Psychedelic Drugs in Biomedicine

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Psychedelic drugs, such as lysergic acid diethylamide (LSD), mescaline, and psilocybin, exert profound effects on brain and behavior. After decades of difficulties in studying these compounds, psychedelics are again being tested as potential treatments for intractable biomedical disorders. Preclinical research of psychedelics complements human neuroimaging studies and pilot clinical trials, suggesting these compounds as promising treatments for addiction, depression, anxiety, and other conditions. However, many questions regarding the mechanisms of action, safety, and efficacy of psychedelics remain. Here, we summarize recent preclinical and clinical data in this field, discuss their pharmacological mechanisms of action, and outline critical areas for future studies of psychedelic drugs, with the goal of maximizing the potential benefits of translational psychedelic biomedicine to patients.

Origins of Psychedelic Medicine

Humans have used psychedelic drugs for spiritual and religious purposes for centuries, if not millennia. The recent history of these compounds, however, began with the isolation of mescaline, the psychoactive alkaloid produced by the peyote cactus, by Heffter in 1898, and the discovery of the effects of LSD by Hoffman in 1943 [1] (Figure 1). Unlike many other drugs discovered over the past 100 years, the effects of psychedelics were first characterized in humans before animals. LSD, psilocybin, mescaline, and other classic serotonergic psychedelics (Figure 1, inset) exert powerful effects on human behaviors and thought, most prominently resulting in altered perception and changes in consciousness. In 1955, LSD was first tested in mice, producing an unusual behavioral response (walking backwards) similar to a normal mouse on an inclined plane [2]. The following year, LSD was given to rats trained to climb a rope [3]. Between then and now, psychedelics have been studied in a wide range of animal models, from rodents to fish to flies.

Due to the profound effects of psychedelic drugs on behavior, a wave of studies into the potential medicinal uses of these drugs began in the late 1950s and lasted throughout the next decade (Figure 1). However, the association of these drugs with counterculture, poor understanding of their powerful effects, and concerns over misuse ultimately led to strict scheduling. LSD, psilocybin, and mescaline were placed in the most restrictive drug categories (Schedule I in the US), criminalizing their possession and use but also creating political, societal, cultural, and fiscal impediments to scientific research into the underlying biology of psychedelic states. In fact, nearly all research, even in animal models, halted with the passage of the Controlled Substances Act of 1970 in the US, and similar legislation in other countries, declaring psychedelics dangerous substances with no medical value [4].

However, the appeal of psychedelic drugs did not completely abate during the 1980s through to the early 2000s, leading to a revival of research that is rapidly increasing our knowledge of
their mechanisms [5]. In the 1980s, the rigorous study of psychedelics in rodents began again in a few laboratories. At that time, research was primarily centered around using psychedelics like LSD as psychotomimetics, as well as examining their effects on locomotor activity, learning, memory, and other cognitive processes. Although the target of psychedelics, the serotonin 5-HT<sub>2A</sub> receptor, had not yet been cloned, it had become evident that the effects of these agents likely had something to do with the serotonin system, and that such studies using psychedelics as tools could clarify the role of serotonin in normal neurobiology and behavior. Around the same time, pharmacological tools like the selective 5-HT<sub>2</sub> receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI), and the antagonist ketanserin, became available, allowing the 5-HT<sub>2</sub> receptor to be identified as the target for psychedelics necessary and sufficient for their behavioral effects. The radiolabeled versions of these compounds were instrumental in mapping 5-HT<sub>2</sub> receptor expression in the brain, where high levels were identified in specific cortical areas [6]. When individual receptors were cloned, and additional pharmacological tools were developed that could distinguish among the three 5-HT<sub>2</sub> receptor family subtypes, the 5-HT<sub>2A</sub> receptor was determined to be the key target for psychedelic-mediated behaviors. For example, the behavioral effects of psychedelics like LSD and DOI are abrogated in the <i>HTR2A</i>−/− knockout mouse [7]. Finally, ketanserin, which is a relatively selective 5-HT<sub>2A</sub> receptor antagonist in humans, blocks the psychedelic effects of both psilocybin and LSD clinically [8].

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**Figure 1. History of Psychedelic Research.** We present a brief timeline of psychedelic biomedicine in general (A) and PubMed entries for the terms LSD and psilocybin to illustrate the effect of the 1970 Controlled Substances Act on psychedelic research (B). Inset: chemical structures of major psychedelic drugs mentioned in this review including LSD, psilocybin, mescaline, DMT (principal component of ayahuasca), and DOI. Abbreviations: DMT, dimethyltryptamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; fMRI, functional magnetic resonance imaging; LSD, lysergic acid diethylamide; PET, positron emission tomography.
Clinical trials of LSD and psilocybin have shown preliminary efficacy in treating nicotine [9] and alcohol addiction [10,11], depression [12], and end-of-life anxiety [13]. Human neuroimaging studies in healthy volunteers exposed to LSD [14], psilocybin [15], and ayahuasca [16], have recently been completed, whereas animal studies have shed light on the behavioral and physiological effects of these drugs [8]. These results were met with cautious optimism, as the prolonged furlough of psychedelic science has left many unanswered questions regarding the precise effects of psychedelics on the brain. Here, we summarize the current state of psychedelic clinical and animal research, evaluate the potential usefulness of psychedelics as therapeutic agents, and discuss their potential mechanisms of action at molecular, cellular, and circuitry levels. Although some drugs [e.g., Δ9-tetrahydrocannabinol (THC), ketamine, and 3,4-methylenedioxymethamphetamine (MDMA)] may produce similar states, and are sometimes called psychedelics in the literature, they do not share the same principal pharmacological site of action. Similar to previous authoritative reviews [8,17], we limit our discussion to classic psychedelic compounds that exert their major neurological effects via the 5HT2A receptor (Box 1). However, as MDMA shares serotonergic affinity profiles with many classic psychedelic drugs, we refer the reader to a recent review of the potential usefulness of MDMA in treating psychiatric disorders [18].

Effects of Psychedelic Drugs in Animal Models

In rodents, psychedelic drugs that are potent and selective agonists at the 5-HT2A receptor, such as DOI, induce anxiolytic-like effects [19,20]. Psychedelics can also enhance fear conditioning (observed as increased freezing behavior), and facilitate faster extinction of fear memory depending on if drug is administered during the acquisition or the extinction phase [21], and increase impulsivity in a dopamine D2 receptor-dependent manner [22]. Drug discrimination tasks show that discrimination of LSD with a 30-min preinjection period is blocked by 5-HT2A antagonists, whereas the cue from a 90-min preinjection is blocked by D2 antagonists [23]. These data point to a biphasic temporal response for LSD, with the initial phase mediated by 5-HT2A receptors, and the second phase by D2 and possibly other dopamine receptors. The biphasic response to LSD is unique among psychedelics, and the pharmacological mechanism of this later increase in dopaminergic activity has yet to be fully determined. Interestingly, animals chronically treated with LSD develop persistent behavioral abnormalities, which remain long after the drug is discontinued, reminiscent of those observed in other models of psychosis that include increased locomotion, hyper-reactivity, and anhedonia [24]. Additionally, other receptor systems, such as the trace amine-associated receptors (TAARs), necessitate further investigation with regard to psychedelic drug action (Box 2).

Box 1. Psychedelics Principally Act via 5-HT2A Receptors

The 5-HT2A receptor is a G-protein-coupled receptor (GPCR) that is encoded by the human HTR2A gene. It is the most widely expressed serotonin receptor in mammals and found in nearly every tissue type examined including muscle, endothelial, immune, and endocrine. In the brain, it is principally distributed in serotonin-rich terminal areas, but found in all regions examined at some level. Downstream signaling pathways from 5-HT2A receptor include the phospholipase C and inositol triphosphate signaling cascades, which result in a rise in intracellular calcium (Ca2+), arachidonic acid formation, extracellular signal-related kinase signaling, and β-arrestin signal transduction [7,47,74]. LSD and other psychedelic agonists at 5-HT2A show biased activation of specific downstream signaling cascades, a phenomenon known as functional selectivity. For example, LSD leads to a greater activation of β3-arrestin signaling compared to nonpsychedelic lysergamides [47]. A recent crystal structure of 5-HT2B receptor-bound LSD showed that LSD binds to a specific motif within the serotonin receptor that, when mutated, abolishes the LSD-induced induction of β3-arrestin [47]. Although many psychedelic drugs promiscuously activate numerous GPCRs and other signaling pathways, antagonist-assisted studies in both humans and model organisms have identified 5-HT2A as the main site of action responsible for the behavioral effects of psychedelics [7,8,28,29,75,76]. Although the 5-HT2A receptor is responsible for many effects of psychedelics, LSD and similar drugs also bind other serotonin receptors (such as 5-HT1A and 5-HT2C) and show dopaminergic and adrenergic effects at high doses.
Box 2. Novel TAARgets of Psychedelic Drug Exposure

The mind-altering activity of naturally occurring phenethylamine and tryptamine-related psychedelics, such as mesocodeine and psilocybin, has inspired the synthesis of several synthetic derivatives [77]. Tryptamine and phenethylamine both belong to the group of endogenous compounds termed trace amines that are hypothesized to act as neuromodulators or neurotransmitters [78]. Imbalances in trace amines may be involved in the pathology of various psychiatric disorders. For example, abnormal levels of phenethylamine have been hypothesized to contribute to schizophrenia [79]. In 2001, discovery of the trace amine-associated receptors (TAARs) GPCR subfamily rekindled interest in the biology and pharmacology of these compounds [80]. Intriguingly, DOI, dimethyltryptamine, and LSD can functionally activate the TAAR1 receptor [81, 82]. Further analysis revealed that TAAR1 generally acts as a brake on dopaminergic activity, thus paving the way for the development of TAAR1 agonists as novel psychoactive drugs with potential antipsychotic, antidepressant, and antiaddictive properties [83, 84]. Therefore, it is possible that TAARs may contribute to the potential clinical efficacy of psychedelics, and merit further scrutiny in both clinical and preclinical models.

Another commonly used rodent phenotype is the head twitch response (HTR) in mice, which includes rapid side-to-side rotational head movements (distinct from tremors or head weavings). Although administration of psychedelic drugs produces this behavior, and drugs like lisuride (that are described as non-hallucinogenic 5-HT2A receptor agonists) do not, HTR is also highly strain-dependent [25]. Complicating the interpretation of HTR, nonpsychedelics can produce HTR, and drugs like lisuride have affinity for several serotonin receptors, dopamine receptors, and are only very weak agonists of the 5-HT2A receptor. Nevertheless, HTR is absent in 5HT2A receptor knockout mice [7]. Interestingly, HTR induced by psychedelic administration is partially diminished in mGlur2R- [26] and 5HT2C knockout mice [25], suggesting a potential role for other receptor subtypes in mediating psychedelic-induced HTR.

The gold standard for measuring psychedelic-related behavior is the two-lever drug discrimination assay, where the ability of a test drug to substitute for the interoceptive cue produced by a rodent trained to recognize a known psychedelic in humans like LSD has high predictive validity with respect to subjective effects in humans [23]. Rodent models of psychedelic exposure are also used to answer other complex mechanistic questions. For example, how do different neurotransmitter receptors functionally interact with the 5-HT2A receptor, and how does 5-HT2A receptor activation alter molecular and genetic aspects of neuronal function? Additionally, how do individual psychedelic drugs differ from each other? The combination of medicinal chemistry, behavioral studies, and computational modeling have identified key residues of the binding pocket as well as intrahelical interactions necessary for activation of the 5-HT2A receptor. This work is ongoing in several laboratories to understand more fully structure–function relationships at the receptor, which will continue to inform fundamental G protein coupled receptor (GPCR) pharmacology. Importantly, the contemporary study of psychedelics is not limited to mammalian models, and has recently been applied to other species, such as zebrafish (Danio rerio) and fruit flies (Drosophila melanogaster), which are

Box 3. Of Fish and Flies: Recent Model Organisms to Study Psychedelic Drugs

Psychedelics like DOI have been instrumental tools to study how serotonin modulates fly behavior. For example, activation of 5-HT receptors with DOI, LSD, and psilocybin alters fly physiology similarly to humans, affecting visual processing, social interaction, aggression, circadian behaviors, and learning and memory. Given the high conservation of genes, physiology, and metabolic processes between flies and mammals, it is likely that discoveries made in the fly regarding the molecular and genetic effects of 5-HT receptor agonists will translate to a better understanding of basic serotonergic processes in humans. Zebrafish (Danio rerio) are a popular model for psychoactive drug screening due to their low cost and high-throughput potential [85]. Acute LSD administration increases zebrafish top swimming behavior in the novel tank test and increases time in the light compartment in the light–dark box test, indicative of reduced anxiety-like behavior [86]. LSD additionally decreases zebrafish shoal cohesion and increases whole-body cortisol levels, indicating altered sociality and endocrine regulation [86, 87] (also see similar top swimming evoked by mescaline [88]). Likewise, a recent study of behavioral effects of ayahuasca in zebrafish reported anxiolytic-like effects at lower, but reduced swimming activity and higher anxiety at higher, doses [89]. Taken together, the high sensitivity of fruit flies and zebrafish to psychedelic drugs emphasizes the developing utility of these novel model organisms in translational psychedelic biomedicine.
rapidly emerging as novel sensitive model organisms for translational psychedelic research and drug screening (Box 3).

Here, it is important to note the limitations inherent in using animal models to study a phenomenon, namely, the psychedelic state, which is probably unique to humans. For example, it is still unknown what aspect of the psychedelic drug experience (if any) is most recapitulated by the head twitch response, or what internal cues of an animal are informing lever-press responses in the drug discrimination assay. We will likely never know if a rodent, fish, or fly is actually hallucinating. Regardless, the behavioral effects in rodent and other models have allowed researchers to perform crucial tasks and gather highly relevant data that have revealed and provided important biological insights into the effects of psychedelics in humans.

Effects of Psychedelic Drugs on Human Cognition and Behavior

Recent psychedelic research has systematically probed the subjective effects of psychedelics on cognition and behavior in humans. This line of inquiry has necessitated the creation and validation of novel clinical scales to assess the effects of these drugs, such as the Altered States of Consciousness (APZ) questionnaire [27]. Generally, APZ probes three domains – oceanic boundlessness (positive effects on mood and wellbeing); anxious ego dissolution (altered self-awareness and transcendence of typical experience); and visionary restructuralization (visual effects). Typically, serotonergic psychedelics increase all these measures except anxiety [28–30]. A recent LSD study on healthy adults revealed increased positive mood and visual imagery alterations that peaked 1–5 h after administration versus placebo [30]. LSD additionally moderately and transiently increases heart rate, blood pressure, body temperature, and plasma levels of cortisol, prolactin, oxytocin, and epinephrine, with no effect on circulating norepinephrine [30]. Psilocybin produces similar increases in positive mood and a bias toward positive stimulus evaluation that is attenuated by pretreatment with the antagonist ketanserin, which is selective for the 5-HT2A receptor in humans [28]. The bias toward positive stimulus appraisal seen in both LSD and psilocybin studies may be caused by reduced amygdala reactivity during negative/fearful stimulus presentation in individuals given the respective psychedelic drugs versus a control state [31,32]. As the amygdala is strongly implicated in negative affective states, such as anxiety and depression, its modulation may contribute to the beneficial effects of psychedelic drugs in treating these disorders.

Imaging studies have further probed the neural substrates of psychedelic drug exposure [33], including widespread alterations in blood oxygen level-dependent (BOLD) signal in the brain by acute psilocybin [15], as well as increases in 18-fluorodeoxyglucose uptake in specific regions, together indicating that blood flow and metabolism are altered in the brain. Of particular note is decreased BOLD signal in the thalamus, which may contribute to altered sensory processing during the peak subjective effects of psychedelics. Resting state functional connectivity (RSFC) analyses of psilocybin imaging data have shown that the psychedelic state is characterized by the emergence of both short-lived and persisting connectivity networks between brain regions that normally do not show significant functional association [34]. Ayahuasca administration also significantly decreases activation of, and connectivity within, hubs of the default mode network (DMN); a network of brain regions showing a high degree of functional connectivity at rest, thought to underlie introspection [16]. LSD increases cerebral blood flow in the visual cortex and RSFC between the visual cortex and other brain regions not normally involved in visual processing, and these increases positively correlate with subjective changes in visual imagery [14]. Decreased RSFC between the parahippocampal gyrus and proximal regions (including the retrosplenial cortex) correlate with subjective measures of ego dissolution, implicating this neural circuit in self-awareness and reflexive processes [14]. Paralleling psilocybin and ayahuasca data, LSD also decreases network segregation between established circuits in the
brain, such as the DMN [14]. Collectively, these important neuroimaging studies continue to unravel potential circuitry underlying complex clinical effects of psychedelic drugs.

**Perspectives on Psychedelics in Modern Biomedicine**

**Psychedelic Drugs for Treatment of Anxiety and Depression**

As a result of the robust effects of psychedelics on mood, there is a sustained scientific interest in their potential for treating depression and anxiety, and several small clinical trials have recently reported promising results. For example, a single dose of psilocybin given in a program of psychotherapy is beneficial in patients with anxiety related to terminal cancer [13]. Cancer patients also demonstrated rapid increases in positive affect after psilocybin administration, with 60–80% showing improved anxiety and depression measures at a 6.5-months follow-up [35]. High-dose psilocybin treatment of cancer patients also reduced clinician- and patient-rated levels of anxiety and depression that persisted for at least 6 months in 80% of patients [36]. Interestingly, psilocybin reliably evokes mystical and spiritually meaningful experiences in patients; the degree of which correlates with the personality domain of openness [37,38]. Moreover, testing psilocybin in patients with treatment-resistant depression markedly reduced depression symptoms when measured 3 months later [12]. The efficacy of psilocybin in such severely affected cohorts suggests that psychedelic drugs may prove a novel pharmacotherapy for patients who suffer from mild to moderate depression and anxiety.

**Psychedelic Drugs for Treatment of Addiction**

Addiction exerts a large toll both on the individual and society [39]. As psychedelic drugs often exert long-lasting changes to behavior and cognition [12,35], these compounds have been tested for the potential to disrupt established patterns of addictive behavior. Recent meta-analyses of data on LSD as a treatment for alcoholism from the 1950s to 1960s showed its beneficial effect on alcohol abuse compared to various placebo and control conditions [11]. Only one small modern pilot study has examined the effects of a psychedelic drug on alcoholism, exposing patients with alcohol use disorder (AUD) to psilocybin-assisted psychotherapy [10], and reporting increased abstinence and fewer drinking days several weeks after treatment. Notably, within-individual improvement in patterns of alcohol misuse, particularly with regard to craving, correlates with the subjective intensity of the effects of psilocybin in the first drug administration session, suggesting that subjective experience plays an important role in the long-term behavioral changes induced by psychedelic drugs [10]. Another small clinical trial was recently performed to test the impact of psilocybin on smoking cessation, and found that 80% of patients were smoke-free 6 months after receiving two or three doses of psilocybin along with cognitive behavioral therapy (CBT) [9]. Analyses of this cohort also showed that the mystical qualities induced by the psilocybin sessions positively correlated with confidence of abstaining from tobacco, and negatively correlated with craving and temptation to smoke [40]. These promising pilot clinical trials for alcohol and nicotine abuse have led to expanded trials, now underway, and show promise for the therapeutic future of psychedelics in the treatment of addiction.

**Potential Neurobiological Mechanisms of Psychedelic Drugs**

Although the pharmacological profile of psychedelic drugs, including their agonism at the 5-HT2A receptor, is well known, it remains unclear exactly how these drugs cause such widespread and long-lasting effects on brain function and behavior at modest doses. Here, we focus on four classes of underlying effects of psychedelics, ranging from cellular and molecular effects in the brain to behavioral manifestations, which may help to explain the potential beneficial outcomes of psychedelic exposure on a number of etiologically varied psychiatric illnesses (Figure 2, Key Figure). Although some of these concepts have been discussed recently [41], here we place them in a novel context and expound on a hierarchy of the effects of psychedelics moving from molecular genetics to neurons to neural circuits to behavior.
Psychedelics Cause Robust Changes in Neuronal Transcription

Given the increasing popularity of transcriptome-wide methods such as RNA-sequencing and gene expression microarrays, only a few studies have investigated brain gene expression in response to psychedelics. The first unbiased genomic studies examined the effects of LSD in rat brain prefrontal cortex (PFC), and found that only a small number of genes were upregulated, and none downregulated. These included Krox20/Egr2 90 min after exposure, along with serum glucocorticoid kinase (Sgk1), inhibitor of nuclear factor κB (IκB-α), neuron-derived orphan receptor 1 (Nor1), and Ania3 [42]. All of these gene products play a role in synaptic plasticity. For example, Ania3, which itself is a splice isoform of Homer [43], complexes with the postsynaptic scaffolding protein Homer1a and regulates dopaminergic and glutamatergic signaling. The same study showed that LSD increases expression of cFos and activity-regulated cytoskeleton-associated protein (Arc) in the PFC. cFos is a transcription factor and a critical marker of neuronal activity, whereas Arc is a crucial synaptic protein [44]. A follow-up experiment confirmed that many, but not all, of these effects can be abolished or attenuated by 5-HT2A antagonist pretreatment [45], and that three additional genes are upregulated in the PFC of LSD-exposed rats: MAP kinase phosphatase 1 (Mkp1), core/enhancer binding protein B (C/ebp-B), and ILAD1 [later renamed arrestin domain-containing 2 (Arrdc2)] [46]. Notably, C/EBP-B is also involved in scaling of synaptic strength, and Arrdc2 is homologous to the arrestin family of proteins that are involved in the receptor-mediated response to psychedelics [47].

In a subsequent study by another group examining the mouse somatosensory cortex, LSD and DOI, but not the nonhallucinogenic 5-HT2A receptor agonist lisuride, upregulated genes of early growth response protein 1 (Egr1), 2 (Egr2), and Period-1 (Per1) 1 h after administration [48]. Egr1 and Egr2 are immediate early genes that encode for transcription factors regulating multiple genes, and Egr1 specifically is involved in memory encoding and synaptic organization.
Although these studies have mostly focused on cortical regions, expression changes examined in the hippocampus and thalamic areas for some of these genes indicate regional differences in response. For example, Nor1 is increased in PFC and thalamic regions, but not the hippocampus and Arc is increased several-fold more in the PFC than hippocampus, and not detected in the thalamic region. Expression in other areas, like amygdala, remain to be tested. As many of the genes that are upregulated by psychedelics are rapidly induced immediate early genes, and their corresponding proteins perform actions at or near the synapse, psychedelics likely alter neurotransmission and synaptic morphology that could underlie both acute behavioral changes and those that may persist long after the initial drug exposure. Notably, the PFC and somatosensory cortex of rodents show considerable homology to those of humans [51], indicating that these results may translate well across species. Additionally, other brain regions indicated in the human response to psychedelics such as the amygdala also show between-species homology, although there are notable circuit-level differences [52].

Psychedelics Differentially Activate Distinct Cellular Pathways and Brain Regions

Recently, flow cytometry was used to isolate and characterize brain cells that directly respond to psychedelics. Surprisingly, only ~5% of cortical excitatory neurons become transcriptionally active in response to either DOI or LSD [53]. Although we cannot be certain that these neurons have actually depolarized in response to psychedelics, there is a general consensus that transcriptional activation of immediate early genes likely signifies that a neuron has become electrically active. Gene expression analysis indicates that the activated population of neurons express ~4-fold higher levels of Htr2a mRNA than the nonactivated neurons do [53]. Interestingly, in both the activated and nonactivated populations, 5-HT2A receptor internalization occurs. This subpopulation of excitatory neurons that becomes transcriptionally active in response to 5-HT2A receptor agonists has been termed the trigger population, and their activation is hypothesized to be necessary to initiate the cascade of cellular events ultimately leading to disintegration of the default mode network of the brain and hallucinogenic behaviors [53]. Importantly, connections between immediate early gene expression, neural circuit alteration, and behavior have been previously established. For example, rodent circuit-level alterations in the response to cocaine, specifically in corticolimbic BOLD functional magnetic resonance imaging (fMRI) measures, are observed after treatment with the histone deacetylase (HDAC) inhibitor sodium butyrate, which acts epigenetically to activate gene expression [54]. HDAC inhibitors additionally facilitate the updating of fear memories from the distant past by activating a specific set of immediate-early genes involved in synaptic regulation [55].

Although the psychedelic-responsive neurons are a relatively small fraction of the total neuronal population, they reside in regions densely connected to many other brain structures including the medial PFC and claustrum. High-quality imaging of the mouse claustrum shows a high degree of connectivity to the cortex, hippocampus, and other regions, and that the projection neurons emanating from the claustrum may serve as a ‘superhub’ to coordinate neuronal responses across numerous discrete circuits [56]. Here, we further extend this notion, to include the ability of the widespread projections of the trigger population to modulate synaptic connections, possibly via the induction of immediate early genes such as Arc and cFos. These neuron-level effects likely inform the robust changes in functional connectivity induced by psychedelic drugs, mentioned earlier. In addition to their effects on small subsets of excitatory neurons, psychedelics lead to activation of 5–10% of select types of inhibitory gamma-aminobutyric acid (GABA)-ergic interneurons, as well as non-neuronal cells like astrocytes [53]. Interestingly, the gene expression profile induced by psychedelics in a given type of cell differs by the region of the brain where it resides [53]. These regional differences in gene expression may result in subtle differences in plasticity between structures, and underlie regional differences in network connectivity observed by imaging studies. To summarize, psychedelics
appear to directly activate only small populations of excitatory neurons, which then recruit subsets of inhibitory GABAergic interneurons and non-neuronal cells like glia and astrocytes in regionally distinct patterns to modulate brain activity.

Psychedelic Drugs Induce Entropic Brain Activity

The human brain mapping studies of psychedelic drugs reveal distinct effects based on the exact dose, compound, time course, and imaging methods used. However, some broad conclusions can be drawn based on the common effects of psychedelic compounds on key measures, such as cerebral blood flow and functional connectivity. For instance, both LSD and psilocybin alter occipital cortex oscillatory power [14,57], and LSD exposure elevates V1 visual cortical blood flow and RSFC with brain areas that do not normally connect to V1. These effects correlate with the degree of visual perceptual changes seen following LSD exposure [14], and may contribute to visual hallucinations and related disturbances evoked by psychedelics. One of the most striking findings in RSFC patterns after LSD exposure is the ability of psychedelics to markedly alter established patterns of connectivity within the brain. Compared to placebo, LSD reduces the within-network stability of established brain networks (such as the DMN, visual, auditory, and sensorimotor networks), while also decreasing the separateness or ability to distinguish between these networks [14]. The breakdown between conventional pathways of neuronal communication, as well as the emergence of novel RSFC patterns, is in line with a recent hypothesis that psychedelics lead to entropic activity within the brain [58]. Specifically, psychedelics may induce a brain state whereby established resting state networks break down, and novel local connectivity hubs form between regions that show little connectivity in a baseline state, with stable long-range functional associations also formed over considerable neural distance [34,59]. The concept of psychedelic-induced entropic brain activity has recently emphasized altered connectivity [41], including disruption of established resting state networks and failure of hub regions, in various states of psychopathology [60]. Such broadband alteration in RSFC and the induction of hub failure may allow the brain to re-enter a state of widespread global plasticity, whereby the maladaptive patterns responsible for the manifestation of psychiatric illness can be reset [41]. If true, this may be one of the reasons why psychedelic drugs show promising (albeit preliminary) efficacy in treating psychiatric illnesses of different etiology. Thus, the upregulation of synaptic plasticity genes, as well as the activation of cells like microglia that may act to reduce inflammation within the brain, can serve as one molecular mechanism underlying the increase in entropic brain activity by psychedelic drugs. Understanding the precise molecular etiology of psychedelic-induced states will shed greater light on their ability to alter perception profoundly and will offer insights into their potential therapeutic effects.

Psychedelic Drugs Induce Mystical Experiences Related to Therapeutic Potential

Despite the accumulated body of knowledge of molecular, cellular, and circuit-level changes seen in the brains of animals and humans exposed to psychedelics, some important, higher-order affected domains remain unclear. For decades, psychedelics have been known to trigger powerful experiences of a mystical/spiritual nature, but only recently has this psychological phenomenon been examined with appropriate scientific rigor [61]. One of the most striking observations is the lasting effect that the mystical experience has on the patient. For example, 2 months after psilocybin administration, patients rated the experience highly on measures of personal significance and reported higher positive affect attributed to the drug experience [61]. Many of these positive effects on wellbeing were still present when measured 14 months after drug administration [62]. In line with these studies in healthy volunteers, other studies of psychedelics in brain disorders, such as addiction, also measured the mystical qualities of the experience. As already noted, the mystical nature and spiritual significance of psilocybin treatment positively correlated with decreased craving to smoke in patients with tobacco addiction [40], with nearly all individuals rating the psilocybin experience as one of the top five most spiritually meaningful experiences in their lifetimes [63]. The subjective mystical nature,
intensity, and level of personal significance assigned to the experience correlate with long-term positive outcomes in both healthy individuals and patients with tobacco addiction. Likewise, ayahuasca treatment increases traditionally measured mindfulness capabilities by reducing reactivity and judgmental processing of stimuli [64]. Therefore, the ability to induce an exceptionally meaningful experience and to increase measures of mindfulness may both contribute to the noted increases in wellbeing and mood following exposure to these drugs.

Although serious adverse reactions to psychedelic drugs have not been seen in modern, controlled-environment human trials, more research is needed to compare the effects of different doses and treatment schedules. Hallucinogen persisting perception disorder (HPPD), colloquially referred to as acid flashbacks, is characterized by intermittent perceptual disturbances that mimic the psychedelic state that are experienced long after the drug has left the body [65]. Notably, the prevalence of HPPD is largely unknown due to the understudied nature of psychedelics in general. An online questionnaire found that 4.2% of respondents who had used psychedelics in the past reported persistent visual disturbances that were severe enough that the patients would consider treatment [66], although this is possibly an overestimate given that people with visual issues were more likely to complete the survey. Determining the neural substrates involved in acid flashbacks may shed further light on the neurological processes affected by psychedelics. Another question regards the relationship between the biological effects of psychedelics, including the alterations in gene expression and neuronal activity, with the psychological phenomenon of mystical experiences. It is possible that these two concepts show a positive correlation, such that increased subjective ratings of the personal and spiritual meaning of the psychedelic experience reflect a greater increase in synaptic activity-related gene expression, which causes more robust entropic brain activity. However, it is also possible that other factors contribute to the psychological ratings of the experience, and that internal states modulate ongoing brain activity and biology. Although these two domains are interconnected, and likely to a degree that we cannot begin to appreciate at the present juncture, future work should focus on correlating biological endpoints to subjective ratings of the psychedelic experience.

Beyond the Brain: Peripheral Effects of Psychedelics
Mind–body interaction is an essential feature of many human disorders, and peripheral mechanisms of psychedelic action should be considered as well. At physiological concentrations, serotonin itself acting via 5-HT₃ receptors is primarily proinflammatory, leading to increased expression of inflammatory cytokines [67]. Surprisingly, when psychedelics are tested in cell culture models of inflammation they have potent anti-inflammatory effects. All psychedelics tested, including LSD, completely inhibit the inflammation produced by tumor necrosis factor (TNF-α) in rat aortic smooth muscle cells [68]. The increased production of icam1, vcam1, il6, and activation of inducible nitric oxide synthase was completely suppressed. (R)-DOI is a particularly potent anti-inflammatory drug in this assay. Experiments indicated that the suppression of inflammation induced by TNF-α is rapidly occurring at the signal transduction level and not involving changes in gene expression, and that treatment with psychedelics suppresses inflammation already induced by TNF-α treatment [68]. Significantly, (R)-DOI also produces anti-inflammatory effects in human cells in vitro, suggesting that this finding may translate to humans.

These in vitro experiments were followed up by studies in whole animals. Once again, (R)-DOI demonstrated potent anti-inflammatory effects and blocked the production of proinflammatory cytokines and chemokines induced by TNF-α [69]. Significantly, drug levels far below the threshold necessary to produce hallucinogenic behaviors produce maximal anti-inflammatory effect. Therefore, psychedelics potentially represent the first-in-class bioavailable small molecule TNF-α inhibitors that act to inhibit inflammation through intracellular mechanisms at
relevant targets, rather than biologics on the market that act essentially to sequester TNF-α from circulation.

A study with radiolabeled 2,5-dimethoxy-4-bromopropylamine (DOB), a psychedelic phencyclidine closely related to DOI in structure, revealed a substantial fraction was sequestered in the lungs of human volunteers [70]. When tested, (R)-DOI demonstrated potent effects to prevent the development of asthma in a mouse model [71]. At low sub-behavioral doses of inhaled nebulized (R)-DOI, key physiological symptoms of asthma including peribronchial inflammation, eosinophilia, mucus overproduction, and airway hyper-responsiveness to methacholine were completely prevented. Molecular examination of lung tissues and immune cells indicated that (R)-DOI suppresses T helper 2 cell recruitment, and production of several proinflammatory cytokines and chemokines including interleukin (IL)-5, IL-6, and granulocyte–macrophage colony-stimulating factor [71]. Surprisingly, levels of other cytokines believed to be involved in the development of asthma, like IL-4, were unchanged and remained elevated, despite the absence of any asthma-like symptoms in the mice [71]. Although previous work demonstrated profound inhibition of the effects of TNF-α, it is unclear whether blockade of TNF-α is a significant factor in the ability of (R)-DOI to prevent inflammation associated with asthma. Importantly, psychedelics other than (R)-DOI have been demonstrated to have antiasthmatic effects not only in mice but also in rats (unpublished data), indicating that the effect is not unique to this particular drug, or to mice, but may extend to several other compounds that activate 5-HT2A receptors in other mammalian species including humans. These data suggest that unlike general glucocorticoids, which act as immunosuppressants, psychedelics selectively target subsets of proinflammatory pathways in key tissues relevant to inflammatory disorders like asthma. A key feature of this activity is that, at least for (R)-DOI, these drugs require only small sub-behavioral doses that are orders of magnitude less than what is necessary to affect perception and behavior. It will be interesting to determine how these anti-inflammatory effects observed in rodent models translate to the treatment of human disease. With about 60% of the adult population suffering from some form of inflammatory disorder where preclinical data with (R)-DOI exists suggesting therapeutic efficacy (e.g., asthma, cardiovascular disease, metabolic disorder, and inflammatory bowel disease), the development of psychedelics to treat peripheral inflammation at sub-behavioral levels has the potential to have a far greater impact on human health than in the treatment of psychiatric disorders.

Concluding Remarks

In summary, we hypothesize that the induction of synapse-related gene expression in key neuronal populations identified by molecular studies and the robust alterations observed in neural circuits by imaging studies are highly interrelated processes (Figure 2). The ability of psychedelic drugs, such as LSD and psilocybin, to cause disruption of established neural connectivity and the emergence of novel functional associations may underlie the therapeutic efficacy of psychedelics in a wide variety of psychiatric illnesses [41]. The results of psychedelic research using animal models, such as the induction of specific synapse-associated genes, puts the human neuroimaging findings of markedly altered functional connectivity into a new context. Therefore, biomedical research into psychedelics should take on a more translational nature (Figure 3), in which reverse translation (or bedside to bench) plays a prominent role. Questions remain as to the exact cellular and molecular mechanisms underlying the response to psychedelic drugs. For example, why do different brain regions have different transcriptional responses in what appear to be the same cell type? What roles do interneurons and glia play in hallucinogenic behaviors? Investigators can therefore prioritize brain regions that are altered by psychedelic exposure in fMRI studies, such as the visual cortex, hippocampus, and amygdala, to further elucidate what molecular and cellular mechanisms may underlie the broad network changes seen in those regions.
Clearly, many questions remain regarding the biology of these compounds and their use in the clinic (see Outstanding Questions). Drug development in psychiatry has lagged dramatically behind other biomedical disciplines [72,73], and many of the most commonly used psychiatric medications were discovered serendipitously and often also possess complex adverse effects. Therefore, given the promising results seen in early phase clinical trials with psychedelic drugs for the treatment of addiction, anxiety, and depression, the research community must validate the efficacy of these drugs in more rigorous trials to achieve a better understanding of their mechanisms in the body.

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