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REVIEW ARTICLE

Molecular Mechanisms of Psilocybin and Implications for the Treatment of Depression

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Abstract

Therapeutic deficiencies with monoaminergic antidepressants invites the need to identify and develop novel rapid-acting antidepressants. Hitherto, ketamine and esketamine are identified as safe, well-tolerated rapid-acting antidepressants in adults with treatment-resistant depression, and also mitigate measures of suicidality. Psilocybin is a naturally occurring psychoactive alkaloid and non-selective agonist at many serotonin receptors, especially at serotonin 5-HT_{2A} receptors, and is found in the *Psilocybe* genus of mushrooms. Preliminary studies with psilocybin have shown therapeutic promise across diverse populations including major depressive disorder. The pharmacodynamic mechanisms mediating the antidepressant and psychedelic effects of psilocybin are currently unknown but are thought to involve the modulation of the serotonergic system, primarily through agonism at the 5-HT_{2A} receptors and downstream changes in gene expression. It is also established that indirect effects on dopaminergic and glutamatergic systems are contributory, as well as effects at other lower affinity targets. Along with the direct effects on neurochemical systems, psilocybin alters neural circuitry and key brain regions previously implicated in depression, including the default mode network and amygdala. The aim of this review is to synthesize the current understanding of the receptor pharmacology and neuronal mechanisms underlying the psychedelic and putative antidepressant properties of psilocybin.

Key Points

Psilocybin is a psychoactive alkaloid with psychedelic and putative antidepressant effects. Its actions are proposed to be primarily mediated by agonism at serotonin 5-HT_{2A} receptors and downstream changes in gene expression.

Psilocybin modulates the serotonergic system and indirectly affects the dopaminergic and glutamatergic systems.

Psilocybin alters neural circuitry between areas such as the default mode network and amygdala, which may mediate antidepressant effects.

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1 Introduction

Major depressive disorder (MDD) is a prevalent multifactorial mood disorder and a leading cause of long-term disability worldwide [1]. Much of the socioeconomic burden associated with MDD is attributable to treatment-resistant depression (TRD), characterized by failure to achieve full remission following treatment with two conventional antidepressants [2, 3]. In 2013, TRD was reportedly responsible for a 40–50% increase in direct and indirect medical care costs when compared with treatment-responsive depression [4]. Conventional first-line antidepressants, including selective serotonin reuptake inhibitors (SSRIs), norepinephrine reuptake inhibitors (NRIs), and serotonin-norepinephrine reuptake inhibitors (SNRIs), often exhibit a therapeutic delay of at least 2 weeks [5], and are associated with treatment-limiting adverse effects (e.g., sexual dysfunction) [6, 7]. In recognition of the inadequacy of conventional first-line antidepressants and the unmet needs of individuals with TRD, along with the recent US Food and Drug Administration and European Medicines Agency approval of esketamine [8],

there is a growing interest in rapid-acting antidepressants (RAADs), characterized by therapeutic efficacy following one or few doses [5]. Although there is no well-characterized time frame of therapeutic action for RAADs, these treatments show therapeutic efficacy within a few days to a week [9]. For example, ketamine, a RAAD, was shown to alleviate depressive symptoms within hours of administration, which stands in contrast to monoamine-based antidepressants that require at least 4 weeks before therapeutic benefits are exhibited [10–12].

Although the pathophysiology and neurobiology of depression are not completely understood, the traditional 'serotonin hypothesis' of MDD asserts that a deficit of central serotonin subserves depressive symptoms [13]. Recent studies have suggested additional mechanisms wherein factors associated with depression such as chronic stress can result in increased levels of extracellular glutamate and overactivity of *N*-methyl-D-aspartate (NMDA) receptors. This imbalance in glutamatergic neurotransmission ultimately results in excitotoxic effects and subsequent neuronal atrophy in brain regions associated with depression, including, but not limited to, the prefrontal cortex (PFC) and hippocampus [14–22]. It is important to note, however, that these recent studies stand in contrast to the widely accepted theory of glutamatergic activity, in which neuronal atrophy in the brain reward circuitry results in decreased glutamatergic synaptic excitation [19]. The foregoing findings are proffered as explanatory for the antidepressant properties of ketamine, an NMDA antagonist proven effective for the rapid-onset treatment of TRD and MDD with suicidality [8]. However, the non-enduring efficacy of ketamine in many patients who are acute responders, as well as the absence of sufficient remission in most patients taking ketamine, invites the need for identifying and developing alternative RAADs [23, 24]. As such, there is a renewed interest in the potential role of classical psychedelics for the treatment of TRD.

Classical psychedelics include three distinct groups of hallucinogens: tryptamines, including psilocybin; lysergamides such as lysergic acid diethylamide (LSD), and phenethylamines such as mescaline [25]. Of these, psilocybin has generated the most interest because of its proposed similarity to the rapid-acting properties of ketamine as well as its low physiological toxicity and abuse liability [26]. In addition, psilocybin has a short acute window of 2–6 h, which contributes to more manageable and inexpensive clinical trials, differing greatly from mescaline and LSD with acute windows of 6–8 h and 8–20 h, respectively [27, 28]. Although preliminary studies have supported the efficacy of psilocybin [29–31], a recent phase II clinical trial comparing the relative antidepressant effects of psilocybin with the SSRI escitalopram found no significant differences in antidepressant effects between these two agents. In the aforementioned study, patients in the psilocybin group received two separate 25 mg doses of psilocybin 2 weeks apart plus 6 weeks of daily placebo. Those in the escitalopram group received two separate 1 mg doses of psilocybin 3 weeks apart plus 6 weeks of daily oral escitalopram. In addition, both the psilocybin and escitalopram groups received psychotherapy. Subsequently, the psilocybin group reported a mean change of -8.0 ± 1.0 points in 16-item Quick Inventory of Depressive Symptomatology-Self-Report (QIDS-SR-16) scores from baseline, whereas the escitalopram group reported a mean change of -6.0 ± 1.0 points ($p = 0.17$). The foregoing results provide important insights regarding the efficacy of psilocybin, specifically, that psilocybin did not perform better than a conventional monoaminergic antidepressant. However, due to the nature of the study design and limitations in the analytic methodology employed (e.g., analyses of secondary outcomes were not corrected for multiple comparisons), the relative antidepressant efficacy of psilocybin remains incompletely understood [32].

Psilocybin is a naturally occurring psychedelic found in the *Psilocybe* genus of mushrooms [30]. It exists as a prodrug that is dephosphorylated upon administration in the stomach, intestines, kidneys, and blood through the action of alkaline phosphatases and esterases into its active form, psilocin [33]. Psilocybin and other tryptamines are structurally similar to serotonin. Notably, the indole ring at the fourth position of the tryptamine structure of psilocin is reportedly responsible for the hallucinogenic effects associated with the drug [26]. In the liver, further metabolism of psilocin through demethylation and oxidative deamination by monoamine oxidase (MAO) or aldehyde dehydrogenase reduces the hallucinogenic effects [33]. Although numerous studies have positioned psilocybin as an emerging RAAD, the exact mechanisms responsible for its putative antidepressant and anxiolytic effects remain incompletely characterized. In addition, the role of the psychedelic experience in mediating antidepressant effects is unknown [34, 35].

Herein, the aim of this review is to present the current understanding of the putative antidepressant mechanisms of psilocybin. This review is not intended to synthesize the extant evidence regarding the efficacy of psilocybin for the treatment of depression and other mental disorders (reviewed elsewhere) [36], but rather to outline key mechanistic properties that may mediate its RAAD activity and psychedelic effects. The overarching aim is to provide a synthesis of the pharmacology, creating an important scaffold for identifying derivatives for future drug discovery and development.

2 Receptor Pharmacology

2.1 5-HT_{2A} Receptor Agonism and Downstream Effects

The psychedelic effects of all classical psychedelics are mediated by full or partial agonism at serotonergic 5-hydroxytryptamine 2A (5-HT_{2A}) receptors (5-HT_{2A}Rs) [25, 37]. The actions of psilocybin on the 5-HT_{2A}Rs and the possible involvement of these receptors in mediating the hallucinogenic effects of psilocybin were first reported by Glennon et al. in 1984, who noted a significant correlation between the binding affinities for 5-HT_2 receptors and the dose that produced 50% of the maximal effect (ED_{50}) [38].

5-HT_{2A}Rs are highly expressed in the visual cortex, thus, receptor activity in visual cortical neurons may be sufficient to mediate psychedelic effects, specifically the propensity for visual hallucinations associated with psilocybin [25]. Increased expression of 5-HT_{2A}Rs may also underlie disease states associated with visual hallucinations, including, but not limited to, schizophrenia and Parkinson's disease [39]. Accordingly, overactivity in cortical 5-HT_{2A}Rs is likely contributory to the characteristic visual hallucinations associated with psilocybin. When compared to ketamine employed as a dissociative anesthetic, psilocybin elicits significant visual hallucinatory effects but lacks strong negative effects associated with the psychedelic experience (e.g., loss of physical integrity, pronounced anxiety, emotional withdrawal) [40, 41].

Multiple 5-HT_{2A}R knockout and receptor antagonism experiments support the role of these serotonin receptors in mediating the psychedelic effects of psilocybin. Administration of the 5-HT_{2A}R antagonist, ketanserin, to healthy humans attenuated hallucinatory effects following psilocybin administration. In comparison, antagonism at other 5-HT_2 receptors, such as the 5-HT_{2C} receptors, did not completely attenuate psilocybin-induced hallucinatory effects [42, 43]. Likewise, administration of psilocybin to 5-HT_{2A}R knockout mice resulted in no head-twitch response, likely corresponding to attenuated hallucinatory effects. In addition, re-expression of 5-HT_{2A}Rs in cortical pyramidal neurons was able to successfully restore hallucinogen-induced head twitching [44, 45]. The results of these mice studies strongly suggest that the hallucinatory effects of psilocybin are mediated by 5-HT_{2A}Rs; however, other factors characteristic of a psychedelic experience (e.g., locomotor responses, anxiolytic effects, alterations in time perception) were not investigated [46].

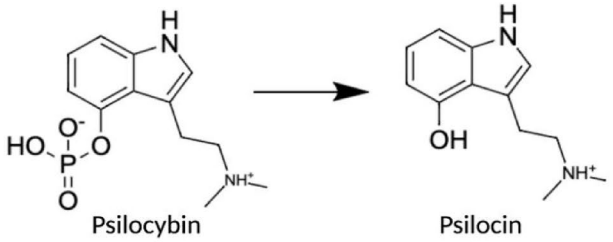
Downstream effects at the 5-HT_{2A}Rs are mediated by secondary messenger signaling and alterations in gene expression [25]. Multiple studies have suggested that hallucinogenic 5-HT_{2A}R agonists elicit different downstream mechanisms when compared with non-hallucinogenic 5-HT_{2A}R agonists [47]. It is important to note that binding to other 5-HT_2 and non-5-HT_2 receptors also contributes a role in mediating the psychopharmacological actions of psilocybin. Experiments conducted by González-Maeso et al. determined that activation of phospholipase C-β is elicited by both 5-HT_{2A}R hallucinogens (e.g., LSD) and non-hallucinogens; however, activation of phospholipase C-β through coactivation of heterotrimeric $G_{q/11}$ and pertussis toxin-sensitive $G_{i/o}$ proteins is unique to hallucinogens, including psilocybin. In addition, coactivation of $G_{i/o}$ requires $G_{\beta\gamma}$ subunit-mediated activation of Src [48]. These $G_{i/o}$ proteins are further coupled to metabotropic glutamate receptor 2 ($mGlu_2$) receptors, ultimately forming a co-expressed 5-HT_{2A}/$mGlu_2$ complex [47]. Formation of the 5-HT_{2A}/$mGlu_2$ complex has been shown to be a key component in the hallucinatory effects of certain 5-HT_{2A}R agonists.

In mGlu2-knockout mice, administration of the serotonergic hallucinogens 4-iodo-2,5-dimethoxyphenyl-isopropylamine (DOI) and LSD did not induce head-twitch behavior [49]. Additionally, administration of DOI in $mGlu_2$-knockout mice over-expressing a chimeric $mGlu_2$ construct which cannot be complexed with 5-HT_{2A}Rs in the frontal cortex did not restore head-twitch behavior [50, 51]. Binding of hallucinogens to the foregoing complex ultimately resulted in downstream G protein signal transduction and unique gene effects. In contrast, non-hallucinogenic 5-HT_{2A}R agonists induced the foregoing events through a different signal transduction cascade. This difference in G protein activation and specific signaling pathways between 5-HT_{2A}R hallucinogens and non-hallucinogens is referred to as the 'agonist trafficking of receptor signaling theory' [52].

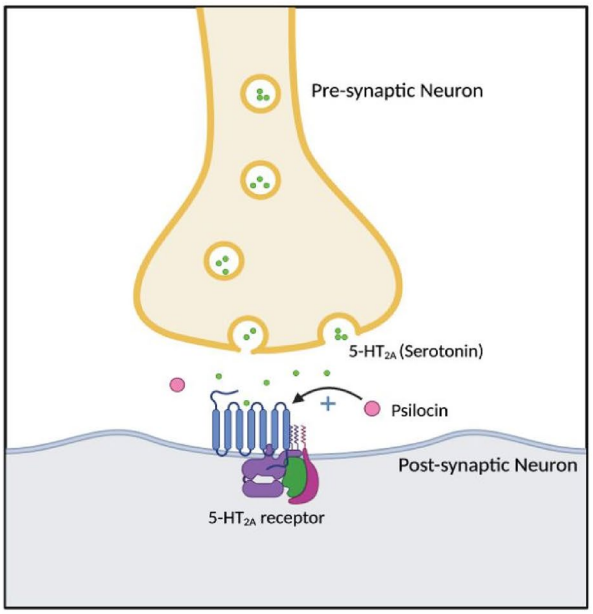
Administration of LSD to several 5-HT_{2A}R-expressing brain regions is associated with increased expression of early growth response genes; *egr-1* and *egr-2,* as well as *c-fos, jun-B, period-1, gpcr-26, fra-1, N-10,* and *I-κBα*, and decreased expression of *sty-kinase* [41]. Alterations in the expression profiles of *egr-1* and *egr-2* are unique to 5-HT_{2A}R hallucinogens, whereas increased *c-fos* expression occurs upon administration of both hallucinogenic and non-hallucinogenic 5-HT_{2A}R agonists (e.g., R-lisuride). The foregoing gene expression findings may present key mediators in 5-HT_{2A}R agonist-induced hallucination, such that *egr-1* and *egr-2* expression may be necessary for the induction of hallucinogenic effects, whereas *c-fos* expression corresponds only to neuronal activity at the 5-HT_{2A}Rs and is not sufficient to modify downstream pathways that produce hallucinatory effects [41, 48]. Expression of *egr-1* has previously been implicated in neuronal plasticity [53]. Electrical stimulation of the perforant pathway and subsequent induction of long-term potentiation (LTP) resulted in increased expression of

A

① Psilocybin dephosphorylation to psilocin by alkaline phosphatases and esterases

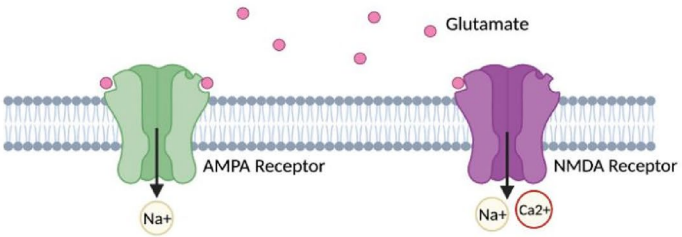


② Psilocin agonism at 5-HT_{2A} receptors resulting in downregulation

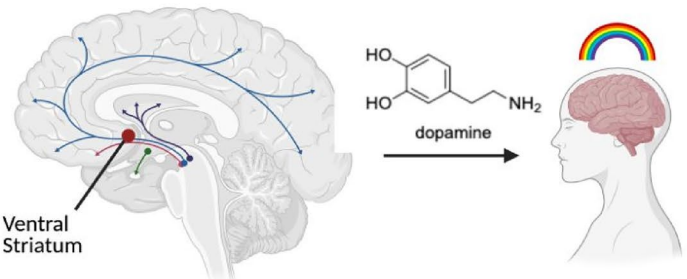


B

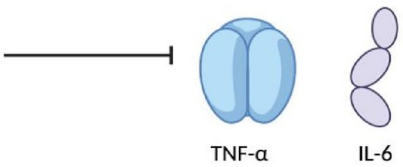
5-HT_{2A} agonism promotes glutamate release from pyramidal neurons in Layer V of PFC leading to antidepressant and anxiolytic effects. Glutamatergic modulation of AMPA and NMDA receptors on cortical pyramidal neurons results in upregulation of BDNF expression.



Increased dopamine concentrations at the ventral striatum causing psychosis



Inhibition of TNF-α and IL-6 release resulting in anti-inflammatory properties



◂**Fig. 1** Proposed mechanism of action for psilocybin in the treatment of major depressive disorder. **A** The proposed sequence of molecular mechanisms of psilocybin. Psilocybin is a prodrug that is dephosphorylated to the active compound, psilocin. Psilocin then binds to 5-hydroxytryptamine 2A (5-HT_{2A}) receptors, eliciting downstream effects including downregulation of 5-HT_{2A} receptors. **B** Other proposed downstream effects of psilocybin 5-HT_{2A} receptor agonism do not occur in a specified sequence. These effects include glutamatergic modulation of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors, increased brain-derived neurotrophic factor (BDNF) expression and dopaminergic activity, as well as inhibition of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) release [120].

egr-1 in the ipsilateral granule cell neurons. As such, *egr-1* expression occurs in conditions conducive to synaptic enhancement (e.g., LTP), suggesting that psilocybin may activate key neuroplastic pathways underlying its putative antidepressant effects [53, 54].

Hallucinogenic 5-HT_{2A}R agonists also show differences in signaling cascades, when compared with non-hallucinogenic 5-HT_{2A}R agonists, through varied β-arrestin-2 expression and subsequent β-arrestin-2-dependent mechanisms. Multiple studies conducted by Schmid et al. have demonstrated that serotonin-induced head twitching in mice is normally partially mediated through β-arrestin-2 interactions. In comparison, the hallucinogenic 5-HT $_{2A}$R agonist DOI induces head twitching independent of β-arrestin-2 interactions [55, 56]. Taken together, the foregoing findings present important differences in signaling cascades between hallucinogenic and non-hallucinogenic 5-HT_{2A}R agonists.

Although the visual hallucinatory effects of psilocybin are largely associated with increased activity in 5-HT_{2A}Rs of the visual cortex, previous studies have suggested that overexpression of 5-HT_{2A}Rs is present in patients with MDD, with expression correlating positively to the severity and duration of depression [39]. As a result, downregulation of 5-HT_{2A}Rs may be associated with the putative antidepressant and anxiolytic properties of psilocybin [36]. However, the mechanism of 5-HT_{2A}R overexpression in depressed patients is not well characterized. In accordance with the foregoing observation, re-expression of 5-HT_{2A}Rs in the PFC in 5-HT_{2A}R knockout models (i.e., rescue experiments) restored anxiety symptoms [57]. Collectively, these findings suggest a possible role for 5-HT_{2A}R downregulation and desensitization in mitigating depressive and anxious symptoms [58].

The downregulation of 5-HT_{2A}Rs by psilocybin may be mediated via brain-derived neurotrophic factor (BDNF). A mouse model experiment conducted by Trajkovska et al. noted a decrease in 5-HT_{2A}Rs in mice over-expressing BDNF. The preceding result suggests possible downstream expression of BDNF following the binding of psilocybin, ultimately leading to downregulation of 5-HT_{2A}Rs [59]. This relationship is further supported by findings that glutamatergic modulation of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and NMDA receptors on cortical pyramidal cells subsequent to 5-HT_{2A}R agonism has been shown to increase expression of neurotrophins, including BDNF [36]. Glutamatergic modulation of NMDA receptors and increased BDNF are the predominant proposed antidepressant mechanisms of ketamine, thus suggesting the parallel involvement of this mechanism in mediating antidepressant effects of psilocybin [60].

An inflammatory state characterized by a preponderance of pro-inflammatory cytokines, most notably tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6), has also been implicated in the pathogenesis of depression and measures of anhedonia [61–66]. Such findings correlate with the accepted mechanism of TNF-α in inducing IL-6 synthesis through phosphorylation of NFκB and activation of the mitogen-activated protein kinase (MAPK) pathway via phosphorylation of p38 MAPK [67, 68]. Multiple studies have shown that treatment with proinflammatory cytokines, including TNF-α and IL-6, induce depression-like behavior assessed via forced swim tests [69, 70]. In addition, other studies have indicated a positive correlation between TNF-α and IL-6 levels and depressive scores [71, 72]. Moreover, IL-6 and TNF-α antagonists have previously been proven efficacious in treating depressive symptoms [73]. Murine studies have shown that agonism at the 5-HT_{2A}R by DOI results in downstream inhibition of TNF-α and subsequent inhibition of IL-6 release [74]. Furthermore, agonism at the 5-HT_{2A}R by psychedelics including LSD, *N*,*N*-dimethyltryptamine, and psilocybin has demonstrated similar results [75–77]. Another study conducted by Nkadimeng et al., which investigated properties of the comorbidity of heart failure and MDD, demonstrated decreased damage to cardiomyocytes by TNF-α upon administration of psilocybin [78]. Taken together, these results suggest that 5-HT_{2A}R agonism by psilocybin and other psychedelics mediates antidepressant effects via inhibition of TNF-α and IL-6 release (see Fig. 1).

2.2 Other Receptors and Modulation of Serotonergic, Dopaminergic, and Glutamatergic Systems

Psilocin also has moderate affinity (Psilocin dissociation constant [K_i] <10,000 nM) for non-5-HT_2 receptors including, but not limited to, 5-$HT_{1A/B/D/E}$, 5-HT_5, 5-HT_6, 5-HT_7, alpha$_{2A/B}$, and dopamine D3 (D_3) receptors (see Table 1). It also has weak affinity for other 5-HT receptors, including 5-$HT_{3/5/6/7}$ and imidazoline 1 receptors [41, 79]. Although it has previously been proposed that psilocybin also has low affinity for dopamine D_2 (D_2) receptors, it has subsequently been noted that 5-HT_{2A}R agonism leads to increased dopamine levels in the ventral striatum resulting in hallucinogenic-like symptoms including

Table 1 Binding affinity of psilocybin and psilocin to 5-HT and other monoamine receptors

Receptor	Psilocybin K_i (nM) [118, 119]	Psilocin K_i (nM) [79]
SERT	> 10,000	3801.0
5-HT_{1A}	> 10,000	49.01
5-HT_{1B}	> 10,000	219.6
5-HT_{1D}	2119	36.4
5-HT_{1E}	194.8	52.2
5-HT_{2B}	98.7	4.6
5-HT_{2A}	> 10,000	107.2
5-HT_3	> 10,000	> 10,000
5-HT_5	6181.0	83.7
5-HT_6	413.5	57.0
5-HT_7	597.9	3.5
α1A	> 10,000	> 10,000
α1B	> 10,000	> 10,000
α2A	> 10,000	1379.0
α2B	> 10,000	1894.0
α2C	> 10,000	> 10,000
D_1	> 10,000	> 10,000
D_2	> 10,000	> 10,000
D_3	> 10,000	2645.0
D_4	> 10,000	> 10,000
D_5	> 10,000	> 10,000

5-HT 5-hydroxytryptamine, *5-HT_{1A}* 5-hydroxytryptamine 1A receptor, *5-HT_{1B}* 5-hydroxytryptamine 1B receptor, *5-HT_{1D}* 5-hydroxytryptamine 1D receptor, *5-HT_{1E}* 5-hydroxytryptamine 1E receptor, *5-HT_{2B}* 5-hydroxytryptamine 2B receptor, *5-HT_{2A}* 5-hydroxytryptamine 2A receptor, *5-HT_3* 5-hydroxytryptamine 3 receptor, *5-HT_5* 5-hydroxytryptamine 5 receptor, *5-HT_6* 5-hydroxytryptamine 6 receptor, *5-HT_7* 5-hydroxytryptamine 7 receptor, *α1A* alpha-1A adrenergic receptor, *α1B* alpha-1B adrenergic receptor, *α2A* alpha-2A adrenergic receptor, *α2B* alpha-2B adrenergic receptor, *α2C* alpha-2C adrenergic receptor, *D_1* dopamine 1 receptor, *D_2* dopamine 2 receptor, *D_3* dopamine 3 receptor, *D_4* dopamine 4 receptor, *D_5* dopamine 5 receptor, *K_i* dissociation constant, *nM* nanometer, *SERT* sodium-dependent serotonin transporter

depersonalization and euphoria [80]. Interestingly, pretreatment with the D_2 receptor antagonist haloperidol followed by psilocybin administration produced only a 30% reduction in euphoria, derealization, and depolarization, with no reduction in visual hallucinations [43, 44]. In contrast, addition of a mixed 5-$HT_{2A/C}$R and D_2 receptor antagonist, risperidone, reduced psilocybin-induced psychotic effects [41]. These findings may suggest an indirect role of the dopaminergic system in eliciting hallucinations, as interactions between serotonergic and dopaminergic systems have been established [33]. Other studies have implicated the necessity of D_2 antagonism for reducing psychotic effects independently of 5-HT_{2A}R activity [41]. As such, the exact mechanism of dopaminergic modulation in mediating psychosis remains unclear.

Modulation of the serotonergic and glutamatergic systems by psilocybin has also been reported. The actions of psilocybin on the serotonergic system are similar to those of SSRIs, occurring via inhibition of the sodium-dependent serotonin transporter (SERT). The foregoing mechanism leads to decreased serotonin reuptake, elevated serotonin levels in the synaptic cleft, and subsequent increases in serotonergic neurotransmission [79]. Agonism at 5-HT_{2A}Rs may also result in activation of glutamatergic systems. 5-HT_{2A}R activity subsequently increases the activity of pyramidal neurons in layer V of the PFC [81, 82]. Studies attribute the increase in activity to a glutamate-dependent interaction; early studies have implicated activation of presynaptic 5-HT_{2A}Rs on glutamatergic thalamocortical afferents projecting to the PFC [83]. Recent studies suggest a different mechanism whereby activation of postsynaptic 5-HT_{2A}Rs on pyramidal neurons may lead to increased glutamatergic action [44]. Although contrasting views have been proposed, it can be surmised that alterations in glutamate release contribute to the putative rapid antidepressant effects associated with psilocybin.

Other RAADs such as ketamine also exhibit similar modulatory effects on the glutamatergic system, including but not limited to, $mGlu_{2/3}$ antagonism [84]. The effects on the $mGlu_{2/3}$ receptors are paralleled by the action of psilocybin on 5-HT_{2A}/$mGlu_2$. Binding of psilocybin to this complex likely results in inhibition of $mGlu_2$ activity. For example, administration of the $mGlu_{2/3}$ agonist LY354740 counteracts excitatory effects in cortical pyramidal neurons. Similarly, administration of DOI in the presence of LY354740 was not able to restore head-twitch behavior in mice whereas administration of DOI with the $mGlu_{2/3}$ antagonist LY341495 increased head-twitch behavior [85, 86].

Psilocybin also acts as a partial agonist with moderate binding affinity for the 5-HT_{1A} receptor ($K_i = 49.0$ nM), specifically at 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (DRN) and median raphe nucleus [87, 88]. Accordingly, activity at the 5-HT_{1A} receptor may lead to increased levels of serotonin and serotonergic modulation [88]. Decreased DRN size has been associated with MDD as the DRN is the largest serotonergic nucleus and a significant contributor to the serotoninergic innervation of the forebrain [89]. Alterations in the DRN may result in changes to normal neural communication and functional connectivity, ultimately allowing new connections and signals to be relayed [88]. Interestingly, psilocybin lessens 5-HT_{1A} activity through partial agonism; however, in comparison to downregulation of 5-HT_{2A}Rs upon binding, this does not occur with 5-HT_{1A} receptors. Rather than contributing to the antidepressant effects of psilocybin, dampening of 5-HT_{1A} activity may conduce its hallucinatory effects. This has been supported by studies demonstrating that antagonism at 5-HT_{1A} receptors (as well as 5-$HT_{2A/C}$ and dopamine D_2 receptors) restored

normal electroencephalographic changes that occurred upon psilocybin administration [79, 90].

3 Alterations in Neural Circuitry

Currently accepted neural models of MDD are characterized by overactivity of the amygdala due to altered connectivity in the default mode network (DMN), particularly in the medial prefrontal cortex (mPFC) [45, 91, 92]. Numerous functional magnetic resonance imaging studies have presented findings consistent with these models and suggest psilocybin induces decreased activity in brain regions and networks associated with MDD, including the amygdala and DMN, which may ultimately underlie its therapeutic effects. A study conducted by Carhart-Harris et al. reported that psilocybin decreased cerebral blood flow and venous oxygenation to the ventral medial prefrontal cortex (vmPFC), thalamus, as well as anterior and posterior cingulate cortices (ACC and PCC, respectively) immediately following intravenous infusion (i.e., during the psychedelic state) [93]. Decreased blood flow in the aforementioned regions is correlated with decreased activity and as such implies decreased functional connectivity. In addition, cerebral blood flow to the thalamus and ACC was found to be positively correlated with the intensity of the psychedelic experience. Collectively, these findings suggest that psilocybin can normalize activity in the default mode network and restore normal neural connectivity in patients with MDD. Activation of 5-HT_{2A}Rs within the thalamus and mPFC may also decrease thalamic activity, leading to decreased consciousness, alertness, and sensory signals, which contribute to the psychedelic experience [94].

Previous studies have demonstrated the occurrence of overactivity in the amygdala in response to negative stimuli in patients with MDD [95–97]. Using evaluation of affective pictures (i.e., facial expression) conducted in patients with MDD post-psilocybin administration, multiple studies have demonstrated attenuated right amygdala responses to negative stimuli and associated induction of positive affective states [25, 26]. However, an open-label study on patients with TRD reported contrasting findings, revealing that psilocybin increased right amygdala activity in response to fearful and happy faces at 1 day post-treatment [98]. A separate study presented findings consistent with prior models wherein decreased functional connectivity between the vmPFC and right amygdala was observed, resulting in antidepressant effects such as lowered rumination at 1-week post-psilocybin administration. The decreased connectivity, however, was associated with the occurrence of increased activity in the amygdala in response to fearful and neutral faces [37]. Inconsistent findings reported in the literature may correspond to an alternative mechanism for the antidepressant effects of psychedelics compared with conventional antidepressants; however, further research is required.

Widely accepted models of MDD have reported alterations in neuroplasticity, including reduced or maladaptive neuroplasticity due to neuronal atrophy, specifically in the PFC [99]. In vitro and in vivo studies have also demonstrated increased neuritogenesis and spinogenesis in the PFC upon 5-HT_{2A}R agonism [100]. Conventional antidepressants may work to restore and enhance neuroplasticity [101–103]. It has been proposed that 5-HT_{2A}R activation increases cortical neural plasticity [100, 104]. These models are consistent with the downstream alterations in gene expression occurring upon 5-HT_{2A}R agonism, most notably upregulation of BDNF. The role of BDNF and other neurotrophins in the pathogenesis of MDD is well understood, although their role in neuroplasticity remains heavily debated. Current models suggest that BDNF increases neuroplasticity by promoting neuronal proliferation and survival [105]. More specifically, studies have suggested that BDNF contributes an essential role in LTP; it is required for late LTP in hippocampal neurons, mediated by binding to its receptor, tropomyosin receptor kinase B (TrkB) [100, 105–107]. Activation of the BDNF-TrkB signaling pathway results in downstream activation of other signaling cascades, including the Ras/MAPK and phosphoinositide 3 kinase (PI3K) pathways. In addition, upon binding, TrkB recruits phospholipase Cγ, leading to activation of the calcium/calmodulin kinase pathway. Activation of the Ras/MAPK and PI3K signal transduction cascades ultimately increases intracellular calcium levels, resulting in further activation of important transcription factors involved in mediating changes to synaptic gene expression, such as cAMP response element binding protein (CREB) [105, 107]. Several BDNF-TrkB knockout mice experiments have supported the role of the BDNF-TrkB signaling pathway in mediating LTP. Notably, BDNF mutant and TrkB knockout mice were shown to have impaired LTP in the hippocampal CA3–CA1 region [108–112]. A study by Zhang et al. demonstrated that lipopolysaccharide-induced inflammation in mice resulted in a depression-like phenotype due to alterations in the BDNF-TrkB signaling pathway within the CA3 and dentate gyrus regions of the hippocampus as well as in the PFC and nucleus accumbens [113]. Taken together, the foregoing findings highlight a possible role of the BDNF-TrkB signaling pathway in mediating the antidepressant effects of psilocybin upon 5-HT_{2A}R agonism.

4 Conclusions

In consideration of the molecular mechanisms presented herein, administration of psilocybin may be a potentially efficacious treatment for MDD and TRD. The mechanisms discussed may underlie diverse physiological and behavioral

Table 2 Data from 13 experimental studies involving psilocybin administration to healthy human subjects and/or patients with MDD

First author, year	Study title	Main objectives	Main findings
Mason et al., 2020 [121]	Me, myself, bye: regional alterations in glutamate and the experience of ego dissolution with psilocybin	Characterization of glutamatergic activity in key brain regions during psilocybin-induced psychedelic state via ultra-high field (7T) proton MRS	Psilocybin administration increased levels of glutamate in the mPFC, which was associated with a negative experience of ego dissolution. Conversely, decreased glutamate in the hippocampus was associated with a positive experience of ego dissolution when compared with placebo.
Gouzoulis-Mayfrank et al., 1998 [122]	Effects of the hallucinogen psilocybin on habituation and prepulse inhibition of the startle reflex in humans	Evaluate effects of psilocybin on PPI and habituation of the startle reflex	Psilocybin administration increased PPI with no effect on habituation.
Carhart-Harris et al., 2017 [123]	Psilocybin for treatment-resistant depression: fMRI-measured brain mechanisms	Evaluate effects of psilocybin treatment on the brain via fMRI measures of CBF and BOLD RSFC	Psilocybin treatment decreased CBF in the amygdala, which was associated with antidepressant effects. Administration of psilocybin increased RSFC within the DMN.
Carter et al., 2005 [124]	Using psilocybin to investigate the relationship between attention, working memory, and the serotonin 1A and 2A receptors	Evaluate effects of psilocybin on attention, working memory, and PFC activity	Psilocybin decreased attentional tracking ability with no effects on spatial working memory. Ketanserin did not attenuate psilocybin-induced effects on attention.
Varley et al., 2020 [125]	Serotonergic psychedelics LSD & psilocybin increase the fractal dimension of cortical brain activity in spatial and temporal domains	Measure effects of psilocybin on neural activity complexity using fractal dimension of brain functional activity, specifically of cortical functional connectivity networks, and BOLD time-series	Psilocybin resulted in increased fractal dimension of functional connectivity networks correlating to complex patterns of neural activity.
Kometer et al., 2012 [126]	Psilocybin biases facial recognition, goal-directed behavior, and mood state toward positive relative to negative emotions through different serotonergic subreceptors	Assess effects of psilocybin and 5-HT_{2A}R activity on emotional processing biases	Psilocybin administration was associated with increased positive mood and goal-directed behavior in response to positive cues, and attenuated negative facial expression recognition. Ketanserin attenuated psilocybin-induced positive mood and decreased recognition of negative facial expression.
Bernasconi et al., 2014 [127]	Spatiotemporal brain dynamics of emotional face processing modulations induced by the serotonin 1A/2A receptor agonist psilocybin	Characterization of spatiotemporal brain dynamics following psilocybin administration during emotional face processing	Psilocybin administration was associated with decreased activity in the amygdala, parahippocampal gyrus, and right temporal cortex in response to neutral and fearful faces. There was decreased activity within limbic and right temporo-occipital brain areas in response to happy faces.
Quendow et al., 2011 [42]	Psilocybin-induced deficits in automatic and controlled inhibition are attenuated by ketanserin in healthy human volunteers	Evaluate effects of psilocybin on automatic sensorimotor gating (PPI) and response inhibition as well as attribute effects to 5-HT_{2A}Rs or 5-HT_{1A}Rs	The administration of psilocybin decreased PPI at short lead intervals (30 ms), increased all 5D-ASC scores, and selectively increased errors in the interference condition of the Stroop Test. The foregoing effects were attenuated by pretreatment with ketanserin, and no effects were exhibited following administration of ketanserin alone.

Table 2 (continued)

First author, year	Study title	Main objectives	Main findings
Wackermann et al., 2008 [128]	Effects of varied doses of psilocybin on time interval reproduction in human subjects	Evaluate effects of psilocybin on internal time representation measured using the dual klepsydra model	Graded doses and low doses of psilocybin resulted in increased kappa at 90 minutes following administration, which was correlated with altered subjective time perception.
Carhart-Harris et al., 2012 [93]	Neural correlates of the psychedelic state as determined by fMRI studies with psilocybin	Characterization of transition from normal waking consciousness to psychedelic state upon psilocybin administration via arterial spin labeling perfusion and BOLD fMRI	Psilocybin decreased cerebral blood flow and BOLD signals in the thalamus, ACC, PCC, and mPFC. Psilocybin also decreased positive coupling between the mPFC and PCC.
Wittmann et al., 2007 [129]	Effects of psilocybin on time perception and temporal control of behavior in humans	Evaluate effects of psilocybin on temporal processing, employing tasks of temporal reproduction, sensorimotor synchronization, and tapping tempo	Psilocybin produced 3 main effects: impaired the subjects' ability to reproduce interval durations longer than 2.5 seconds, impaired the subjects' ability to synchronize to inter-beat intervals longer than 2 seconds, and reduced the subjects' preferred tapping rate. Effects attributed to a psychedelic experience, including depersonalization and derealization, were reported.
Vollenweider et al., 2007 [130]	The effects of the preferential 5-HT2A agonist psilocybin on prepulse inhibition of startle in healthy human volunteers depend on interstimulus interval	Evaluate effects of psilocybin on PPI	Psilocybin dose-dependently reduced PPI at short ISI (30 ms), produced no effect at medium ISI (60 ms), and increased PPI at long ISI (120–2000 ms). Psilocybin did not affect startle reactivity or habituation. In addition, psilocybin dose-dependently impaired sustained attention and increased all 5D-ASC scores, with exception of auditory alterations.
Carter et al., 2005 [131]	Modulating the rate and rhythmicity of perceptual rivalry alternations with the mixed 5-HT_{2A} and 5-HT_{1A} agonist psilocybin	Assess rate and rhythmicity of perceptual alternations upon psilocybin administration as measured by binocular rivalry tests	Psilocybin significantly decreased the rate and rhythmicity of perceptual alterations at 90 minutes post-administration.

5D-ASC 5-dimensional altered states of consciousness rating scale, *5-HT_{1A}R* 5-hydroxytryptamine 1A receptor, *5-HT_{2A}R* 5-hydroxytryptamine 2A receptor, *ACC* anterior cingulate cortex, *BOLD* blood oxygen-level dependent, *CBF* cerebral blood flow, *DMN* default mode network, *fMRI* functional magnetic resonance imaging, *ISI* inter-stimulus-intervals, *LSD* lysergic acid diethylamide, *MDD* major depressive disorder, *mPFC* medial prefrontal cortex, *MRS* magnetic resonance spectroscopy, *PCC* posterior cingulate cortex, *PFC* prefrontal cortex, *PPI* pre-pulse inhibition, *RSFC* resting-state functional connectivity

outcomes of psilocybin exhibited in human molecular studies comprising healthy subjects and/or patients with MDD (see Table 2). Notwithstanding, large clinical trials further demonstrating robust efficacy and safety are required to justify widespread implementation. In addition, further mechanistic studies are warranted to establish a model of the neural modulatory effects of psilocybin in order to understand the mechanisms of psychedelics and associated psychedelic experience.

Currently, the most widely accepted molecular model of the antidepressant and psychedelic effects of psilocybin can be attributed to its activity on various 5-HT receptors, notably, agonism at 5-HT_{2A}Rs, instigating downstream changes in neuronal gene expression as well as an overall decrease in functional connectivity between brain regions implicated in MDD, such as the DMN. Ultimately, complex alterations in connectivity between neural networks occur, allowing new connections in the brain to be formed; this phenomenon has been referred to as a 'pharmaco-physiological interaction' [93].

In light of the association between 5-HT_{2A}R agonism and antidepressant effects, it may be useful to consider the potential of other 5-HT_{2A}R agonists for the treatment of MDD. Pimavanserin (Nuplazid), an antipsychotic drug used in the treatment of Parkinson's disease psychosis, has shown promise for the treatment of MDD [114]. In contrast to typical antipsychotics, pimavanserin is not a dopamine receptor antagonist but rather a combined 5-HT_{2A}R inverse agonist and antagonist [115]. Other 5-HT_{2A}R agonists such as mescaline, LSD, *N*, *N*-dimethyltryptamine, and ayahuasca have also demonstrated potential for the treatment of depression and anxiety. For example, a randomized, double-blind, placebo-controlled study reported antidepressant and anxiolytic effects upon administration of LSD in patients with life-threatening diseases [116]. Similarly, an open-label trial reported a significant reduction in depressive symptoms with a single dose of ayahuasca; however, antidepressant effects of ayahuasca cannot be attributed to 5-HT_{2A}R agonism alone as it also inhibits MAO activity [116, 117]. Other studies examining 5-HT_{2A}R psychedelics (e.g., mescaline, *N*,*N*-dimethyltryptamine) should provide additional insights regarding the pharmacodynamics of psychedelics to inform future drug discovery.

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