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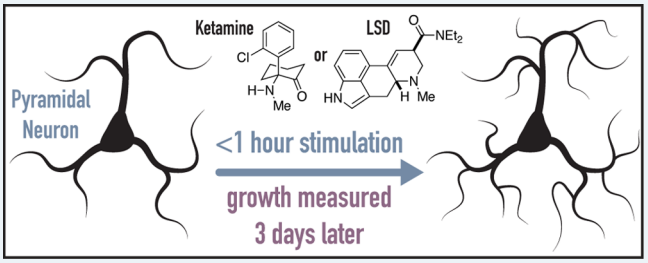
Transient Stimulation with Psychoplastogens Is Sufficient to Initiate Neuronal Growth

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ABSTRACT: Cortical neuron atrophy is a hallmark of depression and includes neurite retraction, dendritic spine loss, and decreased synaptic density. Psychoplastogens, small molecules capable of rapidly promoting cortical neuron growth, have been hypothesized to produce long-lasting positive effects on behavior by rectifying these deleterious structural and functional changes. Here we demonstrate that ketamine and LSD, psychoplastogens from two structurally distinct chemical classes, promote sustained growth of cortical neurons after only short periods of stimulation. Furthermore, we show that psychoplastogen-induced cortical neuron growth can be divided into two distinct epochs: an initial stimulation phase requiring TrkB activation and a growth period involving sustained mTOR and AMPA receptor activation. Our results provide important temporal details concerning the molecular mechanisms by which next-generation antidepressants produce persistent changes in cortical neuron structure, and they suggest that rapidly excreted psychoplastogens might still be effective neurotherapeutics with unique advantages over compounds like ketamine and LSD.



KEYWORDS: *neural plasticity, psychoplastogen, psychedelic, LSD, ketamine, BDNF*

Depression is among the leading causes of disability worldwide, affecting over 300 million people.[1,2] Current treatments, such as the selective serotonin reuptake inhibitor (SSRI) fluoxetine, are only moderately effective, require daily administration for 2−4 weeks before producing beneficial effects, and are associated with a number of side effects leading to discontinuation of treatment regimens.[3,4] Moreover, approximately one-third of patients are unresponsive to these medicines,[4] highlighting the urgent need for new treatment approaches. A better understanding of depression pathophysiology will be necessary to rationally devise more effective therapeutics.

In recent years, it has become clear that depression results from deleterious structural and functional changes in key brain circuits. These include the retraction of dendrites, the elimination of dendritic spines, and the loss of excitatory synapses in the prefrontal cortex (PFC).[5−8] Psychoplastogens, small molecules that promote the rapid regrowth of atrophied cortical dendritic arbors,[9] represent the leading edge of antidepressant neurotherapeutics. They include ketamine,[10−13] scopolamine,[14−17] and serotonergic psychedelics.[18−25] These compounds rapidly promote structural and functional neural plasticity in the cortex[26−28] and produce long-lasting (>24 h) changes in mood and behavior without the need for chronic dosing,[29−34] presumably due to their ability to rewire pathological neural circuitry.

Induced plasticity (iPlasticity) has been proposed as a potential unifying mechanism to explain the efficacy of antidepressants from different chemical classes.[35,36] Traditional antidepressants like fluoxetine are believed to promote cortical neuron growth through transactivation of the tropomyosin receptor kinase B (TrkB),[37−39] the high-affinity receptor for brain-derived neurotrophic factor (BDNF). Psychoplastogens produce more rapid changes in cortical neuron structure, but their mechanisms of action are still opaque. Understanding how psychoplastogens produce enduring changes in cortical neuron structure, despite being rapidly cleared from the body, will be critical if we are to rationally engineer safer and more efficacious medicines for treating depression.

Previously, we demonstrated that psychedelics could increase cortical neuron growth when treated for extended periods of time (24−72 h). Here, we establish that very short stimulation periods (15 min to 6 h) are sufficient for ketamine and lysergic acid diethylamide (LSD), two psychoplastogens from distinct chemical classes, to initiate a neuronal growth

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response characterized by sustained α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and mammalian target of rapamycin (mTOR) activation. This response persists even after the removal of the stimulating ligand. Our results indicate that ketamine and serotonergic psychoplastogens may lead to enduring changes in neuronal structure through a common downstream mechanism of action. Moreover, our results have important implications for central nervous system (CNS) drug development, as they suggest that intentional engineering of neurotherapeutics to be rapidly cleared from the body might be an effective strategy for maintaining efficacy while minimizing side effects.

RESULTS

Transient Stimulation with Psychoplastogens Increases Dendritogenesis, Spinogenesis, and Synaptogenesis. Previously, we demonstrated that treatment of immature cortical cultures (3 days in vitro; DIV3) with psychoplastogens for 72 h significantly increased dendritic arbor complexity as measured via Sholl analysis.[28] However, most psychoplastogens are rapidly cleared from the body (i.e., they have half-lives of several hours),[40,41] with compounds such as *N*,*N*-dimethyltryptamine (DMT) and 5-MeO-DMT having half-lives less than 15 min.[42,43] To determine the shortest period of stimulation necessary to induce neuronal growth, we treated DIV3 cortical cultures with either ketamine or LSD for varying lengths of time, removed the drug, and let the cultures mature until DIV6 (Figure 1A). Remarkably, both drugs were able to robustly increase dendritic arbor complexity after only 15 min of stimulation (Figure 1D), while exogenous BDNF (50 ng/mL) required a full hour to produce effects (Figure S1A). Treatment of cortical cultures with psychoplastogens for 1 h followed by a 71 h growth period resulted in N_{max} values comparable to what we had previously observed using a 72 h treatment paradigm (Figure 1B,C). Concentration−response studies revealed that a concentration of 10 μM produced maximal effects on dendritic growth (Figure S2). When cortical cultures were fixed immediately following 1 h of treatment with psychoplastogens, no changes in dendritic arborization were observed (Figure 1E) indicating that there are distinct stimulation and growth phases.

Next, we determined whether transient psychoplastogen stimulation was sufficient to induce dendritic spine growth, as we have often observed parallels between compound-induced changes in spine density and dendritic arbor complexity. As with dendritic growth, transient stimulation of cortical neurons (DIV19) with ketamine or LSD followed by a growth period in the absence of drug was sufficient to induce large increases in dendritic spine density (Figure 2), with LSD producing effects similar in magnitude to those produced by BDNF (Figure S1B) and ketamine inducing more modest changes. A 6 h stimulation period appears to be optimal for increasing dendritic spine density. In general, longer stimulation periods produce smaller changes in both dendrite and spine growth, which we hypothesize might be due to homeostatic mechanisms resulting from excessive neuronal excitation.[44]

To determine the effect of transient psychoplastogen stimulation on synapse formation, we performed colocalization experiments of pre- (VGLUT1) and postsynaptic (PSD-95) markers. Psychoplastogen-induced increases in synapse density mirrored changes in spine density with a 6 h stimulation/18 h growth period producing the greatest effects (Figure 3). Stopping the experiment after 6 h of stimulation resulted in a much smaller change (Figure 3D), indicating that a growth phase following stimulation is necessary to achieve maximal increases in synapse density. While the effects of psychoplastogens on cortical structural plasticity (i.e., dendrite and spine growth) closely resemble those induced by BDNF, their effects on synaptogenesis are markedly distinct. BDNF induces a noticeably larger (~6-fold increase) and more rapid (15 min stimulation) increase in synaptogenesis (Figure S1C,D) than do psychoplastogens (~2-fold increase, 6 h stimulation). These results have important implications for the therapeutic properties of current and next-generation psychoplastogens. First, they suggest that psychoplastogens (10 μM) and BDNF

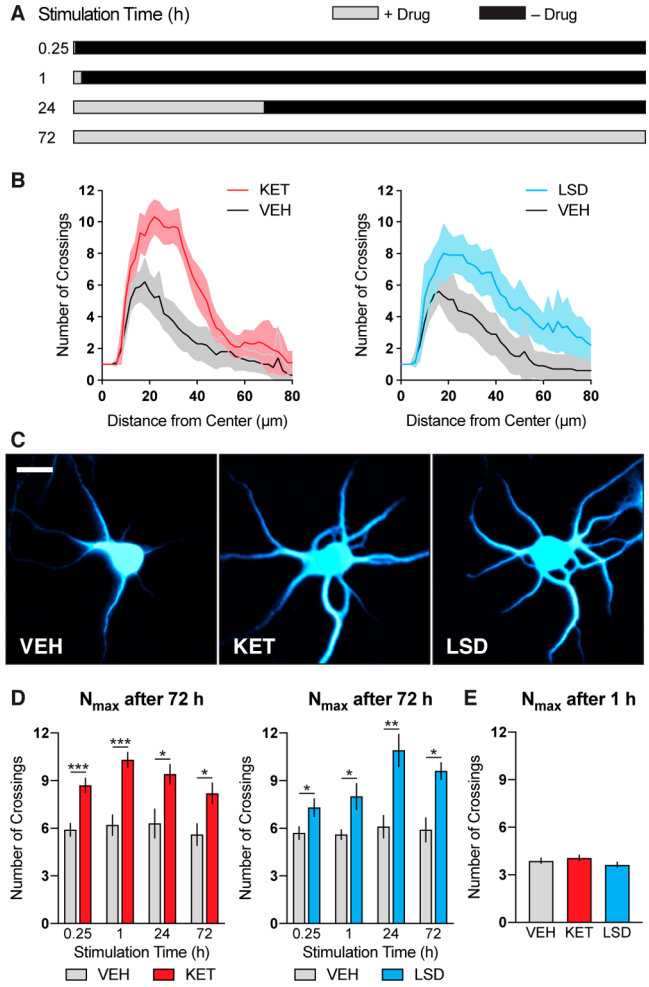


Figure 1. Transient stimulation with psychoplastogens is sufficient to induce dendritic growth. (A) Schematic illustrating experimental design. Cortical cultures (DIV3) were treated with psychoplastogens (10 μM) for short periods of time and then allowed to grow for a total of 72 h. (B) Sholl plots of DIV3 neurons stimulated with psychoplastogens for 1 h and then allowed to grow for 71 h. Shadings indicate 95% CI (C) Representative images of neurons used to make the Sholl plots in B. Scale bar = 10 μm. (D−E) Maximum number of crossings (N_{max}) of the Sholl plots obtained from neurons that experienced both transient stimulation and growth phases (D, n = 10 neurons) or only a 1 h stimulation (E, n = 37 neurons). Data are represented as mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$, as compared to VEH control. For D, multiple t tests were performed, and p values were corrected for multiple comparisons using the Holm-Sidak method. For E, a one-way analysis of variance with Dunnett's post hoc test was utilized. VEH = vehicle; KET = ketamine; LSD = lysergic acid diethylamide. See also Figure S1.

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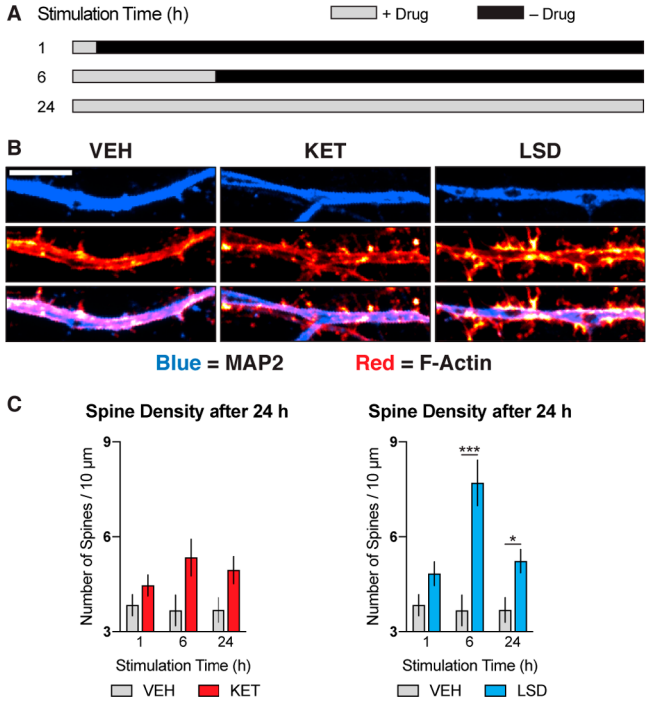


Figure 2. Transient stimulation with psychoplastogens is sufficient to induce spinogenesis. (A) Schematic illustrating experimental design. Cortical cultures (DIV19) were treated with psychoplastogens (10 μM) for short periods of time and then allowed to grow for a total of 24 h. (B) Representative images of secondary dendritic branches stimulated with psychoplastogens for 6 h and then allowed to grow for 18 h. Dendrites and F-actin were imaged using an anti-MAP2 antibody and fluorescent phalloidin conjugate, respectively. In the colocalization channel, dendritic spines can be visualized as red F-actin-rich protrusions emanating from blue dendrites. Scale bar = 5 μm. (C) Quantification of dendritic spine density. Data are represented as mean ± SEM (n = 11−30 neurons). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$, as compared to VEH control following multiple t tests and correction of p values for multiple comparisons using the Holm−Sidak method. VEH = vehicle; KET = ketamine; LSD = lysergic acid diethylamide. See also Figure S1.

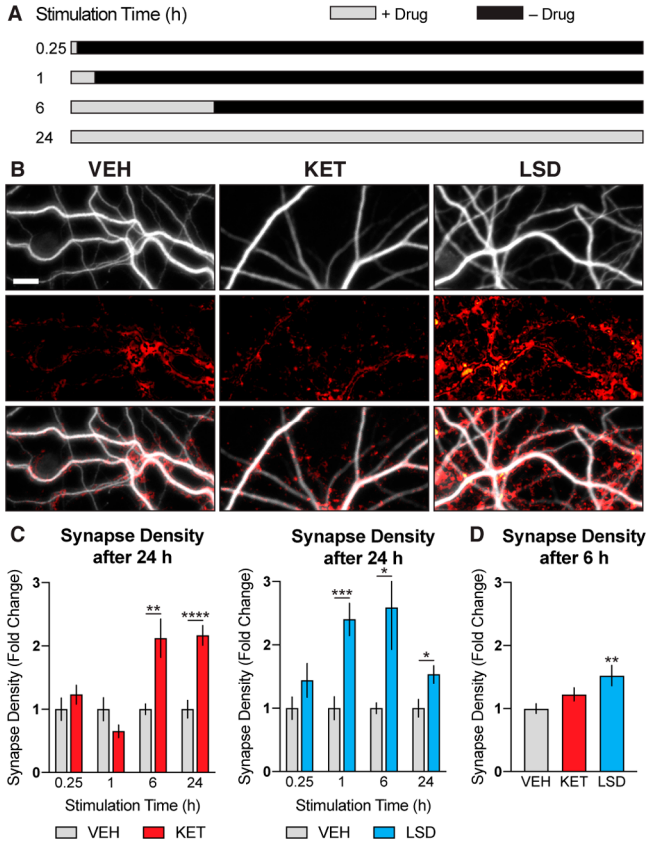


Figure 3. Transient stimulation with psychoplastogens is sufficient to induce synaptogenesis. (A) Schematic illustrating experimental design. Cortical cultures (DIV19) were treated with psychoplastogens (10 μM) for short periods of time and then allowed to grow for a total of 24 h. (B) Representative images of dendritic branches stimulated with psychoplastogens for 6 h and then allowed to grow for 18 h. Dendrites were imaged using an anti-MAP2 antibody (gray). Synapses are represented as colocalization of VGLUT1 and PSD-95 channels (red). Scale bar = 10 μm. (C) Quantification of synapse density (n = 25−27 neurons) from neurons that experienced both transient stimulation and growth phases. (D) Quantification of synapse density (n = 50−54 neurons) from neurons that experienced a 6 h stimulation only. Data are represented as mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$, as compared to VEH control. For C, multiple t tests were performed, and p values were corrected for multiple comparisons using the Holm−Sidak method. For D, a one-way analysis of variance with Dunnett's post hoc test was utilized. VEH = vehicle; KET = ketamine; LSD = lysergic acid diethylamide. See also Figure S1.

(50 ng/mL) produce maximal increases in cortical structural plasticity (dendrite and spine growth) at these concentrations. Our previous work supports such a ceiling effect, as a combination of BDNF and a psychoplastogen did not result in additive or synergistic effects on neuronal growth.[28] Second, these results suggest that a psychoplastogen with BDNF-like synaptogenic efficacy has yet to be discovered, highlighting an important area for future drug discovery. As synaptogenic effects can be observed prior to substantial increases in spine growth (Figure S1), it appears that these two processes are distinct. This conclusion is further supported by the recent discovery of compounds capable of increasing synapse density while concomitantly decreasing spine density.[45]

Psychoplastogen-Induced Growth Requires Sustained AMPA Receptor and mTOR Activation. Having firmly established that transient stimulation with ketamine and LSD is sufficient to induce cortical neuron growth, we next used several pharmacological inhibitors to probe which biochemical signaling pathways are essential during the stimulation and growth periods (Figure 4A). The leading hypothesis concerning the mechanism of antidepressant psychoplastogens involves a compound-induced glutamate burst in the cortex resulting in BDNF secretion, TrkB stimulation, and ultimately mTOR activation.[28,46] Duman and co-workers have proposed that ketamine and scopolamine initiate this glutamate burst by blocking NMDA and muscarinic receptors on inhibitory neurons, respectively,[47] while Aghajanian and co-workers have shown that psychedelics increase cortical glutamate levels by activating 5-HT2A receptors on excitatory neurons.[48] Treating cortical neurons with DNQX, an AMPA receptor blocker, during the initial stimulation phase blocked dendritic growth, as expected (Figure 4B). However, we were surprised to find that treatment with DNQX during the growth period had a similar effect, indicating that sustained AMPA receptor activation is necessary for both the initiation and maintenance of psychoplastogen-induced neuronal growth.

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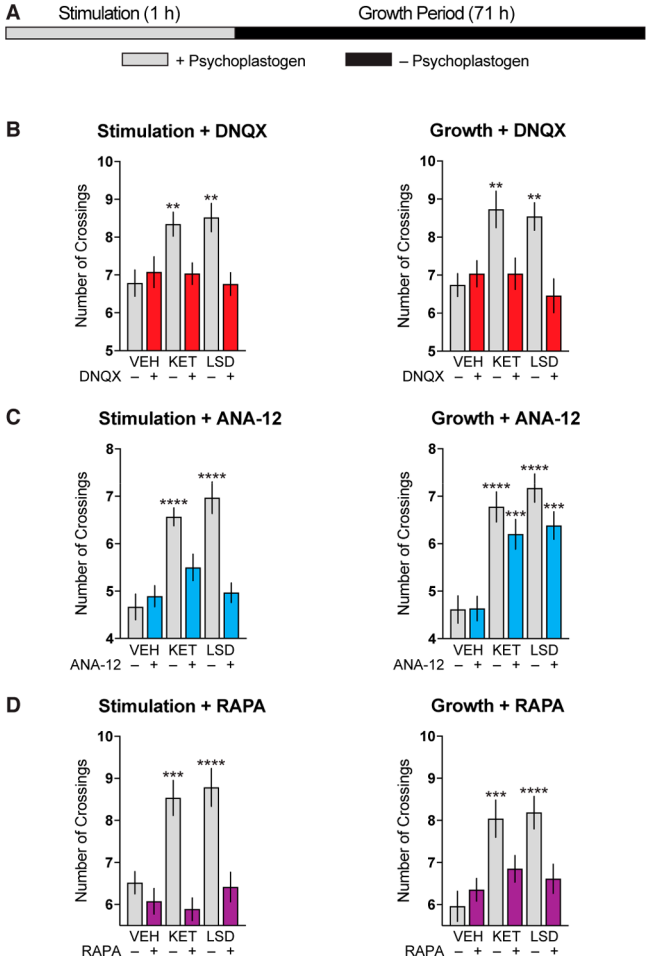


Figure 4. Sustained AMPA receptor and mTOR stimulation is necessary for psychoplastogen-induced dendritic growth. (A) Schematic illustrating experimental design. Cortical cultures (DIV3) were treated with psychoplastogens (10 μM) for 1 h (stimulation phase) and then allowed to grow in the absence of psychoplastogens for 71 h (growth phase). (B–D) Maximum number of crossings (N_{max}) of the Sholl plots obtained after the stimulation and growth phases. Pharmacological inhibitors of AMPA receptors (B), TrkB receptors (C), and mTOR (D) were applied during either the stimulation or growth phases. Data are represented as mean ± SEM (n = 22–37 neurons). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$, as compared to VEH control without inhibitor following a one-way analysis of variance with Dunnett's post hoc test. VEH = vehicle; KET = ketamine; LSD = lysergic acid diethylamide; DNQX = 6,7-dinitroquinoxaline-2,3-dione; RAPA = rapamycin. See also Figure S2.

Originally, we hypothesized that AMPA receptor activation would lead to BDNF secretion, ultimately stimulating TrkB receptors. As BDNF is well known to enhance its own expression through positive autoregulation,[49−52] we hypothesized that sustained TrkB signaling would be necessary for the long-lasting effects of transient psychoplastogen stimulation on dendritogenesis. However, this did not appear to be the case, as treatment with ANA-12, a selective TrkB antagonist,[53] only blocked pyschoplastogen-induced growth when applied during the initial stimulation period and was ineffective when applied in the absence of psychoplastogen during the growth phase (Figure 4C). Conversely, blocking mTOR during either the stimulation or growth periods was sufficient to abrogate neuronal growth (Figure 4D), likely due to the critical role that mTOR plays in the synthesis of structural proteins and ion channels necessary for neural plasticity.[54]

Given the key role of AMPA receptor activation on both the initiation and maintenance of psychoplastogen-induced cortical neuron growth, we next sought to determine if neuronal activity in the absence of a psychoplastogen was capable of producing comparable changes in dendritogenesis. Stimulation of cortical cultures for 1 h with 40 mM KCl, a concentration known to induce glutamate release,[55] did not lead to a growth phenotype comparable to that produced by psychoplastogens (Figure S3). Moreover, the combination of KCl with either ketamine or LSD did not enhance psychoplastogen-induced dendritogenesis (Figure S3). These results suggest that psychoplastogens induce neuronal growth by engaging biochemical signaling mechanisms that are distinct from those activated simply through neuronal depolarization/overexcitation.

DISCUSSION

Accumulating evidence has suggested that activation of AMPA receptors, TrkB, and mTOR are all critical for the effects of ketamine and other psychoplastogens on both cortical structural plasticity and behavior.[26,28,56−64] Recently, an elegant study by Liston and co-workers established a causal link between ketamine-induced spinogenesis in the PFC and antidepressant-like behavior in the tail suspension test.[65] However, the details of psychoplastogen-initiated biochemical signaling leading to sustained changes in neuronal structure/function have remained poorly defined. Here, we demonstrate that transient stimulation with either ketamine or LSD, psychoplastogens from chemically distinct classes, trigger a growth phase that continues in the absence of drug.

Single administrations of psychoplastogens are well-known to produce effects in humans and rodents that persist long after the drug has been cleared from the body.[29−34] Pharmacokinetic studies in rodents have revealed that behaviorally relevant doses of ketamine and DMT yield brain concentrations >10 μM,[66,67] concentrations that are more than sufficient to trigger neuronal growth in culture. Psychoplastogen-induced growth of cortical neurons appears to require initial TrkB activation and is characterized by sustained AMPA receptor and mTOR activation. Though the exact details of this biochemical signaling network still remain elusive, it is clear that mechanisms exist for sustaining growth through an autoregulatory positive feedback loop. For example, initial activation of AMPA receptors is known to induce BDNF secretion, which in turn activates mTOR.[68,69] Activation of mTOR not only leads to the translation of proteins necessary for cell growth but also produces additional BDNF. Finally, BDNF itself is known to induce glutamate release in cortical neurons via a nonexocytotic pathway,[70] which could lead to sustained AMPA receptor activation. Superficially, it would appear that psychoplastogens merely catalyze this growth process by initiating a glutamate burst. However, psychoplatogen-induced neuronal growth cannot simply be the result of transient neuronal excitation, as a high concentration of potassium ions is not sufficient to produce similar changes in cortical neuron structure.

Though we know that psychoplastogens activate mTOR, it is still unclear how compounds with distinct primary targets (NMDA receptors for ketamine and 5-HT2A receptors for LSD) are capable of activating the same biochemical pathways. One hypothesis is that ketamine and LSD both induce

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glutamate bursts through their respective primary targets. Ketamine is believed to increase glutamate secretion by inhibiting NMDA receptors on inhibitory interneurons,[46] while serotonergic psychedelics like LSD have been shown to promote glutamate release by activating 5-HT2A receptors.[47] However, given the fact that increased neuronal activity is insufficient to produce psychoplastogen-like effects on neuronal growth, it is possible that ketamine and LSD could share an unidentified common target responsible for their psychoplastogenic effects. Given recent evidence suggesting that 5-HT1A receptors may play a role in the antidepressant effects of ketamine,[71] coupled with the fact that LSD has high affinity for 5-HT1A receptors, we decided to test the effects of 8-OH-DPAT (a selective 5-HT1A agonist) on dendritic growth. Treating cortical neurons with 8-OH-DPAT did not increase dendritic growth at any concentration (Figure S2), suggesting that 5-HT1A receptors do not play a role in the psychoplastogenic effects of ketamine or LSD. At this point, it seems the most likely mechanism explaining the shared plasticity-promoting properties of psychoplastogenic NMDA receptor and 5-HT2A receptor ligands is related to their shared abilities to promote glutamate secretion through different receptors.

Currently, it is unclear exactly how long psychoplastogen-induced plasticity lasts. Two-photon in vivo imaging experiments have shown that ketamine can increase the rate of dendritic spine formation for up to 2 weeks in rodents,[72] with behavioral effects often observed 1 week following administration.[64] However, not all psychoplastogens are equivalent, with some producing more enduring effects than others. Recently, the antidepressant-like behavioral effects of psilocybin in rodents were shown to last much longer than those produced by ketamine,[73] results that mirror the human clinical data for these two compounds.

Here, we have shown that like BDNF, psychoplastogens activate positive autoregulatory feedback loops leading to sustained neuronal growth. Small molecules offer significant advantages over protein- and peptide-based activators of neuronal growth signaling, as they can readily cross the blood−brain barrier. Moreover, our work suggests the potential for a paradigm shift in CNS drug development toward molecules that are intentionally designed to reach high brain concentrations and be rapidly cleared from the body in order to minimize side effects. One of the major concerns with using drugs like psilocybin and ketamine in the clinic is related to their relatively long-lasting hallucinogenic/dissociative properties, necessitating the hospitalization of patients for extended periods of time. In this regard, shorter acting plasticity-promoting psychedelics, such as DMT,[74] could offer significant advantages by reducing healthcare costs.[75] With the advent of nonhallucinogenic psychoplastogens,[76] it might be possible to produce a plasticity-promoting drug suitable for at home administration. The work described here suggests that infrequent administration of such compounds might be sufficient to produce sustained changes in neural circuitry, thus avoiding potential side effects from chronic daily use, such as those that have plagued patients using traditional antidepressants.

METHODS

Data Analysis and Statistics. Treatments were randomized, and data were analyzed by experimenters blinded to treatment conditions. Statistical analyses were performed using GraphPad Prism (version 8.1.2). Data are represented as mean ± SEM, unless otherwise noted, with asterisks: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; and ****, $p < 0.0001$. Brightness and contrast were adjusted equally for all representative images.

Data Availability. The data sets analyzed as part of this study are available via Mendeley Data: https://data.mendeley.com/datasets/6f5wf65fbf/draft?a=5311108e-7332-4df8-9d4c-86dcdd023cd0.

Drugs. The NIH Drug Supply Program provided (+)-lysergic acid diethylamide (+)-tartrate (2:1) (LSD). Other chemicals/proteins were purchased from commercial sources such as ketamine hydrochloride (KET, Fagron), (±)-8-hydroxy-2-(dipropylamino)tetralin hydrobromide (8-OH-DPAT, Sigma), brain-derived neurotrophic factor (BDNF, Sigma-Aldrich), ANA-12 (MedChem Express), DNQX (Sigma), and rapamycin (Alfa Aesar). For cell culture experiments, VEH = 0.1% (studies assessing the effects of psychoplastogen alone) or 0.2% (studies assessing the effects of psychoplastogen + inhibitor) molecular-biology-grade dimethyl sulfoxide (Acros).

Animals. All experimental procedures involving animals were approved by the University of California, Davis, Institutional Animal Care and Use Committee (IACUC) and adhered to principles described in the National Institutes of Health 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).

Cell Culture. Primary cortical cell cultures were prepared as described previously.[28] Briefly, pregnant Sprague Dawley dams were euthanized at embryonic day 18 (E18), and the cortices of the pups were harvested. Cells were plated on poly-D-lysine-coated plates at specific densities depending on the experiment (vide infra). Cultures were maintained at 37 °C under an atmosphere containing 5% CO_2. Plating media 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 16−24 h, media was exchanged for replacement media consisting of 1× B27 supplement (Life Technologies), 1% penicillin−streptomycin, 0.5 mM glutamine, and 12.5 μM glutamate in Neurobasal. For experiments requiring cells older than 7 days in vitro (DIV7), at 96 h postplating, 50% of media was removed, and feeding media containing 1× B27 supplement, 1% penicillin−streptomycin, 0.5 mM glutamine in Neurobasal was added with an additional 20% volume added to account for evaporation.

Dendritogenesis Experiments. The dendritogenesis experiments reported in Figures 1D and S1A were performed as previously described[28] with the exception that cells were treated with psychoplastogens or BDNF for short periods of time (15 min or 1, 24, or 72 h) prior to exchanging the media for replacement media devoid of psychoplastogen/BDNF. The dendritogenesis experiments reported in Figures 1E, 4, and S2 were performed as previously described[74] with the exception of the treatment conditions.

Spinogenesis Experiments. Spinogenesis experiments were performed as previously described[28] with the exception that cells were treated on DIV19 with psychoplastogens (10 μM) for short periods of time (1, 6, and 24 h) prior to exchanging the media for replacement media devoid of psychoplastogens. Cells were fixed on DIV20 (24 h following

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treatment). Images were taken on a Nikon HCA Confocal microscope a with a 100×/NA 1.45 oil objective.

Synaptogenesis Experiments. Synaptogenesis experiments were performed using 96-well plates coated with poly-D-lysine at a density of 15 000 cells per well. The outer wells were not used to avoid edge effects. On DIV7 and DIV14, 50% of the medium was removed (100 μL) and fresh feeding media containing Neurobasal, 1× B27 supplement (Life Technologies), 1% penicillin−streptomycin, and 0.5 mM glutamine was added (70%, 140 μL). Cells were treated with drugs on DIV19. First, 10 mM DMSO stock solutions were diluted 100-fold in Neurobasal, then diluted 10-fold into each well (1:1000 dilution, final concentration of DMSO = 0.1%). Cells were incubated with drug for 0.25, 1, and 6 h, then media was removed and replaced with fresh feeding media devoid of psychoplastogens. Cells were allowed to grow until a total of 24 h had elapsed. At this time, 80% of the media (160 μL) was removed, and a 50% volume of a 4% aqueous paraformaldehyde (PFA) solution (100 μL) at room temperature was added. The fixative was applied to the cultures for 20 min at room temperature. Cells were then washed two times with Dulbecco's phosphate-buffered saline (dPBS, Life Technologies) and permeabilized with 0.2% Triton X-100 (ThermoFisher, 85111) in dPBS for 20 min at room temperature without shaking. Plates were then blocked with antibody diluting buffer (ADB) containing 2% bovine serum albumin (BSA) in dPBS for 1 h at room temperature without shaking. Then, plates were incubated overnight at 4 °C with gentle shaking in ADB and a chicken anti-MAP2 antibody (1:10 000; EnCor, CPCA-MAP2), a guinea pig anti-vGLUT1 antibody (1:1000; Millipore, AB5905), and a mouse anti-PSD-95 antibody (1:500; Millipore, MABN68). The next day, plates were washed three times in dPBS and once in ADB. Plates were then incubated in ADB at room temperature containing an anti-chicken IgG secondary antibody conjugated to Alexa Fluor 488 (1:500; LifeTechnologies), an anti-guinea pig IgG secondary antibody conjugated to Cy3 (1:500; Jackson ImmunoResearch Inc., catalog no. 706-165-148), and an anti-mouse IgG secondary antibody conjugated to Alexa Fluor 647 (1:500; Jackson ImmunoResearch Inc., catalog no. 715-605-151) for 1 h. Next, plates were washed five times with dPBS, and after the final wash, 100 μL of dPBS was added to each well. All images were obtained using a Molecular Devices ImageXpress Micro XLS Widefield High-Content Analysis System at 9 sites per well using 40× magnification. Image analysis was performed using MetaXpress software. Analysis was completed by first using the MAP2 channel to establish a mask of the neuron using thresholds between 0 and 30 μm. Then, the mask of the neuron was expanded by 1 μm. The size of the objects for the presynaptic and postsynaptic fluorescent images were established, and only signals of 0−1.5 μm punctate were used to generate a mask. To measure synaptic density, the presynaptic and postsynaptic masks were overlaid using the logical operation "and" to retain only signal that colocalized to form the synapse mask. The number of events occurring in this synapse mask was quantified and normalized to the MAP2 channel mask area (number of counts per μm^2). Normalized data were then tested for outliers using the ROUT method in Graphpad Prism (version 8) at Q = 1%. The outlier test was completed to remove artifacts in an unbiased manner. To prepare the representative images shown in Figure 3B, brightness/contrast was adjusted evenly across all images. After this, the vGLUT1 and PSD-95 channels were both processed using the "smooth" function in ImageJ (Fiji, Ver. 1.51v) followed by the plug-in "Image Calculator" with image1 corresponding to vGLUT1 and image2 corresponding to PSD-95. The operation used was "And", and "Create new window" was checked. The resulting window contained all points of colocalization between the two images. This window was also processed through the "smooth" function once more. Next, the MAP2 channel was merged with the colocalization channel for a composite that was falsely colored to distinguish the two channels.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsptsci.0c00065.

Effects of BDNF on neuronal growth; effects of LSD, ketamine, and 8-OH-DPAT on dendritic growth; KCl does not enhance psychoplastogen-induced growth (PDF)

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Author Contributions

CL performed the majority of the dendritogeneis, spinogenesis, and synaptogenesis experiments with assistance from ACG, MVV, and WCD. ACGG and PJL supervised the high-content imaging experiments. DEO conceived the project and supervised experiments. DEO and CL wrote the manuscript with input from all authors.

Notes

The authors declare the following competing financial interest(s): D.E.O. is the president and chief scientific officer of Delix Therapeutics, Inc.

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■ ABBREVIATIONS

VEH	vehicle
KET	ketamine
LSD	lysergic acid diethylamide
DNQX	6,7-dinitroquinoxaline-2,3-dione
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
TrkB	tyrosine kinase B
mTOR	mammalian target of rapamycin
BDNF	brain-derived neurotrophic factor

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