficial layers into which pyramidal cell apical dendrites extend (b). Along dendrites, 5-HT2ARs are found in spines, where they interact with a number of proteins, a small subset of which are shown in (c). These interactions include those with MAP1A, which is involved in receptor trafficking along microtubules within the dendritic shaft (1), PSD-95, which is also necessary for receptor trafficking and proper localization, as well as clustering 5-HT2ARs with other postsynaptic proteins (e.g., NMDARs) (2), heterotrimeric G proteins, whose activation has numerous downstream cellular effects (3), kinases, which phosphorylate the receptor to regulate its activity both tonically and in response to agonist-induced stimulation (4), and β-arrestins, which have roles in receptor desensitization, internalization, and G protein-independent signaling (5). Interactions with CAV1 (6) suggest that this receptor is located within caveolae of the plasma membrane. Relevant abbreviations: 5-HT2AR, 5-hydroxytryptamine receptor 2A; CAV1, caveolin-1; MAP1A, microtubule associated protein 1A; NMDAR, *N*-methyl-D-aspartate receptor; PSD-95, postsynaptic density protein 95, RSK2, ribosomal S-6 kinase 2; PKC, protein kinase C

Psychedelic drugs also induce ERK and glycogen synthase kinase 3 beta (GSK-3β) phosphorylation (Bohn & Schmid, 2010; Schmid et al., 2008). These effects of psychedelics on pERK and GSK-3β phosphorylation are attenuated in PSD95 knockout mice (Abbas et al., 2009) and are differentially modulated in βArr2 knockout mice (Bohn & Schmid, 2010; Schmid et al., 2008). CAV1 deletion also attenuates the actions of psychedelics on c-Fos, Egr1, and calcium mobilization in cortical neurons (Allen, Yadav, et al., 2011). Taken together these findings indicate that psychedelic drugs can differentially modulate signaling and gene transcription and that these actions require intact scaffolding to synapses in vitro and in vivo.

2.5 | Psychedelics, spine formation, and synaptic plasticity

There has been a resurgence of interest in the effects of psychedelics on dendritic spine morphology. In large part, this is because of the frequent observation that conventional antidepressant drugs, including monoamine oxidase inhibitors and selective serotonin reuptake inhibitors, alter dendritic spine morphology following both acute and chronic administration (Benes & Vincent, 1991) (Benuskova, 1991) (Bal-Klara & Bird, 1990; Norrholm & Ouimet, 2001) (Hajszan et al., 2005). Ketamine, a hallucinogenic dissociative with rapid-acting antidepressant effects, similarly induces spine alterations following acute administration (Li et al., 2010). In an elegant study, it was found that elimination of ketamine-induced synaptic changes by use of a membrane-bound photoactivable Rac1 blocks its long-term but not immediate antidepressant effects in mice (Moda-Sava et al., 2019).

Dendritic spine remodeling by a psychedelic drug was first observed in vitro by Jones and colleagues in 2009 (Jones et al., 2009). In that study, the authors found that treatment of cultured rat primary cortical neurons with DOI (1 μM) transiently increased spine morphological parameters, including area, breadth, and length, after 30 min. By 60 min, however, these parameters had returned to their baseline levels. Additional experimentation revealed that DOI increased phosphorylated p21-activated kinase (PAK), a substrate of the Rho guanine-nucleotide exchange factor Kalirin-7, in a concentration-dependent manner. Kalirin-7 has a known role in synaptic remodeling; thus, Jones et al. used a peptide mimic of the Kalirin-7 C-terminus to inhibit its interactions with PDZ scaffold proteins that localize it to membranes, and in doing so showed that both DOI-induced PAK phosphorylation and spine remodeling were blocked. Subsequently, several other studies have demonstrated

that 5-HT2AR activation can enhance spine formation (Mi et al., 2017) (Ohtani et al., 2014) (Yoshida et al., 2011).

More recently, it was shown that psychedelics across chemical classes alter spine morphology in vitro. In a study by Ly et al. (2018) cultured rat primary cortical neurons were treated with the lysergamide LSD (10 μM), phenethylamine DOI (10 μM), or tryptamines DMT (90 μM) or psilocin (10 μM) for 24 h. The authors then performed Sholl analysis (Sholl, 1953) to examine dendritic arborization, finding that all of these drugs increased arbor complexity, including dendritic branch length, crossings, and number, an effect that could be blocked by a high concentration of the 5-HT2AR/CR antagonist ketanserin (100 μM). As BDNF has been implicated in ketamine-induced dendritic changes, the authors examined BDNF transcript and protein levels following neuron treatment with psychedelics, finding that though BDNF transcript levels were unchanged, its protein expression had increased. Given these findings, the authors showed that both antagonism of the BDNF receptor tropomyosin receptor kinase B (TrkB) by the small molecule ANA-12 or inhibition of its downstream effector mechanistic target of rapamycin (mTOR) by the macrolide rapamycin prevented psychedelic-induced dendritic alterations. In a follow-up study, Ly and colleagues (Ly et al., 2018) demonstrated that transient treatment of neurons with LSD for as little as 30 min resulted in increased dendritic complexity measured 72 h later, and that the extent of this increase was time-dependent. Again, the authors showed that TrkB antagonism and mTOR inhibition prevented psychedelic-induced dendritic growth, as well as demonstrating a dependence on α-amino-3-hydroxy-5-methyl-4-isoxasolepropionic acid-type glutamate receptor stimulation via its blockade with 6,7-dinitroquinoxaline-2,3-dione.

The occurrence of psychedelic-induced spine remodeling was first demonstrated in vivo by Shao et al. in mice using two-photon microscopy (DiBerto & Roth, 2021; Shao et al., 2021). Here, the authors demonstrated that mice treated with psilocybin (1 mg/kg) displayed an increased HTR and ameliorated escape avoidance behavior in a learned helplessness paradigm, indicating that this dose produced hallucinogenic-like and stress-coping behaviors. In *Thy1GFP* mice implanted with cranial windows above their cingulate/premotor cortex, the authors chronically monitored spine dynamics in layer V cortical neuron apical dendrites over the course of about one month. They found that a single administration of psilocybin modestly but significantly increased spine parameters, including head width and protrusion length, and that about half of these changes remained stable for one week and about one-third remained stable for one month. Interestingly, examination of cortical regions by confocal fluorescent microscopy from mice killed 24 h after psilocybin treatment revealed differing levels of dendritic remodeling. Perhaps this should not be surprising, given that morphologically similar neurons are phenotypically distinct. While not directly examined, these same authors have proposed that co-expression ratios of 5-HT2ARs and 5-HT1ARs, to which lysergamide and tryptamine drugs bind and activate, may determine the extent of spine remodeling by buffering the excitatory response to psychedelic actions elicited through 5-HT2AR activation (Araneda & Andrade, 1991; Savalia et al., 2021). Interestingly, in this study the authors found that ketanserin pre-administration failed to completely block psilocybin-induced morphological changes, suggesting that this may be facilitated by a non-5-HT2AR mechanism, though other factors, such as poor brain bioavailability of this drug when administered systemically, cannot be ruled out (DiBerto & Roth, 2021).

3 | RECEPTOR STRUCTURE AND SIGNALING

Direct study of the receptors activated by psychedelic drugs has accelerated in recent years. These efforts have brought the chemical diversity of known psychedelic compounds to bear and opened new avenues in this area of study. Additionally, receptor-centric models in quantitative pharmacology including the Black-Leff operational model (Black & Leff, 1983) and the extended ternary complex model (Samama et al., 1993) have been applied to great effect in contextualizing the dynamics of psychedelic agonist activity and 5-HT2AR signaling. To this end, atomic-resolution reconstructions of complexes formed by receptors, drugs, and intracellular signaling machinery have been invaluable. In this section, we describe recent advancements in the molecular study of serotonin receptors and their context in psychedelic science.

3.1 | Receptor structure

The first 5-HT2 family receptor structure to be solved was 5-HT2BR in complex with the promiscuous agonist ergotamine (Wacker et al., 2013) (Wang et al., 2013), which was followed by a 5-HT2BR complex with β-arrestin biased LSD (Wacker et al., 2017). More recently, inactive state structures of the 5-HT2CR (Peng et al., 2018) and 5-HT2AR (Kim et al., 2020; Kimura et al., 2019) have been published, along with the Gαq-coupled active state structure of 5-HT2AR (Kim et al., 2020). While no structures of 5-HT2ARs with its endogenous ligand exist, the serotonin-like indole moiety found in LSD and ergotamine provides a potential indication of the binding mode of serotonin. Residue numbering in this section utilizes the Ballesteros-Weinstein numbering (Ballesteros & Weinstein, 1995). Key interactions with the orthosteric binding pocket include a salt bridge between N7 of LSD and D135(3.32) (5-HT2BR numbering), a residue that is conserved among 5-HT2 family GPCRs, as well as numerous biogenic amine receptors. Nonpolar contacts of the ergoline of LSD include F340(6.51) and F341(6.52), as well as F217(5.38) and V136(3.33). An extended binding pocket (also described in structures of 5-HT1BR and 5-HT2BR) is occupied by the diethylamide moiety of LSD, contacting residues W131(3.28), L132(3.29), L362(7.35), and V366(7.39) (Wacker 2013, Wang 2013). While the indole moieties of LSD and ergotamine are offset by approximately 1.9 Å (a 16° rotation about the interaction with D135(3.32)), their similarities in DRY, NPxxY, and partial activation of PIF motifs are reflective of their bias for β-arrestin signaling over Gαq engagement.

These structures provided the first indications of how psychedelic ligands interact with 5-HT2 family GPCRs. This includes a structural rationale for the exceptionally slow dissociation kinetics of LSD with 5-HT2AR and 5-HT2BR: a unique ligand pocket "lid" formed by extracellular loop 2 (ECL2). Rationally designed mutations based on these structures perturbed the signaling bias of LSD, selectively inhibiting the recruitment of β-arrestin2 to 5-HT2BR (Wacker et al., 2017). While 5-HT2BR is closely related to 5-HT2AR and is indeed a potent interactor of psychedelic drugs, it is not essential to their hallucinogenic activity.

Structures of 5-HT2CR in complex with ritanserin (an inverse agonist of 5-HT2AR, 5-HT2CR, and several other biogenic amine receptors) and ergotamine followed (Peng, 2018). The ergotamine complex of 5-HT2CR reaffirmed the conclusions drawn from the ergotamine-bound 5-HT2BR structure, demonstrating several commonalities in their agonist-bound states. Key residues in the orthosteric binding pocket of 5-HT2BR that interact with ergotamine are conserved in 5-HT2CR, including D134(3.32), V135(3.33), F327(6.51), and F328(6.52) (5-HT2CR numbering). The extended binding pockets of 5-HT2BR and 5-HT2CR in complex with ergotamine diverge somewhat from the 5-HT2BR-LSD structure in order to accommodate the cyclic dipeptide moiety. However, their activation hallmarks are generally consistent among the structures with the exception of 5-HT2CR-ergotamine R152(3.50) of the DRY motif, which is fully extended.

Comparison of the ritanserin- and ergotamine-bound states of 5-HT2CR is enlightening, as this comparison is representative of the various conformations adopted by the receptor during ligand binding and activation. Most dramatic of these changes are the 6.6 Å and 7 Å shifts of helices III and VI, respectively, between the ritanserin and ergotamine complexes. Smaller but equivalently significant rotational differences can also be appreciated in the PIF and DRY motifs. In these 5-HT2CR structures, a translation of 1.7 Å can be observed in the bellwether of activation, the W324(6.48) toggle switch (Figure 4). The positions of these residues in relation to the deep occupancy of ritanserin appear to be critical to the functional effects of the drug. Specifically, interactions between ritanserin and I142(3.40), F320(6.44), and W324(6.48) appear to occlude the ability of these residues to undergo the shifts that would accompany receptor activation. Interestingly, mutation of W324(6.48) to tyrosine abrogates inverse agonism of Gαq signaling while retaining that of β-arrestin, and mutation of I142(3.40) to tyrosine abrogates inverse agonism of β-arrestin signaling and reverses the Gαq signaling into agonism. Taken together, these mutations have important implications for the structural mechanisms of psychedelic drug signaling.

X-ray crystal structures of 5-HT2AR in complex with zotepine and risperidone are conclusively representative of receptor inactive states, and strongly suggest parallels between 5-HT2CR and 5-HT2AR inactive state structures (Kimura, 2019). The results generally accord with prior mutagenesis and molecular modeling studies of 5-HT2ARs (Choudhary et al., 1993) (Choudhary et al., 1995) (Roth et al., 1996) (Roth et al., 1997) (Roth et al., 1997; Westkaemper et al., 1999) (Shapiro et al., 2002) (Shapiro et al., 2000) (Kristiansen et al., 2000). Both compounds are inverse agonists of 5-HT2AR with modest receptor selectivity. These two structures of 5-HT2AR bear great similarity to one another, with their most obvious differences being restricted to the extended binding pocket of their ligands. Indeed, the DRY, NPxxY, and sodium site D120(2.50) motifs are consistent, with the largest shift among the side chains of these motifs being 0.9 Å for N376(7.49) (5-HT2AR numbering). In the orthosteric pocket, interactions with the ligands differ somewhat. The positions of toggle switch W336(6.48) are consistent between the structures, and edge-to-face interactions of this residue exist between the non-chlorinated hemisphere of the dibenzotheipine of zotepine and the benzoisoxazole of risperidone. The chlorinated hemisphere of zotepine extends toward TM5 and the backbone of G238(5.42), while the nucleobase-reminiscent hemisphere of risperidone extends into a pocket formed by TM2, TM7, and the ECL2 lid.

The majority of these similarities exist when comparing the structures of 5-HT2CR-ritanserin to risperidone- or zotepine-bound

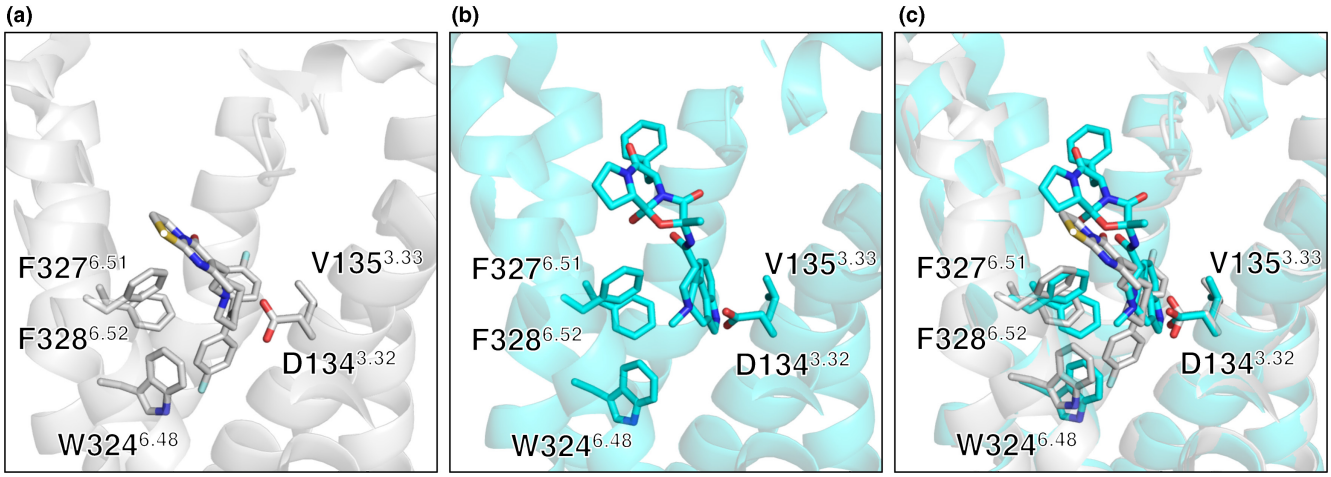

FIGURE 4 Orthosteric binding site of 5-hydroxytryptamine receptor 2C in complex with ritanserin (a), ergotamine (b), and an overlay of the two structures (c)

5-HT2AR. Interestingly, both of the divergent sections of the orthosteric pocket occupied by zotepine and risperidone are also occupied by ritanserin in the 5-HT2CR structure. Similarities also exist in the NPxxY motif and D125(2.50) sodium site of 5-HT2CR. The most notable contrast in activation moieties of 5-HT2CR is a shift of the DRY motif and toggle switch W336(6.48), perhaps attributable to shifts in TM5 and TM6 induced by the conformational restriction of the two fluorobenzene groups of ritanserin. Taken together, the similarities in these structures validate the conclusions drawn from comparison of the active- and inactive-state structures of 5-HT2CR in complex with ergotamine and ritanserin and is suggestive of the binding mode and mechanism of action of β-arrestin-biased psychedelic drugs at 5-HT2 family receptors.

3.2 | Molecular insights into receptor-effector coupling

Another facet of receptor activity is the ability of receptors to couple to multiple intracellular signaling pathways. Conformational differences in receptor-drug complexes are linked to preferential activation of specific signaling pathways, such as Gαq being activated while β-arrestin signaling remains silent. Termed "biased signaling," this has been shown to have a potentially clinically significant impact on the effects induced by GPCR ligands (Che et al., 2021; Che & Roth, 2021; Urban et al., 2007) (Allen et al., 2011) (DeWire & Violin, 2011). Improvements in methodology in structural biology have had an extraordinary impact on understanding the molecular basis of these signaling biases (Che, Dwivedi-Agnihotri, et al., 2021; Che, Roth, et al., 2021; Urban et al., 2007) (Manglik et al., 2016) (Zhuang et al., 2021) (Varadi et al., 2016). The ability to resolve atomic-resolution interactions of activate-state receptors has enabled mechanistic predictions and the identification of residues that are key to signal transduction to particular downstream effectors.

Two structures of 5-HT2AR with LSD or 25CN-NBOH (Kim et al., 2020) cast a brighter light onto the molecular interactions of psychedelics and GPCRs and revealed a novel snapshot of how 5-HT2AR interacts with Gαq. Among psychedelic drugs, 25CN-NBOH is a relatively recently discovered member of a series of compounds related to the NBOMes that have grown in illicit synthesis and usage, despite concerns of toxicity (Poulie et al., 2019). 25CN-NBOH has a high degree of selectivity for 5-HT2AR in vivo and in vitro (Jensen et al., 2017). With recent developments suggesting that β-arrestin recruitment is essential to psychedelic hallucinations, it is an ideal compound for the study of psychedelic drug action (Rodriguiz et al., 2021).

Despite their similarities in potency as psychedelic drugs, comparison of the structures of 5-HT2AR in complex with LSD and 25CN-NBOH reveal important differences in the orthosteric binding pocket and activation motifs (Figure 5). Foremost among these differences is the translocation of the toggle switch W336(6.48) (Kim et al., 2020). In 5-HT2AR-LSD, the toggle switch (and indeed, the majority of the ligand-binding pocket) conformation agrees with that of 5-HT2BR-LSD. Whereas in 5-HT2AR-25CN-NBOH the toggle switch is displaced by the N-benzyl moiety of the ligand, shifting and rotating the sidechain ~80° and an average of 5 Å. This translocation is particularly striking in comparison to the inactive state structures of 5-HT2AR, wherein the space occupied by W336(6.48) has been essentially replaced by the N-benzyl moiety of 25CN-NBOH. In both structures, D155(3.32) and S159(3.36) appear to participate in anchoring an amine of the ligands. However, mutation of these residues reveals that while D155(3.32) is essential to the binding of both drugs, S159(3.36) is far more important for the binding of 25CN-NBOH than LSD (Kim et al., 2020).

These ligand-binding pocket differences play mechanistic roles revealed in the structure of 5-HT2AR with G protein trimer. The indole moiety of LSD overlays the parathoxybenzonitrile of 25CN-NBOH, and forms similarly extensive but electronically distinct interactions (Kim et al., 2020). The added bulk of the nitrile group results in an outward shift of TM5, which propagates down the helix and contributes to an altered conformation of the DRY motif. The DRY motif of 5-HT2AR-25CN-NBOH also participates in the interaction of the receptor with Gαq, and comparisons to 5-HT2AR inactive state structures show clear differences (Figure 6). Rotations of R173(3.50) of the DRY motif and Y380(7.58) of the NPxxY motif into the center of the helical bundle form the cap of the pocket into which the C-terminal helix of Gαq is inserted. A shift of D172(3.49) brings the acidic side chain within 3 Å of Y243(H5.23) of Gαq (Figure 7) (Kim et al., 2020). Both LSD and 25CN-NBOH are β-arrestin biased agonists of 5-HT2AR, but these subtle differences in structure likely play a role in the extent of their bias.

The understanding of the balance among signaling pathways accessible to a receptor has grown a great deal since the early indications of receptors coupling multiple signaling pathways (Roth & Chuang, 1987). The widespread appreciation of the Ternary Complex and Biased Signaling Models (De Lean et al., 1980) (Samama et al., 1993) (Roth, 2016) have cultivated the desire to build molecules with highest affinity for receptor states wherein selected signaling pathways are prioritized or avoided. This has turned out to be a difficult task, though worthwhile in well-validated systems, such as those described in the introduction to this section. In second messenger assays such as phosphatidylinositol hydrolysis and arachidonic acid release, large discrepancies have been observed between 5-HT2AR binding affinity, rat drug discrimination, and human doses (Luethi & Liechti, 2018; Nichols David, 2004). These may be because of as-yet unquantified differences in efficacy and signaling bias of each drug and indicate that these specific second messenger assays are not reliable indicators of psychedelic activity.

3.3 | Receptor selectivity

The coupling of receptors to intracellular signaling components is central to their function, and a delicate balance is struck upon drug

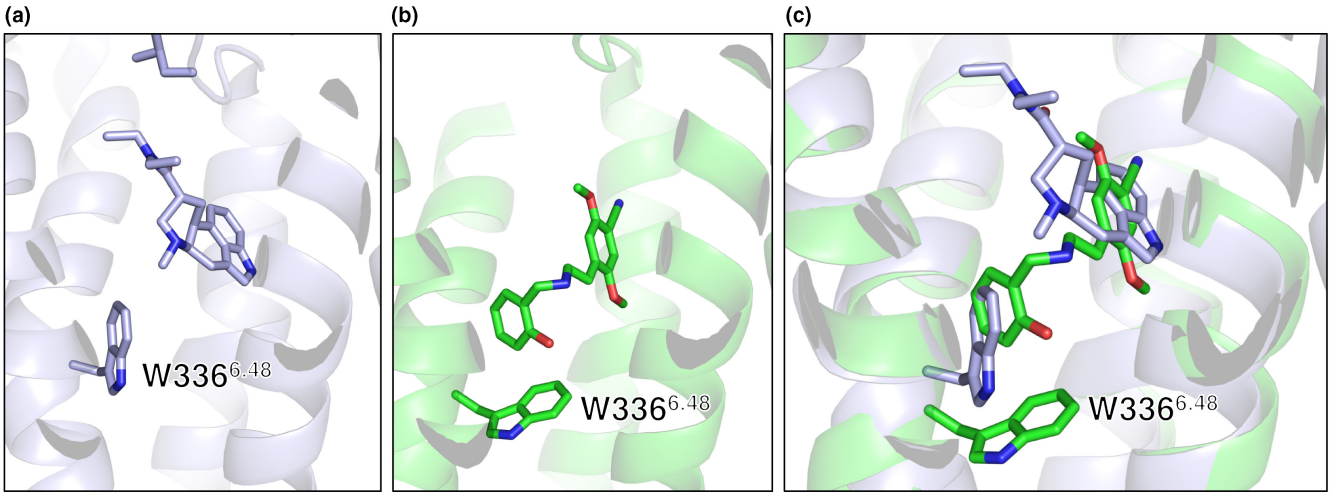

FIGURE 5 Orthosteric binding site of 5-hydroxytryptamine receptor 2A in complex with (a) LSD, (b) 4-[2-[(2-hydroxyphenyl)methylamino] ethyl]-2,5-dimethoxybenzonitrile (25CN-NBOH), and (c) an overlay of the two structures illustrating the position of the W336(6.48) toggle switch

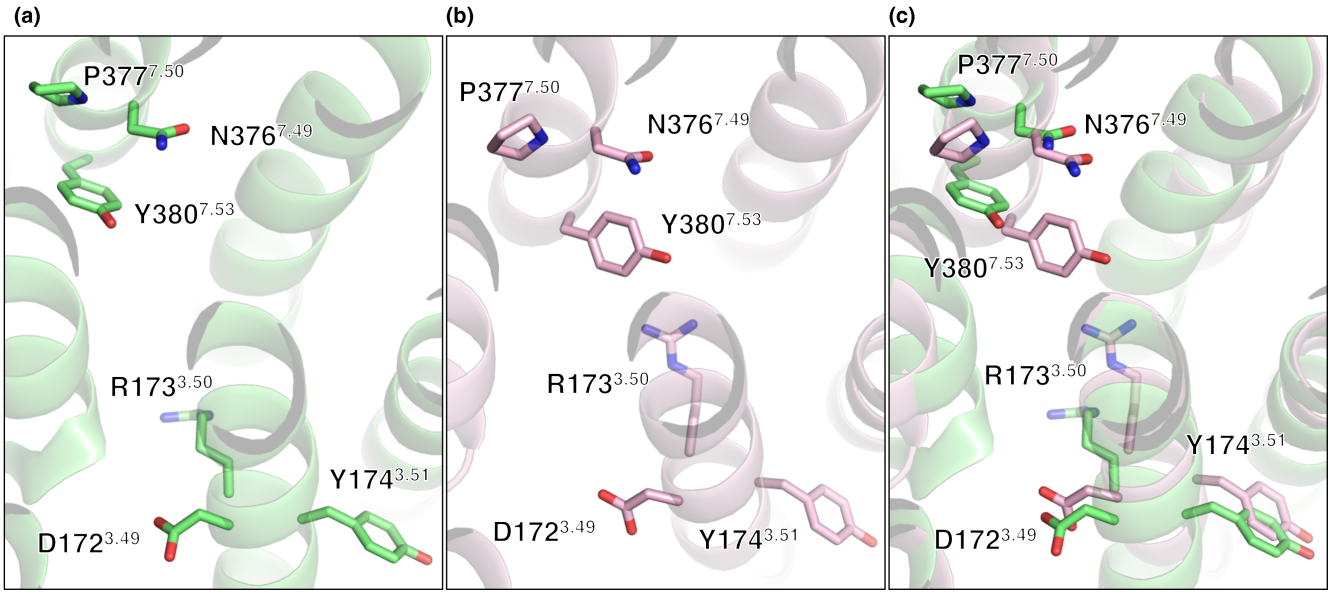

FIGURE 6 Comparison of DRY and NPxxY motifs of 5-hydroxytryptamine receptor 2A bound to (a) 4-[2-[(2-hydroxyphenyl)methylamino] ethyl]-2,5-dimethoxybenzonitrile (25CN-NBOH) and (b) zotepine. (c) Overlay of the two structures

binding. Further structural investigation into the mechanisms of these drug-receptor interactions is critical to understanding how receptors selectively propagate extracellular signals and induce clinically important physiological changes. Studying closely related receptors is also important, as "off-target" effects may moderate the effects of psychedelics, have deleterious clinical consequences, or introduce completely unrelated effects. Further investigation of how the chemical space that constitutes psychedelic drugs interact with the numerous biogenic amine receptors, as well as potential unknown targets, is highly warranted. The suite of structural information currently available marks an important landmark in the understanding of how psychedelic drugs interact with their primary physiological targets.

While the importance of 5-HT2AR has been established, it has been noted that the pharmacology of many psychedelics is complex, as a majority of these compounds interacts with many receptors and transporters in the brain. Among the most promiscuous of psychedelics, LSD has been shown to interact with dopamine, histamine, adrenaline, and trace amine-associated receptors in addition to serotonin family receptors, with important species differences reported (Rudin et al., 2021; Simmler et al., 2016). The number of biological targets engaged by psychedelic drugs varies a great deal and is only weakly correlated with chemotype. While these off-target (non-5-HT2AR) activities are likely dispensable to their hallucinogenic and neuroplastic effects, they will continue to be important considerations for the development of these drugs. Further study

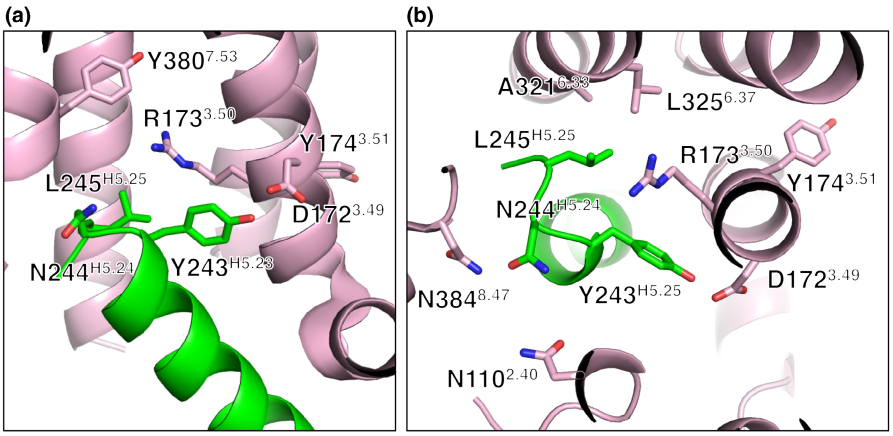

FIGURE 7 Interface of Gαq helix 5 with the intracellular face of 5-hydroxytryptamine receptor 2A in complex with 4-[2-[(2-hydroxyphenyl)methylamino]ethyl]-2,5-dimethoxybenzonitrile (25CN-NBOH). (a) View from the side of the receptor, with the bottom of the frame corresponding to the intracellular space. (b) A top-down perspective, viewing from the extracellular side into the intracellular face

is necessary to determine what physiological consequences are incurred by the activation of non-serotonergic receptors. Human studies such as NCT04558294 and NCT04849013, wherein ketanserin is co-administered with mescaline, will provide critical information about these effects (clinicaltrials.gov).

4 | CONCLUSION

Psychedelics have a long and storied history in basic science research, medical application, and have had a huge societal impact. The earliest hopes held by proponents of psychedelic therapies are currently being explored, and molecular scale understanding of the mechanisms underlying their clinical efficacy is growing in depth and resolution. These insights are actively facilitating further dissection of the effects that psychedelic drugs have on neurochemistry, cognition, and our perception of self. Whether the psychedelic experience is essential to, a side-effect of, or synergistic with the neurological effects of psychedelic drugs remains to be seen. More practically, the biochemical consequences of psychedelic drug action represent a dramatically novel approach to the treatment of mental illnesses. Regardless of the answers to these basic scientific questions, the lives of many people could be improved with these therapies.

CONFLICT OF INTEREST

BLR is an member of the Scientific Advisory Board at MindMed.

AUTHOR CONTRIBUTIONS

STS, JFD, and BLR prepared the article and figures. All authors have approved the content of the article.

DATA AVAILABILITY STATEMENT

All data are available from the authors on request.

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How to cite this article: Slocum, S. T., DiBerto, J. F., & Roth, B. L. (2022). Molecular insights into psychedelic drug action. *Journal of Neurochemistry*, 162, 24–38. https://doi.org/10.1111/jnc.15540