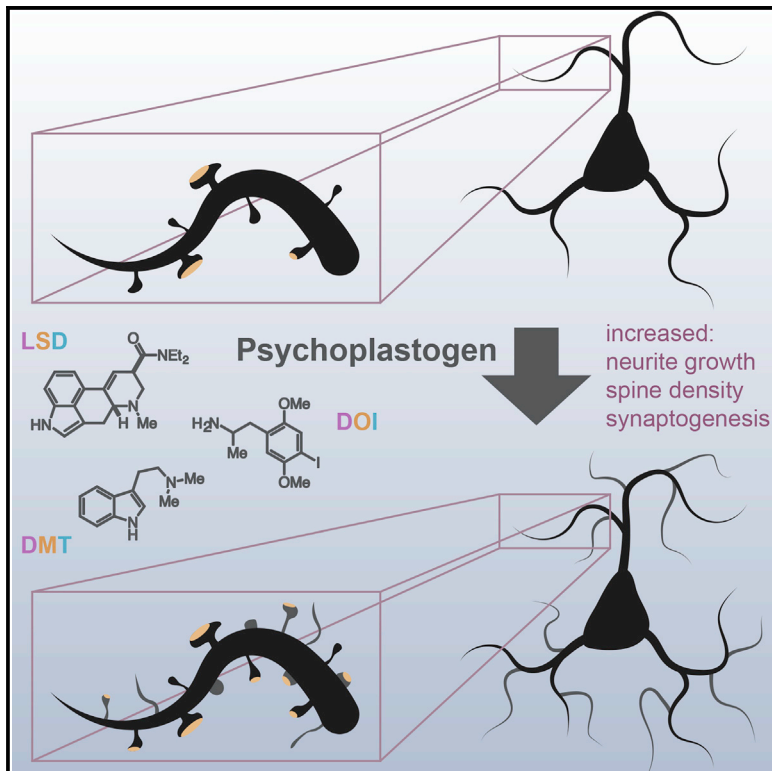


Psychedelics Promote Structural and Functional Neural Plasticity

Graphical Abstract



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In Brief

Ly et al. demonstrate that psychedelic compounds such as LSD, DMT, and DOI increase dendritic arbor complexity, promote dendritic spine growth, and stimulate synapse formation. These cellular effects are similar to those produced by the fast-acting antidepressant ketamine and highlight the potential of psychedelics for treating depression and related disorders.

Highlights

- Serotonergic psychedelics increase neuritogenesis, spinogenesis, and synaptogenesis
- Psychedelics promote plasticity via an evolutionarily conserved mechanism
- TrkB, mTOR, and 5-HT_{2A} signaling underlie psychedelic-induced plasticity
- Noribogaine, but not ibogaine, is capable of promoting structural neural plasticity



Psychedelics Promote Structural and Functional Neural Plasticity

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SUMMARY

Atrophy of neurons in the prefrontal cortex (PFC) plays a key role in the pathophysiology of depression and related disorders. The ability to promote both structural and functional plasticity in the PFC has been hypothesized to underlie the fast-acting antidepressant properties of the dissociative anesthetic ketamine. Here, we report that, like ketamine, serotonergic psychedelics are capable of robustly increasing neurogenesis and/or spinogenesis both *in vitro* and *in vivo*. These changes in neuronal structure are accompanied by increased synapse number and function, as measured by fluorescence microscopy and electrophysiology. The structural changes induced by psychedelics appear to result from stimulation of the TrkB, mTOR, and 5-HT_{2A} signaling pathways and could possibly explain the clinical effectiveness of these compounds. Our results underscore the therapeutic potential of psychedelics and, importantly, identify several lead scaffolds for medicinal chemistry efforts focused on developing plasticity-promoting compounds as safe, effective, and fast-acting treatments for depression and related disorders.

INTRODUCTION

Neuropsychiatric diseases, including mood and anxiety disorders, are some of the leading causes of disability worldwide and place an enormous economic burden on society (Gustavsson et al., 2011; Whiteford et al., 2013). Approximately one-third of patients will not respond to current antidepressant drugs, and those who do will usually require at least 2–4 weeks of treatment before they experience any beneficial effects

(Rush et al., 2006). Depression, post-traumatic stress disorder (PTSD), and addiction share common neural circuitry (Arnsten, 2009; Russo et al., 2009; Peters et al., 2010; Russo and Nestler, 2013) and have high comorbidity (Kelly and Daley, 2013). A preponderance of evidence from a combination of human imaging, postmortem studies, and animal models suggests that atrophy of neurons in the prefrontal cortex (PFC) plays a key role in the pathophysiology of depression and related disorders and is precipitated and/or exacerbated by stress (Arnsten, 2009; Autry and Monteggia, 2012; Christoff et al., 2011; Duman and Aghajanian, 2012; Duman et al., 2016; Izquierdo et al., 2006; Pittenger and Duman, 2008; Qiao et al., 2016; Russo and Nestler, 2013). These structural changes, such as the retraction of neurites, loss of dendritic spines, and elimination of synapses, can potentially be counteracted by compounds capable of promoting structural and functional neural plasticity in the PFC (Castrén and Antila, 2017; Cramer et al., 2011; Duman, 2002; Hayley and Litteljohn, 2013; Kolb and Muhammad, 2014; Krystal et al., 2009; Mathew et al., 2008), providing a general solution to treating all of these related diseases. However, only a relatively small number of compounds capable of promoting plasticity in the PFC have been identified so far, each with significant drawbacks (Castrén and Antila, 2017). Of these, the dissociative anesthetic ketamine has shown the most promise, revitalizing the field of molecular psychiatry in recent years.

Ketamine has demonstrated remarkable clinical potential as a fast-acting antidepressant (Berman et al., 2000; Ionescu et al., 2016; Zarate et al., 2012), even exhibiting efficacy in treatment-resistant populations (DiazGranados et al., 2010; Murrugh et al., 2013; Zarate et al., 2006). Additionally, it has shown promise for treating PTSD (Feder et al., 2014) and heroin addiction (Krupitsky et al., 2002). Animal models suggest that its therapeutic effects stem from its ability to promote the growth of dendritic spines, increase the synthesis of synaptic proteins, and strengthen synaptic responses (Autry et al., 2011; Browne and Lucki, 2013; Li et al., 2010).



Like ketamine, serotonergic psychedelics and entactogens have demonstrated rapid and long-lasting antidepressant and anxiolytic effects in the clinic after a single dose (Bousso et al., 2008; Carhart-Harris and Goodwin, 2017; Grob et al., 2011; Mithoefer et al., 2013, 2016; Nichols et al., 2017; Sanches et al., 2016; Osório et al., 2015), including in treatment-resistant populations (Carhart-Harris et al., 2016, 2017; Mithoefer et al., 2011; Oehen et al., 2013; Rucker et al., 2016). In fact, there have been numerous clinical trials in the past 30 years examining the therapeutic effects of these drugs (Dos Santos et al., 2016), with 3,4-methylenedioxymethamphetamine (MDMA) recently receiving the “breakthrough therapy” designation by the Food and Drug Administration for treating PTSD. Furthermore, classical psychedelics and entactogens produce antidepressant and anxiolytic responses in rodent behavioral tests, such as the forced swim test (Cameron et al., 2018) and fear extinction learning (Cameron et al., 2018; Catlow et al., 2013; Young et al., 2015), paradigms for which ketamine has also been shown to be effective (Autry et al., 2011; Girgenti et al., 2017; Li et al., 2010). Despite the promising antidepressant, anxiolytic, and anti-addictive properties of serotonergic psychedelics, their therapeutic mechanism of action remains poorly understood, and concerns about safety have severely limited their clinical usefulness.

Because of the similarities between classical serotonergic psychedelics and ketamine in both preclinical models and clinical studies, we reasoned that their therapeutic effects might result from a shared ability to promote structural and functional neural plasticity in cortical neurons. Here, we report that serotonergic psychedelics and entactogens from a variety of chemical classes (e.g., amphetamine, tryptamine, and ergoline) display plasticity-promoting properties comparable to or greater than ketamine. Like ketamine, these compounds stimulate structural plasticity by activating the mammalian target of rapamycin (mTOR). To classify the growing number of compounds capable of rapidly promoting induced plasticity (Castrén and Antila, 2017), we introduce the term “psychoplastogen,” from the Greek roots psych- (mind), -plast (molded), and -gen (producing). Our work strengthens the growing body of literature indicating that psychoplastogens capable of promoting plasticity in the PFC might have value as fast-acting antidepressants and anxiolytics with efficacy in treatment-resistant populations and suggests that it may be possible to use classical psychedelics as lead structures for identifying safer alternatives.

RESULTS

Psychedelics Promote Neuritogenesis

Because atrophy of cortical neurons is believed to be a contributing factor to the development of mood and anxiety disorders (Christoffel et al., 2011; Duman and Aghajanian, 2012), we first treated cultured cortical neurons with psychedelics from a variety of structural classes (Figures 1A and S1A) and measured the resulting changes in various morphological features. Using Sholl analysis (Ristanović et al., 2006), we observed that several psychedelics increased dendritic arbor complexity comparably to ketamine, as measured by the area under the curve of the Sholl plots as well as the maximum number of crossings (Figures

1B–1E and S1B–S1E). This increase in arbor complexity appeared to result from large changes in both the number of dendritic branches and the total length of the arbors (Figures 1F, 1H, S1F, and S1H). Psychedelics had a limited effect on the number of primary dendrites and did not alter the length of the longest dendrite (Figures 1G, 1I, S1G, and S1I).

Nearly all psychedelic compounds tested were capable of robustly promoting neuritogenesis, with comparable effects being produced by tryptamines (*N,N*-dimethyltryptamine [DMT] and psilocin), amphetamines (2,5-dimethoxy-4-iodoamphetamine [DOI] and MDMA), and ergolines (lysergic acid diethylamide [LSD]). As a positive control, we treated cells with 7,8-dihydroxyflavone (DHF), a psychoplastogen structurally dissimilar to classical psychedelics (Jang et al., 2010), and found that it also increased dendritic arbor complexity (Figure S2). This neurite outgrowth structural phenotype seems to only be induced by select compounds because serotonin and D-amphetamine, molecules that are chemically related to classical psychedelics and entactogens, exerted minimal to no effects on neuritogenesis (Figure S2).

To establish the relative potencies and efficacies of hallucinogens and entactogens for promoting neurite outgrowth, we conducted 8-point dose-response studies (Figure S3). We defined 100% and 0% efficacy as the maximum number of crossings induced by ketamine (10 μ M) and vehicle (0.1% DMSO), respectively. We chose the 10 μ M concentration of ketamine as the upper limit because this concentration of ketamine is reached in the brain following intraperitoneal administration of an antidepressant dose in rats (Yang et al., 2018). For consistency, we used this same concentration when testing the effects of psychedelics and entactogens, with DMT being the only exception. We used a maximum 90 μ M concentration of DMT in our studies to more closely mimic the brain concentration of DMT in rats treated with an antidepressant dose (Cohen and Vogel, 1972; Cameron et al., 2018). In this neuritogenesis assay, ketamine's half maximal effective concentration (EC₅₀) value was 132 nM. Surprisingly, the majority of the psychedelics and entactogens we tested exhibited significantly greater potency than ketamine, with LSD being particularly potent (EC₅₀ = 0.409 nM). In fact, LSD exhibited activity across 8 orders of magnitude into the low picomolar range (Figure S3).

Notably, the anti-addictive alkaloid ibogaine (Alper, 2001; Belgers et al., 2016) was the only psychedelic tested that had absolutely no effect (Figure S4). This was a surprising result because we hypothesized that ibogaine's long-lasting anti-addictive properties might result from its psychoplastogenic properties. Previous work by He et al. (2005) clearly demonstrated that ibogaine increases the expression of glial cell line-derived neurotrophic factor (GDNF) and that this plasticity-promoting protein is critical to ibogaine's anti-addictive mechanism of action. Because several reports have suggested that noribogaine, a metabolite of ibogaine, might actually be the active compound *in vivo* (Zubaran et al., 1999; Baumann et al., 2000, 2001), we decided to test its ability to promote neuritogenesis in cultured cortical neurons. Gratifyingly, noribogaine robustly increased dendritic arbor complexity with an EC₅₀ value comparable to ketamine (Figure S3), providing additional evidence suggesting that it may be the active compound *in vivo*.

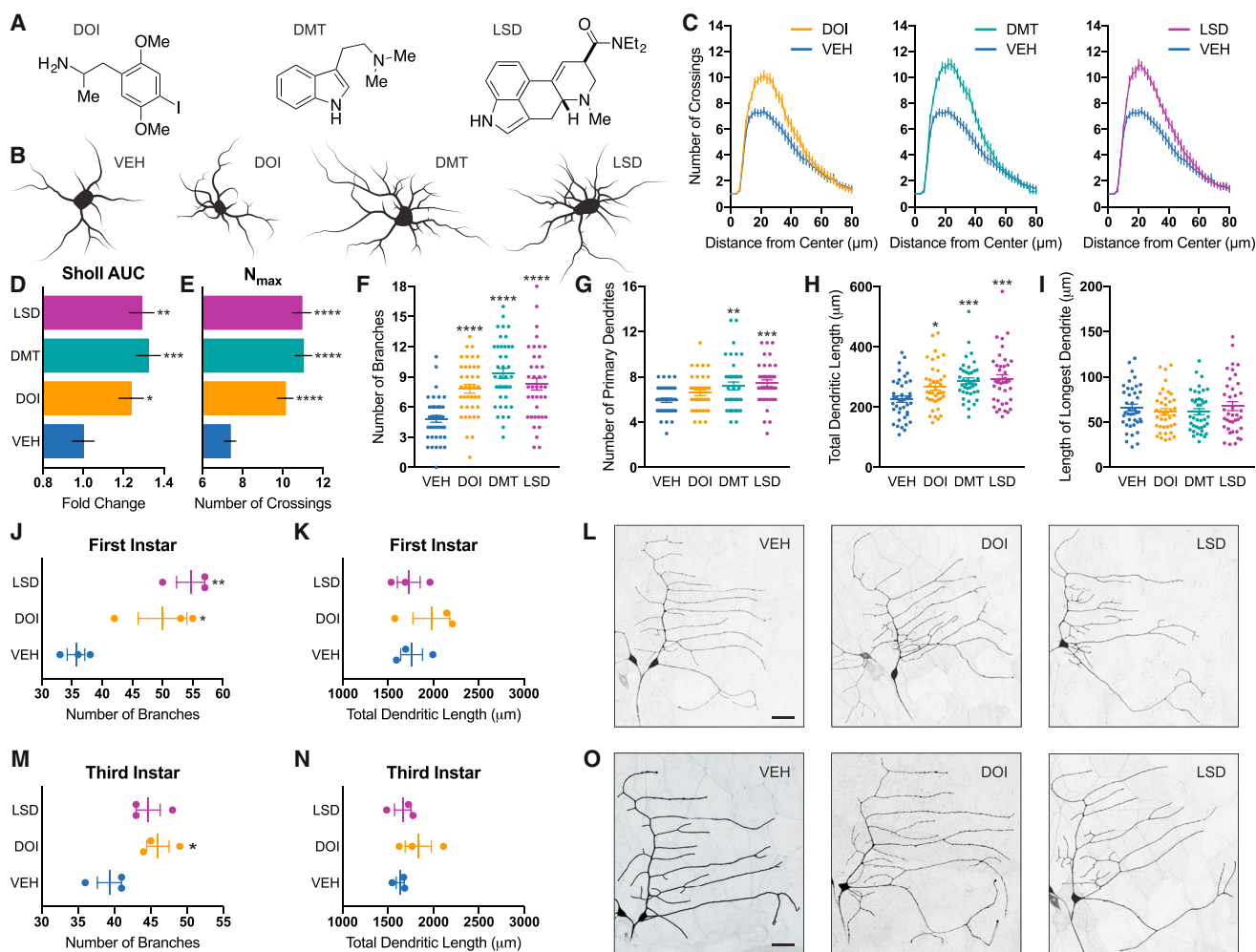


Figure 1. Psychedelics Promote Neuritogenesis both *In Vitro* and *In Vivo*

(A) Chemical structures of psychedelics.

(B) Representative tracings of cortical neurons (DIV6) treated with compounds.

(C) Sholl analysis demonstrates that psychedelics increase dendritic arbor complexity ($n = 39\text{--}41$ neurons).

(D) Area under the curve (AUC) of the Sholl plots in (C).

(E) Maximum number of crossings (N_{\max}) of the Sholl plots in (C).

(F–I) Cortical neurons treated with psychedelics display an increase in the number of branches (F), the number of primary dendrites (G), and the total length of the dendritic arbor (H) but not the length of the longest dendrite (I).

(J and K) Class I neurons from *Drosophila* larvae treated with psychedelics during the first instar display increased branching (J) but not total length of the dendritic arbor (K) ($n = 3$ neurons).

(L) Representative images of neurons from (J) and (K).

(M and N) Class I neurons from *Drosophila* larvae treated with psychedelics during the third instar display increased branching (M) but not total length of the dendritic arbor (N) ($n = 3$ neurons).

(O) Representative images from (M) and (N).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, as compared to vehicle control (VEH). Scale bars, 30 μm . Data are represented as mean \pm SEM. See also Figures S1–S5.

To assess the *in vivo* effects of classical psychedelics on neuritogenesis, we started treating *Drosophila* larvae during the first instar with LSD and DOI. As observed in rodent cortical cultures, both LSD and DOI significantly increased dendritic branching of class I sensory neurons; however, they did not increase the total length of the dendritic arbors (Figures 1J–1L). Because of the striking effects of psychedelics on the structures of immature

neurons, we hypothesized that they might influence neurodevelopment. To test this, we chronically treated zebrafish embryos with compounds for 6 days immediately following dechoriation and assessed gross morphological changes and behavior. We did not observe any differences in head sizes between the treatment groups, nor did we detect any statistically significant differences in activity levels (Figure S5). Next we assessed the

ability of psychedelics to promote neuritogenesis in more mature neurons by starting to treat *Drosophila* larvae during the late second instar. Again, psychedelics increased the branching of class I neurons, although the effect was less dramatic than that observed when treatment was started during the first instar (Figure 1M–1O). Although different developmental stages might be more or less susceptible to the effects of psychedelics, it is also possible that the smaller effect size observed after administering compounds starting at the later time point was simply the result of treating the larvae for a shorter period of time. Regardless, it was quite surprising to observe compound-induced changes in neuronal structure after initiating treatment during the late second instar because class I neurons are stereotyped and typically possess relatively few higher-order branches (Grueber et al., 2002). Moreover, our results demonstrate that psychedelics can promote changes in neuronal structure across vertebrate (rats) and invertebrate (*Drosophila*) species, suggesting that they act through an evolutionarily conserved mechanism.

Psychedelics Promote Spinogenesis and Synaptogenesis

In addition to dendritic atrophy, loss of dendritic spines is a hallmark of depression and other neuropsychiatric disorders (Christoffel et al., 2011; Duman and Aghajanian, 2012), so we next assessed the effects of psychedelics on spinogenesis. We treated mature rat cortical cultures for 24 hr with DOI, DMT, and LSD as representative compounds from the amphetamine, tryptamine, and ergoline classes of psychedelics, respectively. All three compounds increased the number of dendritic spines per unit length, as measured by super-resolution structured illumination microscopy (SIM) (Figures 2A, 2B, and S6), with LSD nearly doubling the number of spines per 10 μm . Additionally, treatment caused a shift in spine morphology, favoring immature (thin and filopodium) over more mature (mushroom) spine types (Figure 2C). Colocalization of pre- and postsynaptic markers following treatment demonstrated that psychedelics promoted synaptogenesis by increasing the density, but not the size of synapses (Figure 2D–2F). This increase in synapse density was accompanied by an increase in the density of VGLUT1 puncta, but not PSD-95 puncta, following compound administration (Figures 2G and 2H).

Encouraged by our *in vitro* results, we next assessed the effects of a single intraperitoneal dose of DMT on spinogenesis in the PFC of adult rats using Golgi-Cox staining. We chose to administer a 10 mg/kg dose of DMT for three reasons. First, all available data suggested that this dose would produce hallucinogenic effects in rats with minimal safety risks (Glennon et al., 1980, 1983; Glennon, 1999; Gatch et al., 2009; Smith et al., 1998; Appel et al., 1999; Winter et al., 2007; Carbonaro et al., 2015; Helsley et al., 1998; Strassman et al., 1994; Nair and Jacob, 2016). Second, we have previously shown that a 10 mg/kg dose of DMT produces positive effects in rat behavioral tests relevant to depression and PTSD (Cameron et al., 2018). Finally, we wanted to directly compare the effects of DMT with ketamine, and seminal studies conducted by Li et al. (2010) had previously demonstrated that a 10 mg/kg dose of ketamine produced a robust increase in dendritic spine density in

the PFC of rats. We observed a significant increase in the density of dendritic spines on cortical pyramidal neurons 24 hr after dosing with DMT (Figures 2I and 2J). This effect was comparable with that produced by ketamine at the same dose (Figure 2J). Importantly, this DMT-induced increase in dendritic spine density was accompanied by functional effects. *Ex vivo* slice recordings revealed that both the frequency and amplitude of spontaneous excitatory postsynaptic currents (EPSCs) were increased following DMT treatment (Figures 2K–2M). Interestingly, 10 mg/kg and 1 mg/kg doses produced similar responses despite the fact that they are predicted to be hallucinogenic and subhallucinogenic, respectively (Strassman et al., 1994; Nair and Jacob, 2016).

Because the half-life of DMT is exceedingly short (~ 15 min), these results confirm that structural and functional changes induced by DMT persist for hours after the compound has been cleared from the body. Moreover, they demonstrate that DMT produces functional effects on pyramidal neurons of the PFC that mirror those produced by ketamine (Li et al., 2010). Because the PFC is a key brain region involved in extinction learning (Quirk et al., 2006), and both ketamine and DMT have been shown to facilitate fear extinction (Cameron et al., 2018; Girgenti et al., 2017), our results suggest a link between the plasticity-promoting and behavioral effects of these drugs. Because fear extinction can be enhanced by increasing levels of brain-derived neurotrophic factor (BDNF) in the PFC (Peters et al., 2010), and ketamine's behavioral effects have been shown to be BDNF-dependent (Lepack et al., 2014), we next sought to determine the role of BDNF signaling in the plasticity-promoting effects of classical psychedelics.

Psychedelics Promote Plasticity through a TrkB- and mTOR-Dependent Mechanism

The role of BDNF in both neuritogenesis and spinogenesis is well known (Cohen-Cory et al., 2010), and several reports suggest that psychedelics are capable of increasing levels of neurotrophic factors (He et al., 2005; Martin et al., 2014; Nichols and Sanders-Bush, 2002; Vaidya et al., 1997). Therefore, we treated cortical neurons with BDNF, DOI, and a combination of the two to see whether they had any additive or synergistic effects. Dose-response studies using recombinant BDNF (Figures 3A–3C) revealed that a 50 ng/mL treatment increased neuritogenesis to a comparable extent as DOI (10 μM). Moreover, a combination of the two did not confer any added benefit, suggesting that they operate through a related mechanism (Figures 3D–3F). Next, we treated cortical neurons with DOI, DMT, and LSD for 24 hr before measuring BDNF gene and protein expression using droplet digital PCR (ddPCR) and ELISA, respectively. Although psychedelics did not increase the expression of BDNF transcript (Figure 3G), they did result in a 2-fold increase in BDNF protein levels, although this effect was not statistically significant (Figure 3H). When cortical cultures were co-treated with ANA-12 (Cazorla et al., 2011), a selective antagonist of BDNF's high-affinity receptor TrkB, the ability of psychedelics or BDNF to stimulate neuritogenesis and spinogenesis was completely blocked (Figure 4).

Activation of TrkB is known to promote signaling through mTOR (Takei et al., 2004), which plays a key role in structural

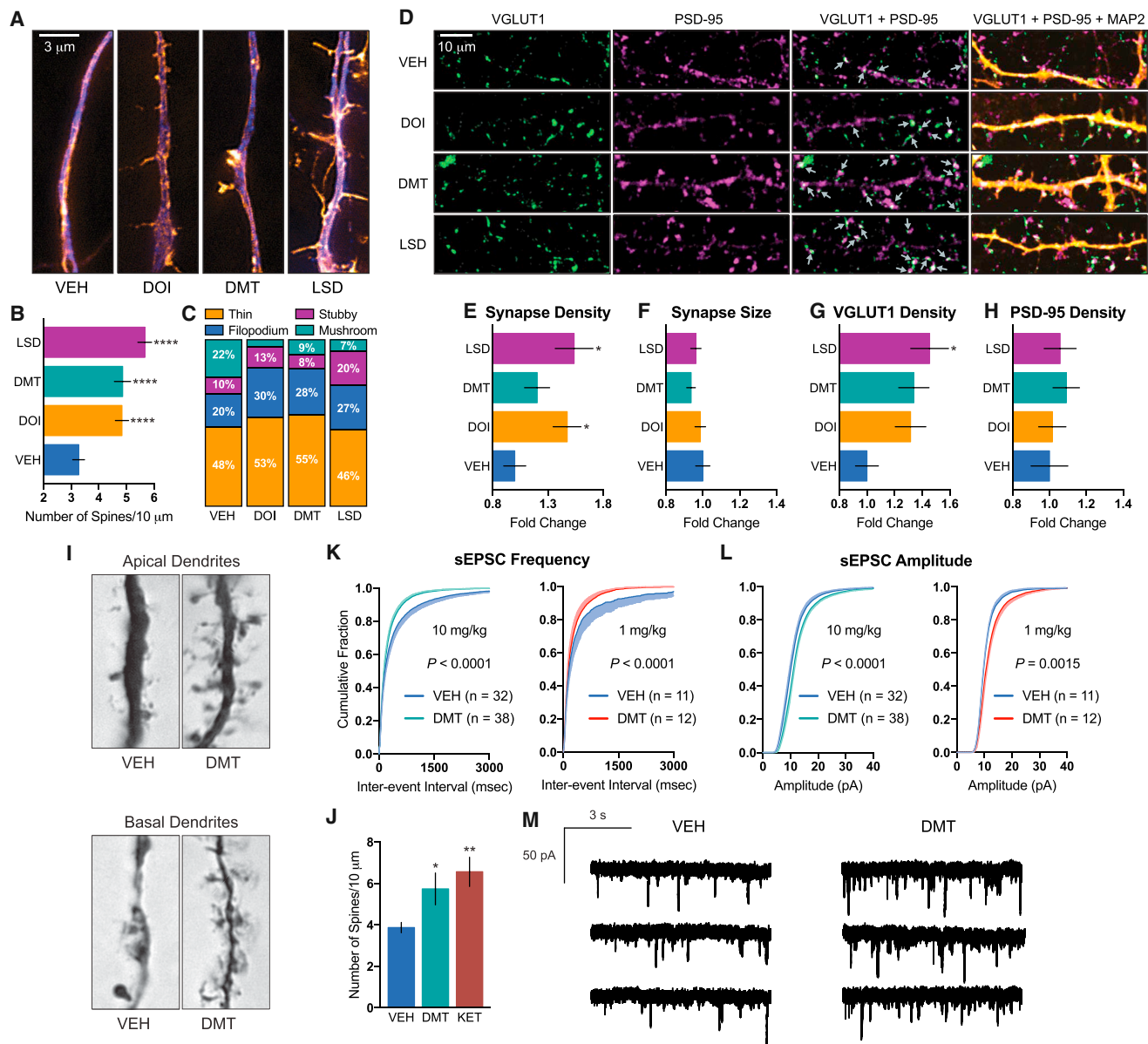


Figure 2. Psychedelics Promote Spinogenesis, Synaptogenesis, and Functional Plasticity

(A) Representative images of cortical neurons (DIV19) treated with compounds for 24 hr, demonstrating that psychedelics increase the number of dendritic spines (blue, MAP2; orange, F-actin).

(B) Quantification of spine density (n = 56–65 neurons).

(C) Relative proportions of spine types following treatment of cortical cultures with psychedelics (n = 16–21 neurons).

(D) Representative images of cortical cultures with psychedelics (n = 16–21 neurons), demonstrating that psychedelics increase synaptogenesis (green, VGLUT1; magenta, PSD-95; yellow, MAP2). White areas in the VGLUT1 + PSD-95 images indicate colocalization of pre- and postsynaptic makers and are indicated by gray arrows.

(E–H) Quantification of synapse density (E), synapse size (F), presynaptic density (VGLUT1) (G), and postsynaptic density (PSD-95) (H) following 24-hr treatment of cortical neurons (DIV19) (n = 39–42 neurons).

(I) Representative images of Golgi-Cox-stained pyramidal neurons from the PFC of rats 24 hr after receiving a 10 mg/kg dose of DMT.

(J) Quantification of spines from (I), demonstrating that DMT (10 mg/kg) increases spinogenesis *in vivo* to a comparable extent as ketamine (10 mg/kg) (n = 11–17 neurons).

(K and L) Whole-cell voltage-clamp recordings of layer V pyramidal neurons from slices obtained 24 hr after DMT treatment (10 mg/kg and 1 mg/kg) demonstrate that DMT increases both spontaneous excitatory postsynaptic current (sEPSC) frequency (K) and amplitude (L) (n = 11–38 neurons from 3 animals).

(M) Representative traces for the 10 mg/kg experiments quantified in (K) and (L).

*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, as compared to vehicle control (VEH). Data are represented as mean ± SEM. See also Figure S6.

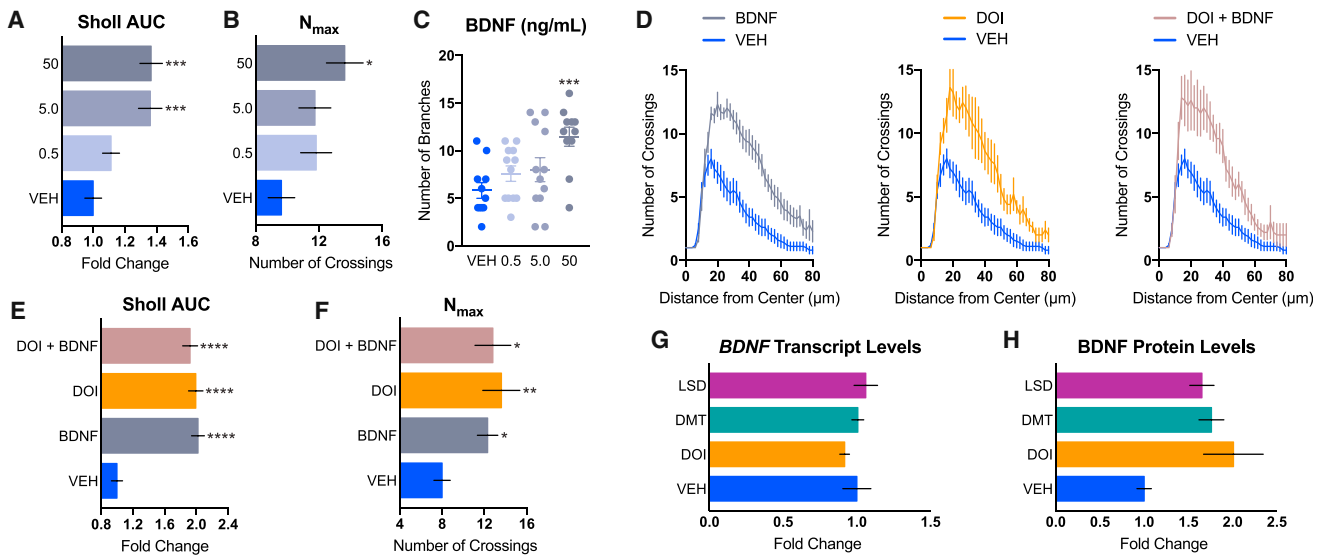


Figure 3. Psychedelics and BDNF Promote Neuritogenesis via a Related Mechanism

(A–C) Dose response of recombinant BDNF on neuritogenesis. AUC of the Sholl plots (A), N_{max} of the Sholl plots (B), and total number of branches (C) of treated cortical neurons (n = 11–12 neurons per treatment, DIV6) indicate that the highest concentration of BDNF (50 ng/mL) is more effective at promoting neuritogenesis than lower concentrations (5.0 and 0.5 ng/mL).

(D) Sholl analysis (n = 5–10 neurons) demonstrating that DOI (10 μM) increases neuritogenesis to a comparable extent as recombinant BDNF (50 ng/mL). A combination of DOI (10 μM) and BDNF (50 ng/mL) did not have any additive or synergistic effects.

(E) AUC of the Sholl plots in (D).

(F) N_{max} of the Sholl plots in (D).

(G and H) Cultured cortical neurons (DIV18) were treated with compounds for 24 hr, and then BDNF gene (G) and protein (H) expression was assessed via ddPCR (n = 4) and ELISA (n = 3–4), respectively.

*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, as compared to vehicle control (VEH). Data are represented as mean ± SEM.

plasticity (Jaworski et al., 2005; Kumar et al., 2005), the production of proteins necessary for synaptogenesis (Hoeffler and Klann, 2010), and the effects of ketamine (Dwyer and Duman, 2013; Li et al., 2010). Treatment with rapamycin, an mTOR inhibitor, completely blocked psychedelic-induced neuritogenesis (Figure 5), thus confirming that mTOR activation plays a role in the plasticity-promoting effects of classical serotonergic psychedelics.

The 5-HT_{2A} Receptor Mediates the Effects of Psychedelics on Structural Plasticity

Finally, we sought to determine whether the 5-HT_{2A} receptor played any role in the plasticity-promoting effects of DOI, DMT, and LSD because this receptor is known to be primarily responsible for the hallucinogenic effects of classical psychedelics (Nichols, 2004, 2016). Furthermore, the psychoplastogenic potencies of these and related compounds correlate well with their 5-HT_{2A} receptor affinities (Figure S3) (i.e., a higher 5-HT_{2A} binding affinity generally predicts more potent psychoplastogenic effects). Control experiments demonstrated that 5-HT_{2A} receptors were expressed on cultured rat cortical neurons at both 6 days in vitro (DIV6) and DIV19 (Figure 6A). Next we found that co-treatment with ketanserin, a selective 5-HT_{2A} antagonist, completely abrogated the ability of DMT, LSD, and DOI to promote both neuritogenesis and spinogenesis (Figures 6B–6F). Ketanserin was also able to block the effects of psilocin as well as the non-classical psychedelic noribogaine and enactogen MDMA (Figure 6G).

These initial experiments were performed using doses of psychoplastogens that produced maximal effects on structural plasticity (circa 10 μM) in combination with a 10-fold excess of ketanserin (100 μM). At these concentrations, we could not rule out the possibility of other receptors contributing to the antagonistic effects of ketanserin. Therefore, we treated cultured cortical neurons with a significantly lower dose of LSD (10 nM) and attempted to block its ability to promote neurite outgrowth using increasing doses of ketanserin (Figure 6H). We found that ketanserin blocks the psychoplastogenic effects of LSD by ~50% when treated at 10 nM. This is consistent with the fact that the binding affinities of ketanserin and LSD for the 5-HT_{2A} receptor are roughly equivalent (low nanomolar). Increasing the concentration of ketanserin to 100 nM, 10-fold higher than the concentration of LSD used in this experiment, completely prevented LSD-induced neuritogenesis. At 100 nM, ketanserin is relatively selective for the 5-HT_{2A} receptor, although, at this concentration, we cannot rule out the possible involvement of 5-HT_{2C}, adrenergic, or histamine receptors.

As a final note, the concentration responses of most psychoplastogens had Hill slopes that deviated from 1.0 (Figure S3), implying polypharmacology. Because psychedelics have relatively high affinities for 5-HT_{2A} receptors, it is likely that the effects of psychedelics are mediated primarily through 5-HT_{2A} receptors at low concentrations and modulated by other targets at high concentrations. Interestingly,

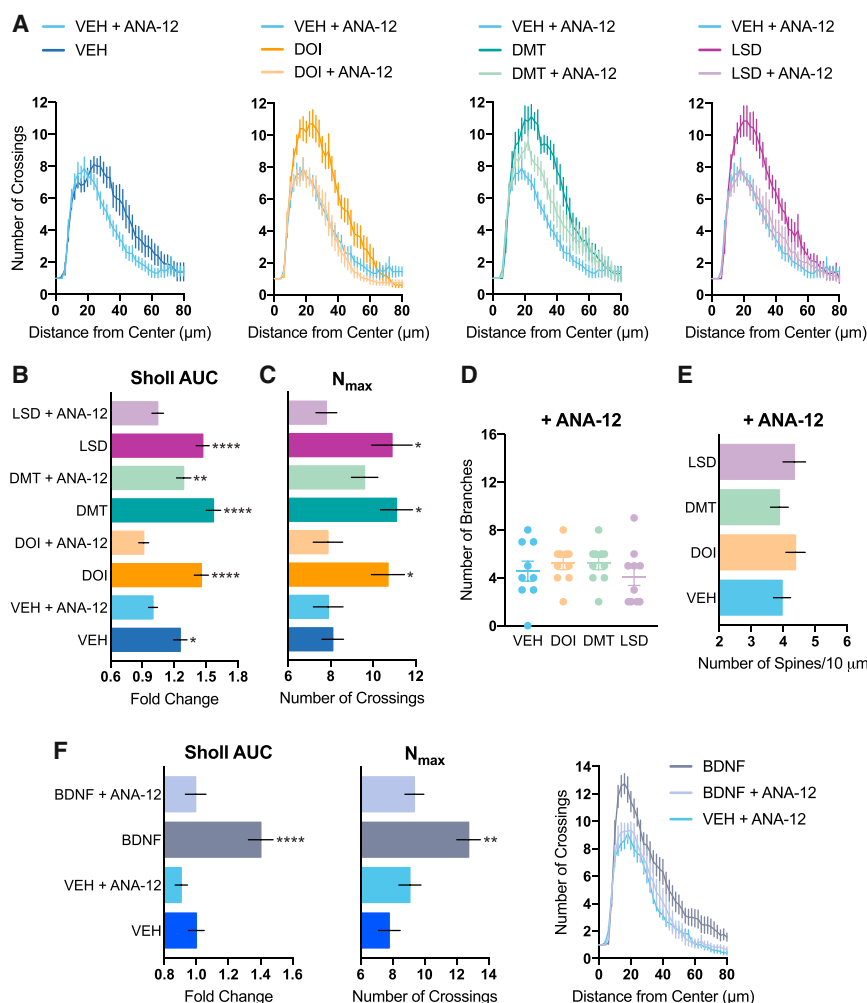


Figure 4. Psychedelic-Induced Changes in Neuronal Structure Are Mediated by TrkB

(A–D) The effects of psychedelics on dendritic arbor complexity are blocked by ANA-12, a selective inhibitor of TrkB, as measured by Sholl analysis of cultured cortical neurons (A) (DIV6). Compound-induced increases in the AUC of the Sholl plots (B), the N_{max} of the Sholl plots (C), and the number of dendritic branches (D) are completely blocked by ANA-12 (n = 8–10 neurons).

(E) The spinogenesis-promoting properties of psychedelics are blocked by ANA-12 (n = 19–21 neurons, DIV19).

(F) Control experiment demonstrating that ANA-12 blocks the effects of BDNF on neuriteogenesis (n = 11–15 neurons).

*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, as compared to vehicle control (VEH) or vehicle + antagonist. Data are represented as mean ± SEM.

Prior to this study, two reports suggested that psychedelics might be able to produce changes in neuronal structure. Jones et al. (2009) demonstrated that DOI was capable of transiently increasing the size of dendritic spines on cortical neurons, but no change in spine density was observed. The second study showed that DOI promoted neurite extension in a cell line of neuronal lineage (Marinova et al., 2017). Both of these reports utilized DOI, a psychedelic of the amphetamine class. Here we demonstrate that the ability to change neuronal structure is not a unique property of amphetamines like DOI because psychedelics from the ergo-

line, tryptamine, and iboga classes of compounds also promote structural plasticity. Additionally, D-amphetamine does not increase the complexity of cortical dendritic arbors in culture, and therefore, these morphological changes cannot be simply attributed to an increase in monoamine neurotransmission.

DISCUSSION

Classical serotonergic psychedelics are known to cause changes in mood (Griffiths et al., 2006, 2008, 2011) and brain function (Carhart-Harris et al., 2017) that persist long after the acute effects of the drugs have subsided. Moreover, several psychedelics elevate glutamate levels in the cortex (Nichols, 2004, 2016) and increase gene expression *in vivo* of the neurotrophin *BDNF* as well as immediate-early genes associated with plasticity (Martin et al., 2014; Nichols and Sanders-Bush, 2002; Vaidya et al., 1997). This indirect evidence has led to the reasonable hypothesis that psychedelics promote structural and functional neural plasticity, although this assumption had never been rigorously tested (Bogenschutz and Pommy, 2012; Vollenweider and Kometer, 2010). The data presented here provide direct evidence for this hypothesis, demonstrating that psychedelics cause both structural and functional changes in cortical neurons.

The identification of psychoplastogens belonging to distinct chemical families is an important aspect of this work because it suggests that ketamine is not unique in its ability to promote structural and functional plasticity. In addition to ketamine, the prototypical psychoplastogen, only a relatively small number of plasticity-promoting small molecules have been identified previously. Such compounds include the *N*-methyl-D-aspartate (NMDA) receptor ligand GLYX-13 (i.e., rapastinel), the mGlu2/3 antagonist LY341495, the TrkB agonist 7,8-DHF, and the muscarinic receptor antagonist scopolamine (Lepack et al., 2016; Castello et al., 2014; Zeng et al., 2012; Voleti et al., 2013). We observe that hallucinogens from four distinct structural classes (i.e., tryptamine, amphetamine, ergoline, and iboga) are also potent psychoplastogens, providing additional lead scaffolds for medicinal chemistry efforts aimed at identifying neurotherapeutics. Furthermore, our cellular assays revealed that several of these compounds were more efficacious (e.g., MDMA) or

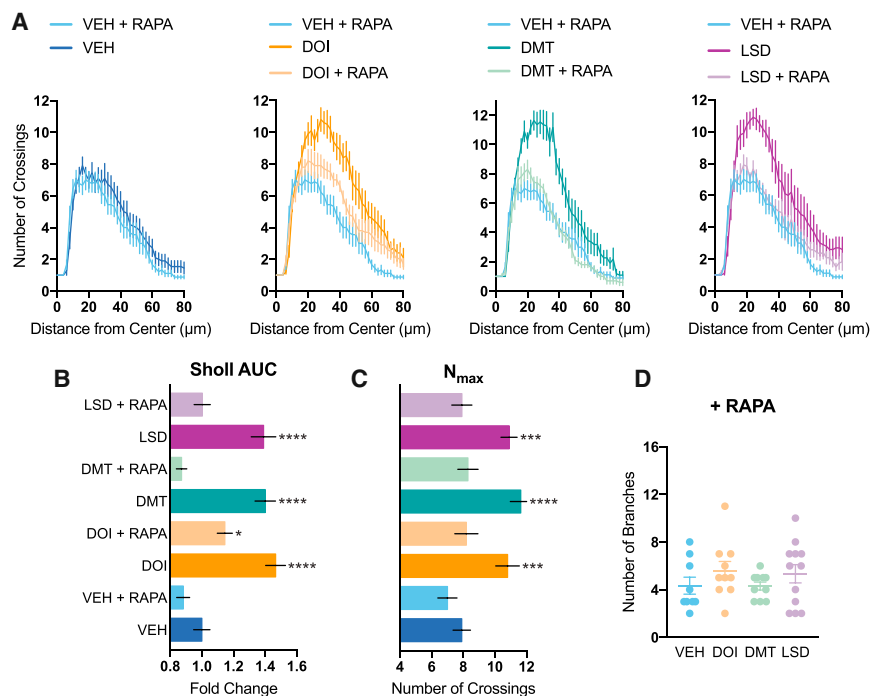


Figure 5. Psychodelic-Induced Changes in Neuronal Structure Are Mediated by mTOR

(A–D) The effects of psychedelics on dendritic arbor complexity are blocked by rapamycin, an inhibitor of mTOR, as measured by Sholl analysis of cultured cortical neurons (A) (DIV6). Compound-induced increases in the AUC of the Sholl plots (B), the N_{max} of the Sholl plots (C), and the number of dendritic branches (D) are completely blocked by rapamycin ($n = 9–12$ neurons).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, as compared to vehicle control (VEH) or vehicle + antagonist. Data are represented as mean \pm SEM.

more potent (e.g., LSD) than ketamine. In fact, the plasticity-promoting properties of psychedelics and entactogens rivaled that of BDNF (Figures 3A–3C and S3). The extreme potency of LSD in particular might be due to slow off kinetics, as recently proposed following the disclosure of the LSD-bound 5-HT_{2B} crystal structure (Wacker et al., 2017).

Importantly, the psychoplastogenic effects of psychedelics in cortical cultures were also observed *in vivo* using both vertebrate and invertebrate models, demonstrating that they act through an evolutionarily conserved mechanism. Furthermore, the concentrations of psychedelics utilized in our *in vitro* cell culture assays were consistent with those reached in the brain following systemic administration of therapeutic doses in rodents (Yang et al., 2018; Cohen and Vogel, 1972). This suggests that neurogenesis, spinogenesis, and/or synaptogenesis assays performed using cortical cultures might have value for identifying psychoplastogens and fast-acting antidepressants. It should be noted that our structural plasticity studies performed *in vitro* utilized neurons exposed to psychedelics for extended periods of time. Because brain exposure to these compounds is often of short duration due to rapid metabolism, it will be interesting to assess the kinetics of psychodelic-induced plasticity.

A key question in the field of psychodelic medicine has been whether or not psychedelics promote changes in the density of dendritic spines (Kyzar et al., 2017). Using super-resolution SIM, we clearly demonstrate that psychedelics do, in fact, increase the density of dendritic spines on cortical neurons, an effect that is not restricted to a particular structural class of compounds. Using DMT, we verified that cortical neuron spine density increases *in vivo* and that these changes in structural plasticity are accompanied by functional effects such as increased amplitude and frequency of spontaneous EPSCs.

forced swim test (Cameron et al., 2018). These results, coupled with the fact that ayahuasca, a DMT-containing concoction, has potent antidepressant effects in humans (Osório et al., 2015; Sanches et al., 2016; Santos et al., 2007), suggests that classical psychedelics and ketamine might share a related therapeutic mechanism.

Although the molecular targets of ketamine and psychedelics are different (NMDA and 5-HT_{2A} receptors, respectively), they appear to cause similar downstream effects on structural plasticity by activating mTOR. This finding is significant because ketamine is known to be addictive whereas many classical psychedelics are not (Nutt et al., 2007, 2010). The exact mechanisms by which these compounds stimulate mTOR is still not entirely understood, but our data suggest that, at least for classical psychedelics, TrkB and 5-HT_{2A} receptors are involved. Although most classical psychedelics are not considered to be addictive, there are still significant safety concerns with their use in medicine because they cause profound perceptual disturbances and still have the potential to be abused. Therefore, the identification of non-hallucinogenic analogs capable of promoting plasticity in the PFC could facilitate a paradigm shift in our approach to treating neuropsychiatric diseases. Moreover, such compounds could be critical to resolving the long-standing debate in the field concerning whether the subjective effects of psychedelics are necessary for their therapeutic effects (Majić et al., 2015). Although our group is actively investigating the psychoplastogenic properties of non-hallucinogenic analogs of psychedelics, others have reported the therapeutic potential of safer structural and functional analogs of ketamine (Moskal et al., 2017; Yang et al., 2015; Zanos et al., 2016).

Our data demonstrate that classical psychedelics from several distinct chemical classes are capable of robustly promoting the growth of both neurites and dendritic spines *in vitro*, *in vivo*, and

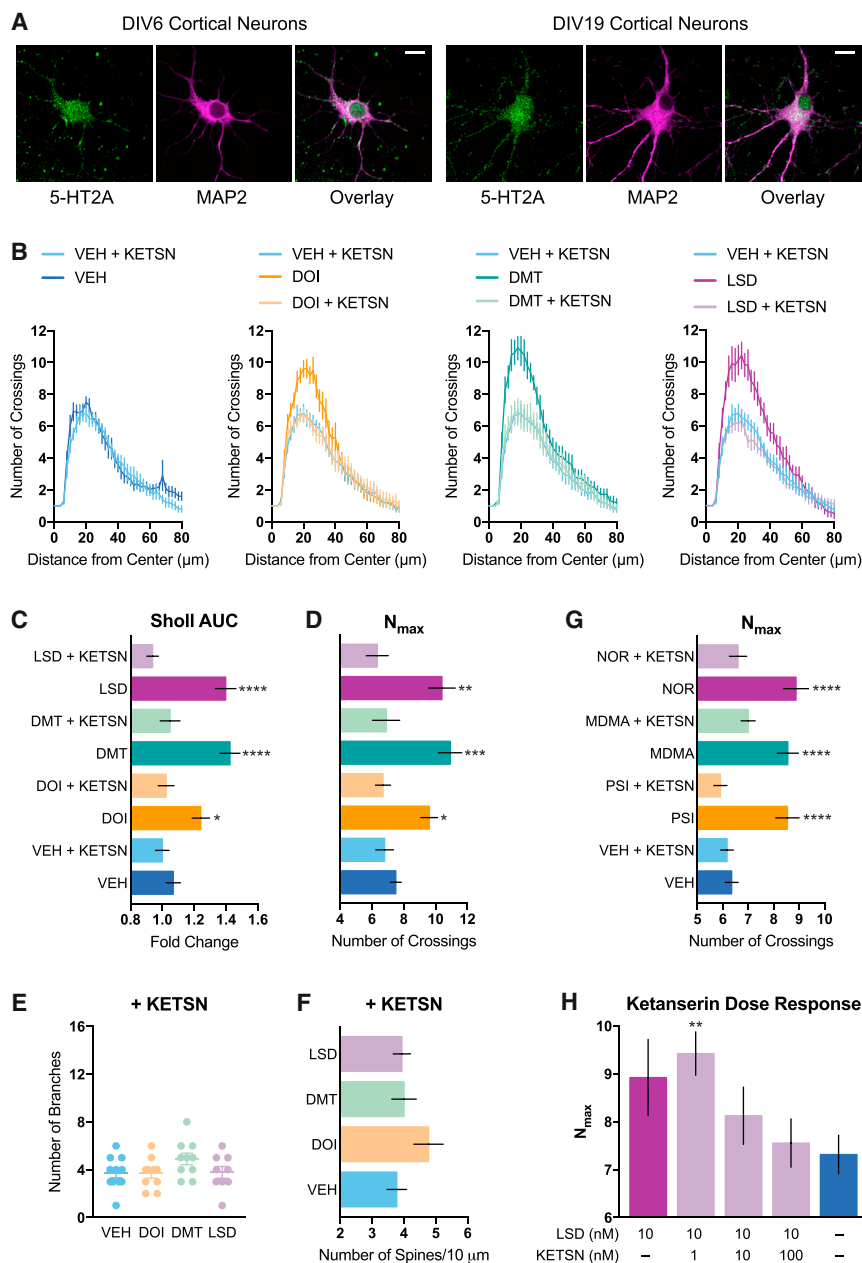


Figure 6. The 5-HT2A Receptor Mediates the Effects of Psychedelics on Structural Plasticity

(A) Rat embryonic cortical neurons express 5-HT2A receptors at both DIV6 and DIV19 (scale bar, 10 μ m).

(B) The effects of psychedelics on increasing dendritic arbor complexity are blocked by co-treating with ketanserin, a selective antagonist of 5-HT2A receptors, as measured by Sholl analysis of cultured cortical neurons (DIV6).

(C–E) Compound-induced increases in the AUC of the Sholl plots (C), the N_{max} of the Sholl plots (D), and the number of dendritic branches (E) are completely blocked by ketanserin (n = 10–11 neurons, DIV6).

(F) The spinogenesis-promoting properties of psychedelics are blocked by ketanserin (n = 19–20 neurons, DIV19).

(G) Ketanserin also blocks the increased N_{max} induced by psilocin, noribogaine, and MDMA.

(H) Ketanserin dose-dependently blocks the psychoplastic effects of 10 nM LSD (n = 9–38 neurons, DIV6).

*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, as compared to vehicle control (VEH) or vehicle + antagonist. Data are represented as mean \pm SEM.

EXPERIMENTAL PROCEDURES

Drugs

For *in vitro* studies, all compounds were dissolved in DMSO and diluted 1:1,000, with the exception that BDNF was dissolved in water. Cells were treated with DMT at a final concentration of 90 μ M (0.1% DMSO), whereas all other compounds were used at 10 μ M (0.1% DMSO) unless noted otherwise. For *in vivo* studies, DMT or ketamine was dissolved in sterile 0.9% saline and administered intraperitoneally at a dose of 10 mg/kg and an injection volume of 1 mL/kg.

Animals

Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA, USA). The age and sex of the animals used are noted under each individual experimental subheading. All experimental procedures involving animals were approved by the University of California, Davis

across species. Importantly, our studies highlight the similarities between the effects of ketamine and those of classical serotonergic psychedelics, supporting the hypothesis that the clinical antidepressant and anxiolytic effects of these molecules might result from their ability to promote structural and functional plasticity in prefrontal cortical neurons. We have demonstrated that the plasticity-promoting properties of psychedelics require TrkB, mTOR, and 5-HT2A signaling, suggesting that these key signaling hubs may serve as potential targets for the development of psychoplastogens, fast-acting antidepressants, and anxiolytics. Taken together, our results suggest that psychedelics may be used as lead structures to identify next-generation neurotherapeutics with improved efficacy and safety profiles.

Institutional Animal Care and Use Committee (IACUC) and adhered to the principles described in the NIH Guide for the Care and Use of Laboratory Animals. The University of California, Davis is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and has an Animal Welfare Assurance number (A3433-01) on file with the Office of Laboratory Animal Welfare (OLAW).

Cell Culture

Primary cortical cultures were prepared using tissue from embryonic day 18 (E18) Sprague-Dawley rats. Cells were plated at various densities on poly-D-lysine-coated plates depending on the specific experiment (Supplemental Experimental Procedures). Plating medium consisted of 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies), 1% penicillin-streptomycin (Life Technologies), and 0.5 mM glutamine (Life Technologies) in Neurobasal (Life Technologies). After 15–24 hr, the medium was removed and exchanged for

replacement medium containing 1 × B27 supplement (Life Technologies), 1% penicillin-streptomycin, 0.5 mM glutamine, 12.5 μM glutamate, and Neurobasal. After 96 hr, 50% of the medium was removed and replaced with feed medium containing 1 × B27 supplement, 1% penicillin-streptomycin, and 0.5 mM glutamine. Once per week, until the cultures had reached sufficient maturity for experiments, 50% of the culture medium was removed and replaced with feed medium, with an additional 20% by volume being added to account for evaporation. For experiments using antagonists or inhibitors, cells were pretreated with ANA-12 (10 μM), rapamycin (100 nM), and ketanserin (100 μM) for 10 min prior to addition of test compounds unless otherwise noted. The final DMSO concentration of these experiments was 0.2%. The neurons used in each cellular experiment were taken from at least two different treatment wells, and the wells were randomized to account for plate effects. All of the cellular experiments were replicated on at least two occasions by two or more experimenters.

Statistical Analysis

Appropriate sample sizes were estimated based on our previous experiences performing similar experiments. Data are represented as mean ± SEM. Statistical analyses were performed using GraphPad Prism (version 7.0a). For analyses involving comparison of three or more groups, a one-way analysis of variance (Dunnett's *post hoc* test) was utilized. No statistics were calculated for the individual points of the Sholl plots. Instead, statistical analyses were performed on the aggregate data (i.e., the area under the curve of the Sholl plot). Probability distributions from electrophysiology experiments were compared using a Kolmogorov-Smirnov test. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, and *****p* < 0.0001 compared with vehicle control or vehicle + antagonist.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and six figures and can be found with this article online at <https://doi.org/10.1016/j.celrep.2018.05.022>.

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AUTHOR CONTRIBUTIONS

D.E.O., C.L., and A.K.M. were responsible for the overall experimental design. C.L. performed the majority of the neuritegenesis, spinogenesis, synaptogenesis, and biochemical experiments described, with A.C.G. and S.S.Z. providing assistance. C.L., L.P.C., and D.E.O. performed the Golgi-Cox staining experiments. L.P.C., J.M.W., and E.V.B. performed the electrophysiology experiments, with J.A.G. supervising these studies. K.M.O.-M. and P.C.W. performed the experiments in *Drosophila*. M.Y.D. and K.F.B. performed the zebrafish experiments. A.S. developed the program for analyzing synapse number and size. M.R.P. supervised the SIM experiments. W.C.D. performed 5-HT2A expression studies and assisted with fluorescence microscopy data analysis. D.E.O. conceived the project, supervised the experiments, and wrote the manuscript with input from all authors.

DECLARATION OF INTERESTS

D.E.O. has submitted a patent application related to this work (PCT/US2017/054277).

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