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5-HT_{2A}Rs in layer 5 pyramidal neurons mediate the neuroplastic but not the hallucinogenic effects of psilocybin

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Abstract

It is unclear how serotonin 2A receptors (5-HT_{2A}Rs) in cortical layer 5 pyramidal neurons (L5 PyrNs) differentially contribute to psilocybin-induced hallucinations versus neuroplasticity. Here we show that psilocybin promotes synapse formation and maturation while accelerating the elimination of pre-existing synapses. Cell type-specific manipulation further demonstrated that 5-HT_{2A}R signaling in L5 PyrNs is necessary and sufficient for psilocybin-induced synaptic remodeling but dispensable for the head-twitch response, a rodent proxy of hallucination.

Main text

Psychedelics are psychoactive substances best known for their ability to induce hallucinations in humans¹. In rodents, they acutely elicit the head-twitch response (HTR), a rapid, paroxysmal headshake well-established as a proxy for a serotonergic psychedelic's hallucinogenic potential in humans². Besides these psychotropic effects, psychedelics are potent promoters of

neuroplasticity, elevating dendritogenesis and synapse formation while altering neurotransmission *in vitro* and *in vivo*³. Psilocybin, a psychedelic derived from *Psilocybe* “magic” mushrooms, receives much research interest as a potential treatment for various neuropsychiatric disorders⁴; its neuroplastic effects are postulated to be pivotal to its sustained therapeutic value⁵. Like other serotonergic psychedelics, psilocybin is an agonist of the serotonin 2A receptor (5-HT_{2A}R), which is abundantly expressed in the brain, particularly in neocortical layer 5 pyramidal neurons (L5 PyrNs)⁶. Pharmacological studies have implicated 5-HT_{2A}R signaling in the hallucinogenic potential of psychedelics², but its role in the neuroplastic effects remains controversial⁷⁻⁹. Researchers have synthesized several plasticity-promoting, non-hallucinogenic psychedelic analogs¹⁰, but the role of L5 PyrNs in psilocybin’s neuroplastic versus hallucinogenic effects remains incompletely understood^{9,11}.

To evaluate psilocybin’s hallucinogenic and neuroplastic effects in wild type (WT) mice, we administered 0.3, 1, or 3 mg/kg psilocybin to ~3 months old C57BL/6J mice. All doses induced robust HTRs; the 1 and 3 mg/kg doses elicited HTRs comparably, but significantly more than the 0.3 mg/kg dose (Fig. 1a). A modest sex difference was observed at the 1.0 mg/kg dose, with females exhibiting slightly more HTRs than males (Extended Data Fig. 1a). HTR counts gradually increased during the first 5 min after injection, peaked at 5-10 min, and declined afterwards (Extended Data Fig. 1b).

We also examined psilocybin’s neuroplastic effects through *in vivo* two-photon (2P) imaging of dendritic spines in *Thy1*-GFP-M mice, which express cytoplasmic green fluorescent protein predominantly in a sparse subset of cortical L5 PyrNs (Fig. 1b, Extended Data Fig. 2a). Mice were imaged at baseline (d0), received psilocybin (0.3, 1, or 3 mg/kg) or saline injection after 1 day (d1), and were re-imaged 24 h later (d2). Compared with saline, 1 and 3 mg/kg

psilocybin increased spine formation significantly to a similar level, whereas 0.3 mg/kg did not; spine elimination was unaffected across all doses (Fig. 1c). Consequently, 1 and 3 mg/kg doses led to a net gain in spine density over the 2d interval (Extended Data Fig. 2b). As both the HTR and neuroplastic effects of psilocybin plateaued at 1 mg/kg, we used this dose in all subsequent studies. In an independent cohort, the same mice were imaged at baseline and after 1 mg/kg psilocybin, which revealed no sex difference in baseline or psilocybin-induced spine dynamics (Extended Data Fig. 2c). A dendrite-level analysis further showed that both baseline and psilocybin-induced spine formation occurred broadly across imaged dendritic segments, rather than concentrated on a subset of highly plastic dendrites (Extended Data Fig. 3).

Next, we investigated the persistence of neuroplasticity induced by a single dose of psilocybin by tracking spine dynamics on the same dendritic segments before and after psilocybin treatment for more than three weeks (Fig. 1d). While spine formation between d0 and d2 significantly increased, neither spine formation nor elimination over a 2d interval at 1- or 3-week post-treatment differed significantly from the pre-treatment baseline (Fig. 1e), suggesting that psilocybin only transiently enhances spine formation. However, when measured over 7d or 21d intervals, spine formation remained elevated in psilocybin-treated mice (Fig. 1f,g). Interestingly, spine elimination in psilocybin-treated mice significantly increased over 21d (Fig. 1g). Consequently, the magnitude of net spine density changes diminished with longer imaging intervals (Fig. 1h). To determine how new spines formed immediately after psilocybin treatment contribute to long-lasting alterations in synaptic circuits, we classified spines as either pre-existing (present on both d0 and d2 of imaging) or newly formed (present on d2 but not on d0) and tracked their fate to d7 and d21 (Fig. 1i, Extended Data Fig. 4a). In control mice, most new spines were unstable: only $40.6 \pm 3.0\%$ survived to d7 and $29.3 \pm 4.1\%$ to d21. After psilocybin treatment,

however, a significantly higher fraction of new spines persisted over the same intervals ($61.3 \pm 3.5\%$ to d7; $48.0 \pm 3.4\%$ to d21). On the contrary, pre-existing spines in psilocybin-treated mice were less likely to survive than their counterparts in control mice (Fig. 1j,k, Extended Data Fig. 4b). These results suggest that, although it only transiently boosts spine formation, psilocybin alters existing neural circuits with a long-lasting impact.

As spine morphology correlates with synaptic strength and dynamics¹², we next examined the morphology and fate of newly formed spines. In control mice, most new spines were stubby ($65.6 \pm 2.9\%$), few were mushroom-shaped ($2.2 \pm 2.2\%$), and the remainder were thin (Fig. 1l). By d7, only $47.6 \pm 11.6\%$ of stubby spines remained, whereas all mushroom spines persisted (Fig. 1m). In psilocybin-treated mice, however, new spines were significantly less likely to be stubby ($46.5 \pm 4.8\%$) and more likely to be mushroom-shaped ($28.4 \pm 1.8\%$; Fig. 1l); the survival rates of both subtypes were comparable to those in saline controls (Fig. 1m). As mushroom spines are considered more mature than stubby spines¹³, our data suggests that psilocybin not only promotes spine formation but also accelerates spine maturation.

To determine whether psilocybin-induced synaptic remodeling also occurs in the stressed brain, we subjected mice to 21d of unpredictable mild stress (UMS) and administered saline or psilocybin (1 mg/kg) 1d after stress cessation. Dendritic spines were imaged before UMS, at the end of UMS, and 1d after saline or psilocybin administration (Fig. 1n). Psilocybin increased spine formation more in post-UMS mice than in unstressed controls (Fig. 1o). Furthermore, while elevated spine elimination persisted beyond stress cessation with saline treatment, psilocybin reduced it to the unstressed control level (Fig. 1p). Consequently, psilocybin induced a greater net gain in spine density in post-UMS mice than in unstressed mice (Fig. 1q). Together, these data

suggest that post-stress psilocybin treatment not only promotes new spine formation but also stabilizes existing spines.

Are new spines induced by post-UMS psilocybin treatment preferentially formed at sites of spine elimination during UMS? In psilocybin-treated mice, a significantly higher fraction of spines lost during UMS were recovered, compared to saline-treated mice ($7.8 \pm 0.4\%$ vs. $4.8 \pm 0.7\%$, $p = 0.0159$, Fig. 1r). However, the fraction of new spines emerging at sites of prior spine elimination was comparable between the two groups (psilocybin $15.3 \pm 0.9\%$ vs. saline $14.8 \pm 1.8\%$, $p = 0.9603$, Fig. 1s). Thus, the enhanced recovery of lost spines following psilocybin treatment likely reflects overall increased spine formation rather than preferential regrowth at prior spine elimination sites. Furthermore, psilocybin-treated post-UMS mice also showed a significantly higher proportion of new spines assuming the mushroom morphology (Extended Data Fig. 5), suggesting that psilocybin also induces rapid structural maturation of new spines in the post-stress brain.

We next investigated how 5-HT_{2A}R signaling in L5 PyrNs contributes to psilocybin's hallucinogenic and neuroplastic effects, by either restricting 5-HT_{2A}R expression exclusively to these neurons or selectively abolishing it in them. First, we used a 5-HT_{2A}R knock-out conditional rescue line (*htr2a*^{stop/stop}), in which a floxed stop cassette prevents *htr2a* transcription. Crossing with Rbp4-Cre mice, which express Cre recombinase in cortical L5 PyrNs of both pyramidal tract (PT) and intratelencephalic (IT) subtypes, restores 5-HT_{2A}R expression in Cre⁺ neurons. Hereafter we refer to *htr2a*^{stop/stop} mice as 5-HT_{2A}R full knock-out (FKO) mice, and Rbp4-Cre⁺; *htr2a*^{stop/stop} mice as conditional rescue (CR) mice. Immunohistochemistry confirmed the abolition and restoration of 5-HT_{2A}R expression in FKO and CR mice, respectively (Fig. 2a). As expected, psilocybin failed to elicit the HTR in FKO mice (Fig. 2b). Interestingly, it failed to elicit the HTR

in CR mice as well (Fig. 2b), indicating that 5-HT_{2A}R signaling in L5 PyrNs alone is insufficient to induce this hallucination-like behavior.

The spine density in both FKO and CR mice was comparable to that in WT mice (Fig. 2c), suggesting no gross deficit in synaptic development. In FKO mice, psilocybin did not alter spine formation or elimination over 2d. In contrast, psilocybin significantly increased spine formation in CR mice over 2d, albeit to a lesser extent than in psilocybin-treated WT mice; spine elimination was unaffected, leading to a net gain in spine density (Fig. 2d,e, Extended Data Fig. 6a). Similarly, psilocybin treatment resulted in higher spine formation and spine density gain over 7d in CR mice than in FKO mice (Fig. 2f). New spines formed in CR mice were more likely to persist to d7 than those in FKO mice (Fig. 2g), with a survival rate comparable to psilocybin-induced new spines in WT mice (Extended Data Fig. 6b). Interestingly, after psilocybin treatment, the survival rate of pre-existing spines in CR mice was comparable to that in FKO mice (Fig. 2g) and higher than that in WT mice (Extended Data Fig. 6c). These findings suggest that the selective expression of 5-HT_{2A}R in L5 PyrNs suffices to promote spine formation and stabilization without affecting pre-existing synaptic connections.

Is 5-HT_{2A}R signaling in cortical L5 PyrNs necessary for either psychedelic-induced HTRs or neuroplasticity? To address this question, we selectively abolished 5-HT_{2A}R expression in L5 PyrNs by crossing a 5-HT_{2A}R conditional knock-out line (*htr2a*^{flox/flox}) with Rbp4-Cre mice to generate Rbp4-Cre⁺; *htr2a*^{flox/flox} mice (hereafter referred to as CKO mice). The *htr2a*^{flox/flox} mice responded to psilocybin with HTRs as expected (Fig. 3a). Surprisingly, CKO mice also exhibited HTRs upon psilocybin treatment (Fig. 3a), indicating that 5-HT_{2A}R signaling in cortical L5 PyrNs is not necessary for the HTR. Spine densities in *htr2a*^{flox/flox} and CKO mice were normal (compare Fig. 3b with Fig. 2c). In CKO mice psilocybin altered neither spine formation nor elimination,

while in *htr2a*^{flox/flox} mice it elevated spine formation and spine density over 2d as expected (Fig. 3c,d). Similarly, psilocybin treatment resulted in higher spine formation and net spine density gain over 7d in *htr2a*^{flox/flox} mice, compared to CKO mice (Fig. 3e). In *htr2a*^{flox/flox} mice, new spines were also more likely to persist to d7 than in CKO mice, but pre-existing spines had the opposite fate (Fig. 3f). Together with the findings above, these results suggest that 5-HT_{2A}R signaling in L5 PyrNs is both necessary and sufficient for psilocybin's neuroplastic effects (promoting synapse formation and stabilization), but neither necessary nor sufficient for psilocybin-induced HTRs.

Our study revealed several features of psilocybin-induced structural dynamics in synaptic circuits. The elevation in spine formation is rapid but transient, consistent with previous reports^{9,14}. Notably, mushroom-shaped spines account for a significantly larger fraction of new spines in psilocybin-treated mice than in controls. Characterized by large heads and expanded postsynaptic densities, which correlate strongly with AMPA receptor content and synaptic strength¹⁵, mushroom spines are believed to host mature and stable synapses¹³. Thus, psilocybin not only promotes synapse formation but also accelerates synapse maturation. Contrary to a previous report¹⁴, we found that psilocybin-induced new spines are preferentially stabilized. This discrepancy may reflect regional differences in spine dynamics, as we imaged the somatosensory cortex, whereas the previous study examined the frontal cortex (Cg1/M2). Moreover, we observed a concomitant elevation in the elimination of pre-existing spines, which leads to a cumulative increase in spine elimination that gradually drives spine density towards the pre-treatment level in the normal brain.

In the post-stress brain, psilocybin exerts a more pronounced structural effect than in the normal brain, inducing a greater amount of spine formation and normalizing the elevated spine elimination that persists after stress cessation, leading to a larger net gain in spine density.

Although psilocybin promotes the recovery of spines lost during stress, new spines do not preferentially emerge at prior elimination sites, which differs from the reported effects of ketamine in corticosterone-treated mice¹⁶. Whereas the prevalent view emphasizes the sustained increase in spine density as a physical substrate for durable antidepressant effects⁵, our findings suggest that psilocybin induces synaptic circuit remodeling in a more nuanced manner that depends on the context (*e.g.*, normal or pathological).

Our findings further support a cellular dissociation between psilocybin-induced behavioral and neuroplastic effects, consistent with a recent report that 5-HT_{2A}R expression in glutamatergic neurons in the medial prefrontal cortex is dispensable for the psilocybin-induced HTR but required for the structural plasticity of PT-type L5 PyrNs⁹. The Rbp4-Cre line labels about 50% of all L5 PyrNs¹⁷, encompassing both PT and IT subtypes¹⁸. In crosses between *Thy1*-GFP-M and Rbp4-Cre lines, almost all GFP+ L5 PyrNs were Cre+ (Extended Data Fig. 7). We expect that most of the imaged GFP+ neurons were PT, as supported by a connectomic study¹⁹ on the whole-brain axonal projections of individual neurons in *Thy1*-GFP-M mice and by a transcriptomic study²⁰ suggesting that about two-thirds of Rbp4-Cre+ neurons are PT.

Recent reports on the necessity of 5-HT_{2A}R signaling in psilocybin-induced neuroplasticity are inconsistent^{7-9,14}. Using cell type-specific knock-out and rescue strategies, we demonstrated that 5-HT_{2A}R signaling in cortical L5 PyrNs is both necessary and sufficient for their synaptic remodeling in response to psilocybin. Selective restoration of 5-HT_{2A}R expression in L5 PyrNs (CR mice) partially rescues psilocybin-induced increases in spine formation and stabilization, whereas selective deletion of 5-HT_{2A}Rs from these neurons (CKO mice) abolishes these effects. Intriguingly, while psilocybin accelerates the elimination of pre-existing spines in WT mice, it fails to do so in both CR and CKO mice. These results suggest that 5-HT_{2A}R signaling in L5 PyrNs

accounts for a substantial component of psilocybin-induced spine formation and stabilization, although non-cell-autonomous contributions from other cell types cannot be neglected. The reorganization of pre-existing neural circuits, on the other hand, results from the synergy between elevated spine formation and the action of psilocybin on other cell types.

Moreover, CR mice fail to exhibit HTRs following psilocybin treatment, contrasting a previous report that selective restoration of 5-HT_{2A}R expression in cortical excitatory neurons suffices to rescue psychedelic-induced HTRs²¹. In addition, CKO mice still exhibit psilocybin-induced HTRs. These results suggest that although L5 PyrNs are the predominant type of cortical neurons expressing 5-HT_{2A}Rs, the HTR likely involves additional cell types and neural circuits. Importantly, although the HTR is widely used as a proxy for the hallucinogenic potential of serotonergic psychedelics, this stereotyped motor output does not measure perceptual experience; our results therefore should not be interpreted as directly addressing the neural basis of psychedelic-induced hallucinations in humans.

Recent developments in medicinal chemistry corroborate the dissociation between 5-HT_{2A}R-related neuroplasticity and hallucination-like behavioral manifestations. Tabernanthalog, a non-hallucinogenic structural analog of 5-methoxy-*N,N*-dimethyltryptamine, promotes cortical dendritic spine formation at baseline and in mouse models of stress²²⁻²⁴; such effects are mediated through pathways involving 5-HT_{2A}R, TrkB, mTOR and AMPA receptor activation as classical hallucinogenic psychedelics²⁴. Similarly, the lysergic acid diethylamide analog 2-Br-LSD, a partial agonist of 5-HT_{2A}Rs, does not induce HTRs but promotes dendritogenesis and spinogenesis in cultured cortical neurons²⁵. Other non- or less-hallucinogenic compounds, including (+)-JRT and zalsupindole, likewise promote cortical spinogenesis and neuritogenesis while minimizing psychedelic-like behavioral effects^{26,27}. Taken together, our findings indicate that psilocybin's

neuroplastic and hallucinogenic effects are dissociable at the cellular level, supporting the potential to develop neuroplasticity-based therapies without undesirable psychotropic effects.

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Author Contributions: J.L. and Y.Z. designed the study; J.J.B., E.K., S.M., and J.L. performed the experiments and data analysis; J.L. and Y.Z. wrote the manuscript.

Competing Interests Statement:

The authors claim no conflict of interest.

Figure Legends

Figure 1. Psilocybin induces dose-dependent hallucinogenic responses and structural plasticity in mouse cortical circuits. **a**, HTRs following different doses of psilocybin treatment. Welch's one-

way ANOVA $W(3,27.83) = 201.8, p < 0.0001$, followed by Dunnett's T3 multiple comparisons
 test. Sal: saline-treated; Psi: psilocybin-treated. **b**, Examples of *in vivo* 2P imaging of 2d spine
 dynamics with saline and psilocybin treatment. Arrows: spine elimination; arrowheads: spine
 formation. Scale bar: 2 μ m. **c**, Structural dynamics of spines over 2d in response to different doses
 of psilocybin. Formation: one-way ANOVA $F(3,16) = 12.43, p = 0.0002$, followed by Tukey's
 multiple comparisons test. Elimination: one-way ANOVA $F(3,16) = 0.7065, p = 0.562$. **d**,
 Timeline of longitudinal 2P imaging and psilocybin administration. **e**, 2d spine dynamics at
 different times before and after psilocybin treatment. Formation: repeated measures one-way
 ANOVA $F(3,12) = 23.03, p < 0.0001$, followed by Tukey's multiple comparisons test. Elimination:
 repeated measures one-way ANOVA $F(3,12) = 0.8061, p = 0.5144$. **f**, 7d spine dynamics in control
 (Ctrl) vs Psi mice. Mann-Whitney test, formation: $U = 0, p = 0.0025$; elimination: $U = 6, p =$
 0.0682. **g**, 21d spine dynamics in Ctrl vs Psi mice. Mann-Whitney test, formation $U = 0, p = 0.0043$;
 elimination $U = 1, p = 0.0087$. **h**, Spine density changes after psilocybin treatment over time.
 Repeated measures one-way ANOVA $F(2,10) = 25.27, p = 0.0001$, followed by Tukey's multiple
 comparisons test. **i**, Schematic of the spine fate analysis. **j**, An example illustrating different fates
 of new spines. Scale bar: 2 μ m. **k**, 7d survival rate in Ctrl vs Psi mice. Mann-Whitney test, new
 spines $U = 0, p = 0.0043$; pre-existing spines $U = 0.5, p = 0.0065$. **l**, Percentage of new spines in
 different morphological categories. Repeated measures two-way ANOVA, main effect of
 morphology $F(2,24) = 30.26, p < 0.0001$; main effect of treatment $F(1, 12) = 0.5357, p = 0.4783$;
 interaction $F(2,24) = 9.65, p = 0.0008$; followed by Šídák's multiple comparisons test (Ctrl vs Psi).
m, Survival rates of new spines in different morphological categories. Note that sample sizes differ
 across morphological categories because mice with no new spines in a particular category could
 not contribute to survival rate calculations for that category. Mushroom spines are shown

descriptively only due to insufficient sample size in the saline group. Mixed-effects model (REML), main effect of morphology $F(1,11) = 9.679, p = 0.0099$; main effect of treatment $F(1,12) = 1.025, p = 0.3312$; interaction $F(1,11) = 0.0048, p = 0.9462$. **n**, Timeline of longitudinal 2P imaging with UMS and psilocybin administration. **o-q**, 2d spine formation (**o**), elimination (**p**), and spine density changes (**q**) in Sal vs Psi post-UMS mice in comparison with unstressed mice (same data as in Fig. 1c). Two-way ANOVA followed by uncorrected Fisher's LSD test. Formation (**o**): main effect of Psi treatment $F(1,15) = 52.72, p < 0.0001$; main effect of UMS $F(1,15) = 8.674, p = 0.01$; interaction $F(1,15) = 5.422, p = 0.0343$. Elimination (**p**): main effect of Psi treatment $F(1,15) = 10.28, p = 0.0059$; main effect of UMS $F(1,15) = 13.23, p = 0.0024$; interaction $F(1,15) = 7.307, p = 0.0164$. Spine density change (**q**): main effect of Psi treatment $F(1,15) = 70.19, p < 0.0001$; main effect of UMS $F(1,15) = 0.5218, p = 0.4812$; interaction $F(1,15) = 16.72, p = 0.0010$. **r**, Percentage of lost spines that regrew in Sal vs Psi post-UMS mice. Mann-Whitney test, $U = 0, p = 0.0159$. **s**, Percentage of new spines that grew at sites of previous spine elimination in Sal vs Psi post-UMS mice. Mann-Whitney test, $U = 9, p = 0.9603$. All data are presented as mean $\pm$ SEM; n = number of mice, males are shown as filled circles and females as open circles. All p -values of Mann-Whitney test are two-tailed. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. Details of statistics are given in Supplementary Table 1. Source data are provided as Source Data Figure 1.

Figure 2. Selective expression of 5-HT_{2A}Rs in cortical L5 PyrNs suffices to restore psilocybin-induced spine formation and stabilization but not HTRs. **a**, Immunohistochemistry showing the abolition and restoration of 5-HT_{2A}R expression in L5 PyrNs in FKO and CR mice, respectively. Scale bar: 500 μ m. **b**, 1 mg/kg psilocybin does not elicit HTRs in FKO or CR mice. **c**, Spine

density on the apical dendritic tufts of L5 PyrNs in WT, FKO, and CR mice. One-way ANOVA $F(2,12) = 0.8753, p = 0.4417$. **d**, Example of *in vivo* 2P imaging of 2d spine dynamics in FKO and CR mice with 1 mg/kg psilocybin treatment. Arrows: spine elimination; arrowheads: spine formation. Scale bar: 2 μ m. **e**, 2d spine formation and density changes in WT, FKO, and CR mice under baseline and with psilocybin treatment. Two-way ANOVA followed by uncorrected Fisher's LSD test. Formation: main effect of treatment $F(1,24) = 61.73$, main effect of genotype $F(2,24) = 34.73$, interaction $F(2,24) = 26.05, p < 0.0001$ for all. Spine density changes: main effect of treatment $F(1,24) = 31.73$, main effect of genotype $F(2,24) = 14.37$, interaction $F(2,24) = 18.22, p < 0.0001$ for all. **f**, 7d spine dynamics in FKO vs CR mice with psilocybin treatment. Mann-Whitney test, formation $U = 0, p = 0.0079$; elimination $U = 11, p = 0.8333$; spine density changes $U = 0, p = 0.0079$. **g**, 7d survival rate of spines in FKO vs CR mice with psilocybin treatment. Mann-Whitney test, new spines $U = 0, p = 0.0079$; pre-existing spines $U = 10, p = 0.6349$. All data are presented as mean $\pm$ SEM; n = number of mice, males are shown as filled circles and females as open circles. All p -values of Mann-Whitney test are two-tailed. $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. Details of statistics are given in Supplementary Table 1. Source data are provided as Source Data Figure 2.

Figure 3. Selective knock-out of 5-HT_{2A}Rs in cortical L5 PyrNs prevents psilocybin-induced spine formation but not HTRs. **a**, 1 mg/kg psilocybin elicits HTRs in *htr2a*^{fl^{ox}/fl^{ox}} (fl/fl) and CKO mice comparably. Mann-Whitney test, $U = 22.50, p = 0.3414$. **b**, Spine density on the apical dendritic tufts of L5 PyrNs in fl/fl and CKO mice. Mann-Whitney test, $U = 12, p > 0.9999$. **c**, Example of *in vivo* 2P imaging of 2d spine dynamics in fl/fl and CKO mice with 1 mg/kg psilocybin treatment. Arrows: spine elimination; arrowheads: spine formation. Scale bar: 2 μ m. **d**, 2d spine dynamics in

fl/fl vs CKO mice under baseline and with psilocybin treatment. Two-way ANOVA followed by uncorrected Fisher's LSD test. Formation: main effect of treatment $F(1, 16) = 63.27$, main effect of genotype $F(1,16) = 41.84$, interaction $F(1,16) = 73.38$, $p < 0.0001$ for all. Elimination: main effect of treatment $F(1,16) = 0.5039$, $p = 0.488$; main effect of genotype $F(1,16) = 0.3373$, $p = 0.5695$; interaction $F(1,16) = 1.837$, $p = 0.1942$. Spine density changes: main effect of treatment $F(1,16) = 21.68$, $p = 0.0003$; main effect of genotype $F(1,16) = 15.30$, $p = 0.0012$; interaction $F(1, 16) = 31.22$, $p < 0.0001$. e, 7d spine dynamics in fl/fl vs CKO mice with psilocybin treatment. Mann-Whitney test, formation $U = 0$, $p = 0.0079$; elimination $U = 6$, $p = 0.1984$; spine density changes $U = 2$, $p = 0.0476$. f, 7d survival rate of spines in fl/fl vs CKO mice with psilocybin treatment. Mann-Whitney test, new spines $U = 2$, $p = 0.0317$; pre-existing spines $U = 2$, $p = 0.0397$. All data are presented as mean $\pm$ SEM; n = number of mice, males are shown as filled circles and females as open circles. All p -values of Mann-Whitney test are two-tailed. $*p < 0.05$, $**p < 0.01$, $***p < 0.0001$. Details of statistics are given in Supplementary Table 1. Source data are provided as Source Data Figure 3.

References

- 1 Nichols, D. E. Psychedelics. *Pharmacol Rev* **68**, 264-355 (2016). <https://doi.org/10.1124/pr.115.011478>
- 2 Halberstadt, A. L. & Geyer, M. A. Effect of Hallucinogens on Unconditioned Behavior. *Curr Top Behav Neurosci* **36**, 159-199 (2018). https://doi.org/10.1007/7854_2016_466
- 3 Hatzipantelis, C. J. & Olson, D. E. The Effects of Psychedelics on Neuronal Physiology. *Annu Rev Physiol* **86**, 27-47 (2024). <https://doi.org/10.1146/annurev-physiol-042022-020923>
- 4 Uyar, A., Forbrich, L., Lueken, U. & Evens, R. Landscape analysis of pre-registered clinical trials involving classical psychedelics. *J Psychopharmacol*, 2698811251371690 (2025). <https://doi.org/10.1177/02698811251371690>
- 5 Liao, C., Dua, A. N., Wojtasiewicz, C., Liston, C. & Kwan, A. C. Structural neural plasticity evoked by rapid-acting antidepressant interventions. *Nat Rev Neurosci* **26**, 101-114 (2025). <https://doi.org/10.1038/s41583-024-00876-0>

- 6 Cummins, B. R., Billac, G. B., Nichols, D. E. & Nichols, C. D. 5-HT(2A) receptors: Pharmacology and functional selectivity. *Pharmacol Rev* **77**, 100059 (2025). <https://doi.org/10.1016/j.pharmr.2025.100059>
- 7 Vargas, M. V. *et al.* Psychedelics promote neuroplasticity through the activation of intracellular 5-HT2A receptors. *Science* **379**, 700-706 (2023). <https://doi.org/10.1126/science.adf0435>
- 8 Moliner, R. *et al.* Psychedelics promote plasticity by directly binding to BDNF receptor TrkB. *Nat Neurosci* **26**, 1032-1041 (2023). <https://doi.org/10.1038/s41593-023-01316-5>
- 9 Shao, L. X. *et al.* Psilocybin's lasting action requires pyramidal cell types and 5-HT(2A) receptors. *Nature* **642**, 411-420 (2025). <https://doi.org/10.1038/s41586-025-08813-6>
- 10 Sharp, T. & Ippolito, A. Neuropsychopharmacology of hallucinogenic and non-hallucinogenic 5-HT(2A) receptor agonists. *Br J Pharmacol* (2025). <https://doi.org/10.1111/bph.70050>
- 11 Muir, J. *et al.* Isolation of psychedelic-responsive neurons underlying anxiolytic behavioral states. *Science* **386**, 802-810 (2024). <https://doi.org/10.1126/science.adl0666>
- 12 Kasai, H., Matsuzaki, M., Noguchi, J., Yasumatsu, N. & Nakahara, H. Structure-stability-function relationships of dendritic spines. *Trends Neurosci* **26**, 360-368 (2003). [https://doi.org/10.1016/S0166-2236\(03\)00162-0](https://doi.org/10.1016/S0166-2236(03)00162-0)
- 13 Bourne, J. & Harris, K. M. Do thin spines learn to be mushroom spines that remember? *Curr Opin Neurobiol* **17**, 381-386 (2007). <https://doi.org/10.1016/j.conb.2007.04.009>
- 14 Shao, L. X. *et al.* Psilocybin induces rapid and persistent growth of dendritic spines in frontal cortex in vivo. *Neuron* **109**, 2535-2544 e2534 (2021). <https://doi.org/10.1016/j.neuron.2021.06.008>
- 15 Holler, S., Kostinger, G., Martin, K. A. C., Schuhknecht, G. F. P. & Stratford, K. J. Structure and function of a neocortical synapse. *Nature* **591**, 111-116 (2021). <https://doi.org/10.1038/s41586-020-03134-2>
- 16 Moda-Sava, R. N. *et al.* Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. *Science* **364**, eaat8078 (2019). <https://doi.org/10.1126/science.aat8078>
- 17 Benezra, S. E., Patel, K. B., Perez Campos, C., Hillman, E. M. C. & Bruno, R. M. Learning enhances behaviorally relevant representations in apical dendrites. *Elife* **13**, RP98349 (2024). <https://doi.org/10.7554/eLife.98349>
- 18 Gerfen, C. R., Paletzki, R. & Heintz, N. GENSAT BAC cre-recombinase driver lines to study the functional organization of cerebral cortical and basal ganglia circuits. *Neuron* **80**, 1368-1383 (2013). <https://doi.org/10.1016/j.neuron.2013.10.016>
- 19 Gong, H. *et al.* High-throughput dual-colour precision imaging for brain-wide connectome with cytoarchitectonic landmarks at the cellular level. *Nat Commun* **7**, 12142 (2016). <https://doi.org/10.1038/ncomms12142>
- 20 Munz, M. *et al.* Pyramidal neurons form active, transient, multilayered circuits perturbed by autism-associated mutations at the inception of neocortex. *Cell* **186**, 1930-1949 e1931 (2023). <https://doi.org/10.1016/j.cell.2023.03.025>
- 21 Gonzalez-Maeso, J. *et al.* Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron* **53**, 439-452 (2007). <https://doi.org/10.1016/j.neuron.2007.01.008>
- 22 Cameron, L. P. *et al.* A non-hallucinogenic psychedelic analogue with therapeutic potential. *Nature* **589**, 474-479 (2021). <https://doi.org/10.1038/s41586-020-3008-z>

- 23 Lu, J. *et al.* An analog of psychedelics restores functional neural circuits disrupted by unpredictable stress. *Mol Psychiatry* **26**, 6237-6252 (2021).
<https://doi.org/10.1038/s41380-021-01159-1>
- 24 Aarrestad, I. K. *et al.* The psychoplastogen tabernanthalog induces neuroplasticity without proximate immediate early gene activation. *Nat Neurosci* **28**, 1919-1931 (2025).
<https://doi.org/10.1038/s41593-025-02021-1>
- 25 Lewis, V. *et al.* A non-hallucinogenic LSD analog with therapeutic potential for mood disorders. *Cell Rep* **42**, 112203 (2023). <https://doi.org/10.1016/j.celrep.2023.112203>
- 26 Tuck, J. R. *et al.* Molecular design of a therapeutic LSD analogue with reduced hallucinogenic potential. *Proc Natl Acad Sci U S A* **122**, e2416106122 (2025).
<https://doi.org/10.1073/pnas.2416106122>
- 27 Dong, C. *et al.* Psychedelic-inspired drug discovery using an engineered biosensor. *Cell* **184**, 2779-2792 e2718 (2021). <https://doi.org/10.1016/j.cell.2021.03.043>

Methods

Experimental animals

C57BL/6J (JAX# 000664) and *Thy1*-GFP-M (JAX# 007788)²⁸ mice were originally purchased from The Jackson Laboratory; the Rbp4-Cre (MMRRC 031125-UCD)¹⁸, mouse line was obtained from Dr. Lu Chen (Stanford University); the 5-HT_{2A}R knock-out conditional rescue mouse line (*htr2a*^{stop/stop})²⁹ was obtained from Dr. David E. Olson (University of California, Davis); the 5-HT_{2A}R conditional knock-out mouse line (*htr2a*^{flox/flox})³⁰ was obtained from Dr. Hail Kim (Korea Advanced Institute of Science and Technology). To generate conditional rescue (CR) and conditional knockout (CKO) mice for *in vivo* imaging, we used the following breeding strategy. To generate CR mice, homozygous *htr2a*^{stop/stop} mice were first crossed separately with *Thy1*-GFP-M mice or with Rbp4-Cre⁺ mice. The resulting offspring were crossed back with homozygous *htr2a*^{stop/stop} mice to generate *htr2a*^{stop/stop};GFP⁺ and Rbp4-Cre⁺; *htr2a*^{stop/stop} mice, respectively. These two lines were then crossed to obtain Rbp4-Cre⁺; *htr2a*^{stop/stop};GFP⁺ (*i.e.*, CR) and Rbp4-Cre⁻; *htr2a*^{stop/stop};GFP⁺ (*i.e.*, full knockout or FKO) offspring. To generate CKO mice, an analogous breeding scheme was adopted for homozygous *htr2a*^{flox/flox} mice, to generate Rbp4-Cre⁺; *htr2a*^{flox/flox};GFP⁺ (*i.e.*, CKO) and Rbp4-Cre⁻; *htr2a*^{flox/flox};GFP⁺ (*i.e.*, floxed control) mice.

Mice of both sexes, aged between 2 and 4 months, were used in the study. Mice were group-housed on a 12 h light/dark cycle (Zeitgeber Time ZT0 = 6 am) at 21°C ambient temperature and approximately 45% humidity in the room. Mice were randomly assigned to experimental groups. All animal experiments were carried out in accordance with animal protocol (# Zuoy2411) approved by the Institutional Animal Care and Use Committee of University of California, Santa Cruz.

Drug preparation and administration

Psilocybin was administered through intraperitoneal injection at a dosage of 0.3, 1, or 3 mg/kg of bodyweight. USP-grade saline (0.9%) was used as the vehicle.

Head-twitch response (HTR) recording and annotation

The mouse was placed in an empty standard mouse cage after psilocybin or vehicle injection. Its behavior was recorded with an iPhone 15 equipped with a 48-MP main sensor camera (fps = 30) for 20 min. Behavioral videos were manually annotated for the HTR using the BORIS software³¹. The annotator was blind to the animal's experimental condition.

Cranial window implantation

We performed cranial window implantation on mice according to established protocols³² with slight modifications. In brief, the mouse was anesthetized with isoflurane (4% for induction, 1.5% for maintenance). Ophthalmic ointment was applied to prevent eye desiccation and irritation; dexamethasone (2 mg/kg bodyweight) was injected into the quadriceps, and carprofen (5 mg/kg bodyweight) was injected intraperitoneally. A circular piece of the skull over the somatosensory

cortex (centered approximately at AP -1.5 mm, ML 1.5 mm) was removed with a trephine (Fine Science Tools, diameter = 2.3 mm) driven by a high-speed micro-drill (Foredom K1070). The cranial window was sealed with an imaging port made of a round glass coverslip (#2, diameter = 2.3 mm) glued to an overlaying annular glass “doughnut” (#1, inner diameter = 2 mm, outer diameter = 3 mm, Potomac Photonics, Inc.). Dental cement (Jet Denture Repair, Lang Dental) was applied over the exposed skull to secure a custom-made stainless-steel head plate onto the skull. The mouse received the antibiotic enrofloxacin (5 mg/kg) and the analgesic buprenorphine (0.3 mg/kg) preemptively and then daily for 2 more days. The mouse was allowed to recover for 3 weeks before imaging experiments started.

***In vivo* two-photon (2P) imaging of dendritic spines and data analysis**

In vivo 2P imaging of dendritic spines was performed on a 2P microscope (Ultima IV, Bruker Co.) equipped with a 40x/NA = 0.8 water immersion objective (Olympus) and an ultrafast 2P laser (Mai Tai HP, Spectra-Physics) operating at 940 nm. The mouse was anaesthetized with an intraperitoneal injection of a mixture of 17 mg/ml ketamine and 1.7 mg/ml xylazine in 0.9% saline (5.0 ml/kg) and mounted on a custom-made stage for imaging. Stacks of images were acquired with a Z-step size of 1 μ m at 4x digital zoom. Relocation of the same dendrites in subsequent imaging sessions was achieved by reference to blood vessels and the dendritic branching pattern. Data analysis was performed on 3D image stacks in FIJI 7³³. In *Thy1*-GFP-M line, L2/3 neurons are occasionally labeled with GFP, and some of their dendritic segments may extend into cortical L1. We therefore selected imaging regions in which no GFP+ L2/3 neuronal somata were visible at the imaging site and focused on dendritic segments consistent with the apical tuft dendrites of L5 PyrNs within the first 150 μ m below the pial surface (which primarily

corresponds to cortical L1). We acknowledge that this approach cannot exclude every possible L2/3 neuron contribution with absolute certainty, but the scarcity of L2/3 neurons labeled in this line (see Extended Data Fig. 2a) together with our selection criterion ensure that the imaged dendritic segments are predominantly the apical tufts of L5 PyrNs. Typically, 200-250 spines were analyzed per animal per session. The number of dendritic segments and initial spine counts of each mouse in each figure are given in Supplementary Table 1. The percentage of spines formed/eliminated was calculated as the number of spines formed/eliminated divided by the total number of spines counted from the previous imaging session³⁴.

Morphological categorization of spines was performed according to criteria described previously³⁵. Specifically, the analyst scrolled through the z-stack to evaluate the presence of a spine neck and the relative size of the spine head. Mushroom spines were defined as protrusions with a spine head width greater than twice the neck width. Stubby spines were defined as short protrusions less than 0.5 μm in length without a discernible neck. Thin spines were defined as protrusions longer than 0.5 μm with a head width less than or equal to twice the neck width. When a spine protruded above or below the dendritic shaft, we classified it as a mushroom spine only if it appeared narrower/dimmer in at least one z-plane between the dendritic shaft and the bulbous spine head, reminiscent of a neck-like structure. Otherwise, it would be classified as a stubby spine (thin spines protruding above or below the dendritic shaft would be hardly visible). We acknowledge that the inferior axial resolution makes morphological classification in this case less reliable, leading to a potential underestimation of mushroom and thin spines. Nevertheless, such cases are relatively few, so we do not expect them to impact the statistical analysis significantly.

We also investigated whether psilocybin-induced spine formation was uniformly distributed across dendritic segments or concentrated on a small subset of dendrites, using the

473 following permutation test implemented in MATLAB R2025b (MathWorks). Let D denote the
 474 total number of dendritic segments, n_i the initial spine count of the i^{th} dendrite, and $N = \sum_{i=1}^D n_i$
 475 the total initial spine count. Let k_i denote the number of new spines formed on the i^{th} dendrite,
 476 yielding a total of $K = \sum_{i=1}^D k_i$ new spines across all dendrites. Under the null hypothesis of
 477 uniform spine formation, the probability of a new spine being formed on the i^{th} dendrite is
 478 proportional to the initial spine count on the dendrite, which gives the null probability distribution
 479 Q , where $Q_i = n_i/N$. In each simulated trial, we randomly and independently distributed exactly
 480 K new spines among the D dendrites according to the probability distribution Q . For each trial, we
 481 calculated the simulated spine formation fraction for the i^{th} dendrite as $P_i^* = k_i^*/K$, where k_i^*
 482 denotes the number of new spines assigned to this dendrite. Note that because k_i^* must be an
 483 integer, P_i^* in general does not equal Q_i . We quantified this deviation from uniformity using the
 484 Kullback-Leibler (KL) divergence between the simulated distribution P^* and the null distribution
 485 Q for each trial:

$$486 \quad D_{KL}(P^*||Q) = \sum_{i=1}^D P_i^* \ln\left(\frac{P_i^*}{Q_i}\right)$$

487 We also computed the KL divergence between the observed empirical distribution P (where $P_i =$
 488 k_i/K) and the null distribution Q . By comparing the empirical KL divergence given by P against
 489 the distribution of KL divergences generated from simulation (10,000 trials, each given by a P^*),
 490 we obtained a p -value. A significant p -value ($p < 0.05$) indicates that spine formation is
 491 significantly more concentrated on a subset of dendrites than would be expected by chance.

492

493 **Unpredictable mild stress (UMS)**

Mice were subjected to UMS for 3 weeks with random stressors presented on a weekly rotating basis²³. The stressors include restraint stress (confinement in a perforated 50 ml Falcon tube for 30 min), home cage space reduction (by placement of extra bedding materials), exposure to a new room, cage-shaking on an orbital shaker, exposure to sudden loud noises, tail suspension, wet bedding, exposure to stranger mice, light exposure during normal dark cycle, social isolation (single housing), tilted cage, island isolation (placement of mice on small wooden blocks surrounded by water in a cage), complete removal of bedding materials from the cage, random air puffs, foreign objects (Lego blocks, wooden blocks, etc.) in the cage, food deprivation, and continuous exposure to loud music.

Immunohistochemistry and fluorescence microscopy

The mouse was transcardially perfused with 4% paraformaldehyde (PFA) in 0.01M phosphate-buffered saline (PBS) as previously described³⁵. The brain was post-fixed in 4% PFA at 4°C overnight. For immunohistochemical detection of 5-HT_{2A}R, the brain was cryoprotected in 30% sucrose solution, then sectioned into 40 µm thick coronal slices with a vibratome (Leica VT1000S). Free-floating slices were rinsed in PBS (10 min x 3), underwent antigen retrieval in 0.01M citrate buffer (sodium citrate 3 mg/ml, citric acid 0.4 mg/ml, pH = 6.0 [adjusted by NaOH]) at 95°C for 5 min, rinsed again in PBS (10 min x 3), and immersed in the blocking solution (5% normal goat serum [NGS], 5% bovine serum albumin, 0.3% Triton X-100 in PBS) at room temperature for 2 h. Brain slices were then stained with a primary antibody against 5-HT_{2A}R (rabbit polyclonal IgG, Immunostar 24288, Lot# 2041001L, 1:320) in a solution of 5% NGS and 0.3% Triton X-100 in PBS at 4°C for 48 h. They were rinsed in PBS (10 min x 3), stained with a biotinylated goat anti-rabbit IgG (H+L) secondary antibody (Vector Laboratories BA-1000-1.5,

Lot# Y0515, 1:1000) in a solution of 5% NGS in PBS at room temperature for 2 h, rinsed again in PBS (10 min x 3), stained with streptavidin conjugated with Alexa 594 (Invitrogen S32356, 1:1000) in a solution of 5% NGS in PBS at room temperature for 2 h, and finally rinsed in PBS (10 min x 3). Slices were counterstained with 4'-6-diamidino-2-phenylindole (DAPI; 1:36,000) for 10 min, rinsed in PBS (10 min x 3), and mounted on slides with Vectashield HardSet antifade mounting medium (Vector Laboratories H-1400-10). Brain slices were imaged on a Zeiss AxioImager Z2 widefield fluorescence microscope with a 10x/NA=0.45 air objective.

For immunohistochemical detection of Cre recombinase, the post-fixed brain was directly transferred into 1x PBS and sectioned into 40 μ m thick coronal slices with a vibratome (Leica VT1000S). Free-floating slices were blocked in PBS containing 5% NGS and 0.5% Triton X-100 at room temperature for 1 h. Slices were then stained with a primary antibody against Cre recombinase (rabbit polyclonal, Synaptic Systems # 257 003, Lot# 3-25, 1:1000) in a solution of 5% NGS in PBS at 4 °C overnight. After rinsing in PBS, slices were stained with an Alexa Fluor 594-conjugated goat anti-rabbit IgG (H+L) cross-absorbed secondary antibody (Invitrogen A-11012, Lot# 1099502, 1:500) at room temperature for 2 h, then counterstained with DAPI (1:36,000) for 10 min, rinsed in PBS (10 min x 3), and mounted on slides with Vectashield HardSet antifade mounting medium (Vector Laboratories H-1400-10). Brain slices were imaged on a Zeiss AxioImager Z2 widefield fluorescence microscope with ApoTome optical sectioning using a 10x/NA=0.45 air objective.

Statistical analysis

All behavioral and spine dynamics were analyzed with the analyst blinded to the experimental conditions. All statistical analyses were performed using GraphPad Prism 11 (GraphPad Software).

No animals or data points were excluded from the analyses. Data from each mouse was treated as a single data point in a group. No statistical methods were used to pre-determine sample sizes, but our sample sizes are similar to those reported in previous publications^{22,23,34,35}. For parametric tests, data distribution was assumed to be normal but normality and equal variance were not formally tested. We report sample sizes, the statistical tests used, and the *p*-values in figure, figure legends and the Supplementary Table 1. Statistical significance is defined as $p < 0.05$.

Data Availability:

Source Data includes all plotted data in individual Excel sheets and is provided with this paper as a Source Data file.

Code Availability:

The custom-written data analysis codes are available from:
<https://github.com/ZuoLabUCSC/branch-specific-spine-formation>.

Methods-only references:

- 28 Feng, G. *et al.* Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron* **28**, 41-51 (2000). [https://doi.org/10.1016/s0896-6273\(00\)00084-2](https://doi.org/10.1016/s0896-6273(00)00084-2)
- 29 Weisstaub, N. V. *et al.* Cortical 5-HT_{2A} receptor signaling modulates anxiety-like behaviors in mice. *Science* **313**, 536-540 (2006). <https://doi.org/10.1126/science.1123432>
- 30 Choi, W. *et al.* Serotonin signals through a gut-liver axis to regulate hepatic steatosis. *Nat Commun* **9**, 4824 (2018). <https://doi.org/10.1038/s41467-018-07287-7>
- 31 Friard, O. & Gamba, M. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol Evol* **7**, 1325-1330 (2016). <https://doi.org/10.1111/2041-210x.12584>
- 32 Holtmaat, A. *et al.* Long-term, high-resolution imaging in the mouse neocortex through a chronic cranial window. *Nat. Protoc.* **4**, 1128-1144 (2009). <https://doi.org/10.1038/nprot.2009.89>

569 33 Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nat*
570 *Methods* **9**, 676-682 (2012). <https://doi.org/10.1038/nmeth.2019>
571 34 Xu, T. *et al.* Rapid formation and selective stabilization of synapses for enduring motor
572 memories. *Nature* **462**, 915-919 (2009). <https://doi.org/10.1038/nature08389>
573 35 Hodges, J. L. *et al.* Astrocytic Contributions to Synaptic and Learning Abnormalities in a
574 Mouse Model of Fragile X Syndrome. *Biol Psychiatry* **82**, 139-149 (2017).
575 <https://doi.org/10.1016/j.biopsych.2016.08.036>
576





