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Resource**Keywords:**

Posted Date: October 29th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11081511/v1>

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Additional Declarations: There is **NO** Competing Interest.

Multi-scale effects of single-dose psilocybin in the macaque brain

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Abstract

Psilocybin holds strong therapeutic promise for treating major depressive disorder. However, the cellular and molecular substrates underlying its action have not been fully elucidated, especially in the primate brain. We combined multimodal MRI, magnetic resonance spectroscopy (MRS), cerebrospinal fluid (CSF) proteomics and metabolomics, and single-nucleus RNA sequencing (snRNA-seq) to map the multiscale temporospatial dynamics induced by a single dose of psilocybin in cynomolgus macaques. Psilocybin increased the integration of the frontoparietal network (FPN) with the default-mode and limbic networks, transiently reduced integration of the FPN with the somatomotor network, and induced global network segregation. Several regions in the cortex, striatum, and thalamus as well as certain of the frontoparietal white matter pathways showed persistent enhancement lasting 1 week. Notably, these changes were accompanied by a CSF multi-omics shift from acute prostaglandin-dominated inflammation to a sustained enrichment of an omega-3 fatty acid-associated anti-inflammatory state together with a bidirectional reorganization of extracellular matrix (ECM)-related signatures, characterized by increased *CD44* and decreased fibulin-family proteins. In addition, we found lasting upregulation of TCA-cycle and NAD(P)-dependent redox enzymes. This was further supported by sustained elevations of glutamate/glutamine and total creatine in the dorsolateral prefrontal cortex (dlPFC), which was found using *in vivo* MRS. At the cellular level, psilocybin promoted *HTR2A* selective upregulation in deep-layer excitatory neurons along with a widespread enhancement of mitochondrial oxidative phosphorylation centered on the complex I subunit *ND3* across diverse excitatory and inhibitory neuronal subtypes in the dorsolateral prefrontal cortex. Glial-state remodeling, including a shift of astrocytes toward ECM remodeling states and microglia toward an anti-inflammatory state, also occurred. Collectively, these findings provide evidence in the primate brain that psilocybin induces functional integration and segregation of brain networks, enhances energy metabolism and synaptic plasticity, and modulates neuroimmune signaling. All the data will be shared as an integrated resource for primate psilocybin research.

Introduction

Psilocybin has been used in human societies for thousands of years^{1,2} because it powerfully alters states of consciousness. In past decades, psilocybin has shown promise for treating major depressive disorder (MDD) and anxiety³⁻⁶. In patients with MDD, a single 25 mg dose of psilocybin combined with psychological support led to significant symptom improvement within days, with effects sustained up to 6 weeks⁷. In treatment-resistant depression, dose-dependent clinical benefits were observed, with 25 mg psilocybin producing greater symptom reduction than lower-dose control conditions over the acute follow-up period⁵. However, the neural mechanism underlying the benefits of psilocybin therapy has not been fully elucidated, partly because little is known about its effects in the primate brain, particularly in the prefrontal cortex, a region centrally implicated in depression⁸.

Macroscopic magnetic resonance imaging (MRI) studies reported that psilocybin can modulate functional connectivity within the default mode network (DMN) and between the DMN and other associative cortical regions⁹⁻¹¹ while increasing the integration of the sensory networks¹². It has also been shown to alter large-scale cortical network organization in depression by reducing network modularity and increasing global functional integration across cortical systems that include prefrontal control networks¹³. Animal studies further showed that psilocybin rapidly induces long-lasting structural plasticity in layer 5 pyramidal neurons of the mouse medial frontal cortex, including an increase in dendritic spine density and size¹⁴. Recent work also demonstrated that medial frontal cortical pyramidal tract (PT) neurons are selectively required for psilocybin-induced sustained structural remodeling and behavioral effects, in a 5-HT_{2A} receptor-dependent manner¹⁵. Collectively, these findings from humans and rodents indicate that the prefrontal cortex (PFC) acts as a critical region for psilocybin's beneficial effects. Non-human primates (NHP), whose prefrontal cortex closely resembles that of humans in structural organization¹⁶, have also been used to study classic psychedelics, characterizing acute behavioral alterations and reinforcing properties. But only a few studies have probed neural activity, such as drug-induced changes in functional connectivity^{17,18}. As a result, the molecular and cellular substrates through which psilocybin acts in the primate brain remain unresolved, and a multi-scale picture integrating imaging, circuit, molecular, and cellular levels is still lacking. This limits our understanding of how psilocybin produces therapeutic benefits in human patients.

To address these gaps, we conducted a longitudinal multimodal study in cynomolgus macaques, *Macaca fascicularis*, to comprehensively characterize psilocybin-induced responses

across behavioral, systemic, neurochemical, molecular, and cellular levels within a unified experimental framework. We first evaluated longitudinal behavioral changes in monkeys after psilocybin administration. Then, multimodal MRI was used to delineate temporospatial alterations in whole-brain functional topology, inter-regional functional connectivity, and microstructural integrity at pre-dose, 24 hours post-dose, and 1 week post-dose intervals. To bridge these macroscopic changes with the underlying neurochemical and molecular dynamics, we combined *in vivo* magnetic resonance spectroscopy (MRS) of the dorsolateral PFC (dlPFC) with longitudinal proteomic and metabolomic profiling of the cerebrospinal fluid (CSF) at the same time points. Given the central role of the dlPFC in the pathophysiology and treatment of depression, we further performed single-nucleus RNA sequencing (snRNA-seq) of the dlPFC in three monkeys at 48 hours post-dose to reveal the neuronal, glial, and intercellular signaling programs underpinning these systems- and molecular-level changes.

Results

Psilocybin-induced behavioral changes in macaque

We first evaluated the behavioral effects of single-dose psilocybin administration in cynomolgus monkeys across multiple behavioral domains. A total of 15 female cynomolgus macaques were assigned as experimental monkeys; of these, 10 received a single intravenous administration of psilocybin (0.44 mg/kg), comparable to doses previously administered to non-human primates (0.3 mg/kg intravenously and 0.4 mg/kg intramuscularly)^{18–20}. Five control monkeys ($n = 5$) did not receive psilocybin. We mainly used the control monkeys for social behavioral testing and brain tissue collection to provide comparisons with the psilocybin-treated monkeys. Social interaction data were obtained from seven psilocybin-treated monkeys, using five control monkeys as interaction partners; two of the control monkeys participated in both sessions. In the first session, five psilocybin-treated monkeys were paired one-to-one with five control monkeys. Subsequently, three psilocybin-treated monkeys and three control monkeys were used for brain tissue collection. Therefore, in the second session, social interaction testing was performed with the remaining two psilocybin-treated monkeys and two control monkeys. In total, social interaction data were obtained from seven psilocybin-treated monkeys. Of the experimental animals, seven completed longitudinal behavioral testing, MRI scanning, and CSF collection, whereas three were used for brain tissue sampling. Three of the five controls were also utilized for brain tissue collection (Figure 1).

Psilocybin produced behavioral changes, the nature of which depended on the time window of assessment. Within the first three days post-dose, sleep architecture and consummatory behavior were most affected. Sleep staging revealed shifts in vigilance states, including a significant increase in deep sleep duration ($P = 0.016$), accompanied by decreases in light sleep ($P = 0.016$) and wakefulness ($P = 0.016$) (Figure S1B). In the sucrose preference test, both water intake ($P = 0.047$) and sucrose solution intake ($P = 0.031$) increased, while sucrose preference itself was unchanged ($P = 0.469$) (Figure S1D). By contrast, postural and locomotor measures assessed at 3–6 days post-dose were largely unchanged. Monkeys showed a significant increase in total sitting time ($P = 0.016$) but no change in hang-cuddled behavior ($P = 0.109$) or hang-vertically-from-hang-cuddled postures ($P = 0.156$) (Figure S1A). A broader set of locomotor measures, including movement velocity, distance traveled, turning behavior, vertical activity, pacing, and posture transitions, did not differ from baseline (Figure S2). This lack of pronounced locomotor change 3–6 days after psilocybin differs from earlier non-human

primate studies, in which psychedelics, including psilocybin, altered activity levels and induced motor changes within hours of dosing¹⁸. Thus, psilocybin's longer term motor effects in primates, which we examined at a later time window, had previously been largely uninvestigated.

Psilocybin also selectively modulated different social behaviors. Reactive social interactions (e.g., receiving grooming, being approached) and spontaneous activity (e.g., sitting, hang cuddling) showed marginally significant changes after psilocybin administration (both P s = 0.078), whereas proactive social behaviors (e.g., initiating grooming, approaching) remained unaffected (Figure S1C). These findings suggest that psilocybin affects specific behavioral domains in cynomolgus monkeys, with the most robust changes in sleep architecture and consummatory behavior within the first three days post-dose.

Psilocybin-induced sustained functional and structural reorganization of brain circuits

To explore brain spatiotemporal dynamic changes following single-dose psilocybin administration, we assessed dynamic alterations in brain functional organization and microstructure across three time points (pre-dose, 24 hours post-dose, and 1 week post-dose). At the global level, psilocybin significantly increased system segregation from baseline to 1 week post-dose ($P < 0.05$, Cohen's $d z = 0.96$) and from 24 hours to 1 week post-dose ($P < 0.01$, Cohen's $d z = 1.89$), indicating strengthened within-network coherence relative to between-network coupling at 1 week (Figure 2A). Moreover, specific between-network couplings involving the frontoparietal network (FPN) were selectively reorganized. Although a recent non-human primate fMRI study reported acute desynchronization within the DMN immediately following psilocybin administration²¹, our longitudinal tracking revealed a clear long-term pattern. Integration between the FPN and the DMN increased significantly at 24 hours and remained elevated at 1-week post-dose relative to baseline ($P < 0.05$, Cohen's $d z = 1.39$). In contrast, FPN-limbic integration (FPN-LIM) showed a delayed, monotonic increase that reached significance only at 1 week post-dose ($P < 0.05$, Cohen's $d z = 1.11$), whereas FPN-somatomotor integration (FPN-SMN) showed a transient decrease at 24 hours ($P < 0.05$, Cohen's $d z = 0.77$) and returned toward baseline by 1 week. Together, these results indicate that psilocybin's effect on the macroscale network architecture is not a uniform shift toward integration or segregation, but rather a selective reorganization in which most between-network couplings weaken (driving the increase in global segregation) while specific FPN-related couplings, particularly to the DMN and limbic networks, are strengthened. This divergence

from previous acute findings²¹ suggests a critical temporal shift from immediate network dissociation to sustained, selective network reconfiguration over a 1 week period.

To explore the relationship between changes in brain network organization and changes in behavior before and after psilocybin administration, we conducted brain-behavior association analyses. Before correction for multiple comparisons, changes in system segregation at 1 week correlated positively with changes in wakefulness but negatively with deep sleep duration (Figure S3). In other words, monkeys with greater increases in deep sleep had smaller increases in system segregation, suggesting that deeper sleep may help stabilize psilocybin's acute effects on brain networks.

Given that global analyses can only reveal overall changing patterns, we further examined specific alterations in functional connectivity between brain regions after dosing using a linear mixed-effects model (Figure 2B). Psilocybin reduced functional connections between the DMN, limbic, dorsal attention and visual networks (Figure 2B), specifically between the left retrosplenial cingulate cortex and left intraparietal cortex (CCr_L-PCip_L), between the right amygdala and left intraparietal cortex (Amyg_R-PCip_L), and between the left frontopolar cortex and right ventral anterior visual cortex (PFCpol_L-VACv_R). In contrast, psilocybin strengthened several functional connections involving the DMN, limbic, and FPN networks. These included connections between the right central temporal cortex and right ventrolateral premotor cortex (TCc_R-PMCvl_R), between the left temporal polar cortex and right ventrolateral premotor cortex (TCpol_L-PMCvl_R), and between the left parahippocampal cortex and left medial premotor cortex (PHC_L-PMCm_L). Additional strengthened functional connections involved the auditory, DMN, and somatomotor regions, including connections between the subgenual cingulate cortex and right secondary auditory cortex (CCs_R-A2_R), between the left primary auditory cortex and left superior parietal cortex (A1_L-PCs_L), and between the left secondary auditory cortex and left medial premotor cortex (A2_L-PMCm_L) (Figure 2B). Importantly, these reduced or increased functional connectivities followed a coherent temporal change trajectory detected at 24 hours post-dose and persisting to 1 week. In addition, a non-monotonic change in functional connectivity between the DMN and nucleus accumbens (PFCdm_L-NAc_L) was observed.

To further explore whether the functional reconfigurations were accompanied by concurrent changes in the underlying microstructural complexity of the gray matter (GM) and white matter (WM), we employed diffusion kurtosis imaging (DKI) to delineate the microstructural changes in the GM and WM. These measures revealed similar changing

patterns. Sustained increases in gray matter mean kurtosis (MK) were observed within the cortico-striato-thalamo-cortical (CSTC) circuit, including the right thalamus and putamen as well as the temporal, auditory, sensory, gustatory, and insular cortices (Figure 2C). Furthermore, we found enhanced microstructure in major association tracts, including the second and third branches of the superior longitudinal fasciculus (SLF II and SLF III), the right anterior thalamic radiation (AR_R), and the middle longitudinal fasciculus (MdLF) (Figure 2D). A tract-based analysis of fractional anisotropy (FA) revealed increased microstructure in the inferior fronto-occipital fasciculus (IFO), inferior longitudinal fasciculus (ILF), perigenual cingulum bundle (CBP), and temporal cingulum bundle (CBT) (Figure 2D). The tract-wise analyses indicated enhanced directional coherence of long-range tracts that mediate temporo-frontal and occipito-frontal information transfer. Together, these structural changes indicate that psilocybin-induced functional remodeling in the primate brain co-occurred with broadly distributed microstructural adaptations across cortical and subcortical GM regions and WM pathways; this may be the structural basis underlying functional network reconfigurations. Importantly, these functional and structural changes converged on prefrontal circuits involving the dlPFC: At the functional level, several of the reorganized couplings involved the frontoparietal network, of which the dlPFC is a central node, while at the structural level the SLF II, which in macaques links the dlPFC with the occipito-parietal and caudal inferior parietal cortices^{22,23}, showed increased mean kurtosis. This convergence of network- and tract-level changes on prefrontal circuits led us to next examine the molecular and cellular alterations of the dlPFC directly.

Longitudinal CSF multi-omics revealed sustained bioenergetic reprogramming and a temporally staged inflammatory-to-remodeling transition

To identify temporally staged molecular changes associated with psilocybin-induced circuit reconfiguration, we performed longitudinal CSF proteomics and untargeted metabolomics analyses at baseline, 24 hours post-dose, and 1 week post-dose. Seventy proteins showed concordant changes at both post-dose time points, comprising 49 persistently increased and 21 persistently decreased proteins (Figure 3A). The persistently increased proteins were dominated by enzymes involved in central carbon metabolism and redox homeostasis. Glycolytic proteins, including PKM and GPI, together with the TCA-cycle-associated enzyme MDH1, showed sustained increases after psilocybin administration. Coordinated increases were also observed in NAD(P)H-dependent metabolic and redox enzymes, including PGD, GSR, IDH1, ADH5, ADH7, and AKR1C1, consistent with enhanced bioenergetic and redox-buffering capacity. The

extracellular matrix (ECM) receptor CD44 showed increased abundance at both post-dose time points, whereas several ECM-associated fibulin-family proteins, including EFEMP1, EFEMP2, and FBLN5, showed concordant decreases. EFEMP1 exhibited one of the largest persistent decreases and was the most strongly decreased protein at 1 week. This reciprocal pattern is consistent with a reorganization of the ECM composition rather than uniform activation of an ECM program. A Gene Ontology enrichment analysis performed across all differentially abundant proteins identified NAD(P)-dependent oxidoreductase activity as the most reproducibly enriched molecular function at both post-dose time points. This enrichment was largely driven by increased metabolic and redox enzymes, including ADH5, CBR1, AKR1B1, MDH1, PGD, IDH1, ADH7, and AKR1C1. Actin filament binding was also enriched at 1 week post-dose, consistent with the later emergence of cytoskeletal remodeling. Together, these findings indicate that psilocybin induces sustained bioenergetic and redox adaptation accompanied by bidirectional remodeling of extracellular structural programs.

Consistent with the proteomic bioenergetic changes, untargeted metabolomics revealed a striking temporal transition in lipid mediator metabolism (Figure 3B). At 24 hours post-dose, prostaglandin synthesis and regulation emerged as the dominant enriched pathways, with 11-HEDE among the top upregulated metabolites. Prostaglandins are canonical cyclooxygenase-derived eicosanoids that serve as central mediators of neuroinflammation, modulating vascular permeability, glial activation, and nociceptive signaling in the CNS, indicating an acute, pro-inflammatory lipid response in the early post-dose period. By 1 week post-dose, this inflammatory landscape underwent a complete reconfiguration. The dominant pathways shifted to alpha-linolenic acid metabolism and omega-3/omega-6 fatty acid synthesis, bioactive lipids that are mainly involved in pro-resolving mediation and neuroprotection. This temporal transition from acute prostaglandin-dominated signaling toward sustained omega-3 fatty acid metabolism suggests a coordinated change from a pro-inflammatory state to a pro-resolving, plasticity-enabling microenvironment. The enrichment of the nucleotide salvage pathway was supported by the upregulation of adenosine kinase (ADK) and adenosylhomocysteinase (AHCY) in proteomic analyses suggesting enhanced purine cycling to support the elevated energetic demands of neural remodeling. Notably, G alpha (q) signaling was found at both 24 hours and 1 week post-dose. Given that the 5-HT_{2A} receptor canonically couples to the G alpha (q) cascade, this result suggests that the downstream metabolic process triggered by 5-HT_{2A} receptor activation persists beyond the acute period to enable neuroplasticity.

To further validate these bioenergetic alterations found in CSF proteomics and

metabolomics, we performed longitudinal *in vivo* MRS targeting the left dlPFC. Linear mixed-effects modeling revealed significantly progressive increases in both the glutamate-glutamine complex (Glx) and total creatine (tCr) from baseline to 1 week post-dose (Figure 3C). The other metabolites (NAA, myo-inositol, total choline, and aspartate) showed no significant longitudinal changes (Figure S4). The sustained elevation of tCr, reflecting changes in the cerebral creatine–phosphocreatine pool involved in cellular energy buffering, was consistent with the bioenergetic alterations indicated by CSF proteomics. The elevated Glx indicates persistent enhancement of excitatory neurotransmission. The cross-modal convergent evidence indicated that psilocybin primarily drives energy metabolism reprogramming, synaptic plasticity, and pro-inflammatory and neuroimmune processes.

Single-nucleus transcriptomics revealed convergent mitochondrial upregulation across dlPFC neuronal subtypes

To elucidate the cellular and molecular alterations of the brain after psilocybin administration, we profiled the transcriptomic landscape of the macaque left dlPFC using snRNA-seq at 48 hours post-dose. Brain tissues were collected from monkeys with and without psilocybin administration ($n = 3$ per group). After quality control and annotation based on canonical marker genes, we defined 18 major cell types, which included excitatory and inhibitory neuronal subtypes as well as principal glial populations (Figures 4A, B; Figure S5A). To identify psilocybin-induced cellular and molecular changes, differential gene expression analyses revealed that the neurons, particularly the excitatory neurons of L2-3 and L3-5 in the dlPFC, showed the most significant transcriptomic changes in monkeys after psilocybin administration, while oligodendrocytes and astrocytes dominated the non-neuronal changes (Figure 4C). A GO analysis of up-regulated genes in neuronal sub-clusters revealed convergent enrichment of mitochondrial and metabolic pathways. Specifically, with the exception of L5/6 IT Car3 neurons, the affected neuronal subtypes consistently up-regulated genes associated with oxidative phosphorylation, aerobic electron transport, and respiratory chain activity (Figure 4D; Figure S5B) suggesting a unified neuronal bioenergetic response to psilocybin. Notably, the mitochondrial signature identified by single-nucleus transcriptomics was consistent with a sustained upregulation of the TCA cycle and pentose phosphate pathway enzymes found in CSF proteomics and with the progressive elevation of total creatine in the dlPFC detected by MRS. A gene ontology enrichment analysis of the downregulated genes revealed widespread functional impairment across brain cell types following psilocybin treatment. Glial cells

predominantly exhibited reduced immune and supportive functions, whereas neurons showed impaired ion transport, energy metabolism, and signal transduction (Figure S5C). Only two downregulated genes, *HMGN3* (a chromatin-associated protein-encoding gene involved in chromatin remodeling and transcriptional regulation) and ENSMFAG00000034226 (Figure S5D-E), were shared across all excitatory neuron subtypes. All the evidence collectively supports a finding that psilocybin triggers systems-level bioenergetic reprogramming, as manifested by neuronal oxidative phosphorylation.

To identify the genes underlying the convergent metabolic signature, we examined the overlap of up-regulated genes across neuronal subtypes and identified a gene, *ND3*, that was significantly and consistently altered across all neuronal populations (Figures 4E, Figure S5F-G). *ND3* encodes a core subunit of mitochondrial respiratory chain complex I, which further supports the predominance of mitochondrial oxidative pathways in the neuronal response to psilocybin. To assess whether *ND3* represents a central component of the psilocybin-associated neuronal metabolic program, we applied high-dimensional weighted gene co-expression network analysis (hdWGCNA) to neuronal cells and identified 11 co-expression modules (Figures S6A-C). Among these, Module 10 was the most strongly up-regulated and was shared across both excitatory and inhibitory neuronal populations. *ND3* was also identified as a hub gene within this module (Figures 4F, Figure S6D-F). As a core subunit of mitochondrial complex I, the Module 10 hub gene *ND3* directly aligns with the CSF proteomic enrichment of NAD(P)-dependent oxidoreductase activity, providing cross-scale evidence for the psilocybin's bioenergetic effect.

Psilocybin selectively engaged deep-layer excitatory neurons and reconfigured microglial inflammatory states

HTR2A is widely recognized as one of the principal molecular targets mediating the effects of psilocybin²⁴⁻²⁷. To identify psilocybin-induced transcriptional changes in different cell types, we first examined the expression of *HTR2A* across each cell type. Although *HTR2A* was widely expressed across neuronal populations in the control group, its upregulation after psilocybin administration was largely constrained to deep-layer excitatory neurons, with the most pronounced increase in L3-5 excitatory neurons ($\log_2FC = 0.84$, $P < 0.01$; Figure 5A-B, Figure S6G-H). In contrast, superficial excitatory neurons and inhibitory subtypes showed no significant change. These results indicate that deep-layer excitatory neurons, particularly the L3-5 population, are the most transcriptionally responsive neuronal populations to psilocybin

in the primate dlPFC.

To assess synapse-related transcriptional changes after psilocybin administration, we computed a composite synaptic function score based on gene sets associated with synaptic transmission, plasticity, and structural remodeling (GO:0007268, GO:0050808, GO:0007269, GO:0048169). After psilocybin administration, synaptic functional scores were highest in deep-layer excitatory populations, particularly L5/6 IT and L3-5 neurons (Figure 5C, Figure S7A-B). The largest increases in synaptic function scores were observed in L5-associated excitatory populations (Figure 5D). Although L5/6 IT Car3 neurons did not share the extensive mitochondrial upregulation observed in other neuronal subtypes, they exhibited a distinct response profile dominated by synaptic and neurotrophin programs, with the most pronounced synaptic enhancement and the most significant brain-derived neurotrophic factor (BDNF) upregulation ($P < 0.01$; Figure 5E).

We next examined neuroinflammation-related transcriptional signatures after psilocybin administration. We derived a composite neuroinflammation index from neuroinflammation gene sets. At the global level, psilocybin caused a reduction in the neuroinflammation score ($P < 0.001$; Figure 5F). This reduction varied across cell types: Inhibitory neurons showed larger decreases in neuroinflammation scores, whereas deep-layer excitatory neurons only exhibited modest changes (Figure S7C-D). These alterations were accompanied by a significant decrease in the expression of the pro-inflammatory cytokine gene *IL6* (Figure S7E).

Although the global decrease in neuroinflammation was largely driven by neurons, glial cells showed a distinct response profile with microglia exhibiting the most pronounced changes (Figure 5G), consistent with their prominent immune-metabolic transcriptional response (Figure S5B). Given the functional heterogeneity of microglia, the microglia were subdivided into five subpopulations and further classified as pro-inflammatory (M1; clusters 2 and 3) and anti-inflammatory (M2; other clusters) states based on established M1/M2 gene-set scores (Figure S7F). Within microglia, both the M1 and M2 subpopulations exhibited elevated inflammatory gene expression after psilocybin administration, indicating context-dependent immune engagement rather than global suppression (Figure 5H, Figure S7G). By assessing the polarization balance using the ratio of M1-to-M2 gene-set scores, we found that the M2 microglia showed a significant decrease in this ratio reflecting enhanced M2 polarization and strengthened anti-inflammatory signaling, whereas the M1 subset remained relatively stable. These results suggest that psilocybin remodels deep-layer excitatory neuronal synaptic programs while simultaneously reshaping microglial states toward a strengthened anti-

inflammatory phenotype in a subtype-specific manner. The microglial polarization toward strengthened M2 states aligns with the temporal shift from prostaglandin-dominated to the omega-3 fatty acid-enriched metabolism observed in CSF.

Coordinated neuron-glia communication remodeling established a plasticity-enabling microenvironment

The concurrent neuronal and glial responses after psilocybin administration suggested coordinated intercellular signaling. To examine how psilocybin reshapes cell-cell communication in the dlPFC, we first mapped macaque genes to their human orthologs and then inferred ligand-receptor interaction networks using CellChat, focusing on pathways related to synaptic plasticity and neuroimmune regulation. Across major cell types in the dlPFC, psilocybin enhanced developmental and adhesion-related signaling, attenuated immune-associated pathways, and increased vascular and ECM communication (Figure S8A).

Neuron-astrocyte communication showed a distinct remodeling pattern. The overall strength of their communication decreased after psilocybin administration (Figure 6A), with the largest reductions observed between L3-5 and L5/6 IT excitatory neurons and astrocytes (Figure S8B). This reduction was primarily attributed to an attenuation of neurexin (NRXN)-mediated signaling (Figure 6B, Figure S8C). Excitatory neurons showed coordinated decreases in *NRXN1*, *NRXN2*, and *NRXN3*-associated signaling toward astrocytes (Figure S8D), whereas inhibitory neurons displayed a more selective reduction in NRXN2-related interactions (Figure 6C). In contrast to the reduced neuronal input to astrocytes, astrocyte-to-neuron signaling increased toward deep-layer excitatory populations, with BMP signaling showing the upregulation from the astrocytes toward the L3-5 and L5 ET neurons (Figure 6D, Figure S8C). *BMP7* was the dominant ligand contributing to this enhanced astrocyte-to-neuron communication (Figure S8E). In addition, L5/6 IT Car3 neurons selectively strengthened their outward neurotrophin signaling networks through the BDNF-NTRK2 ligand-receptor pair (Figure 6E, Figure S8F). Finally, astrocyte-microglia communication was also enhanced after psilocybin administration with NCAM signaling showing the largest enhancement, suggesting remodeling of glial crosstalk (Figure S8G).

Given the important role of astrocytes in forming and supplying energy to synapses, we performed a Monocle2-based pseudo temporal trajectory analysis to infer a cell state transition of astrocytes and identified three astrocyte states (Figure 6F). State 1 predominated in monkeys without psilocybin administration, whereas States 2 and 3 were primarily observed in the

psilocybin group. State 3 showed the most pronounced psilocybin-specific enrichment. Functional enrichment revealed the following associations: State 1 with kinase signaling and cell adhesion, State 2 with synaptic structural support, and State 3 with GABAergic synapse and postsynaptic membrane-related pathways (Figure S9A). State 3 was markedly enriched in a network of pathways, including trans-synaptic signaling, acidic amino acid transport, GTPase regulator activity, telencephalon development, and cell-matrix adhesion (Figure 6G). Notably, CytoTRACE-derived stemness scores were significantly higher in both States 2 and 3 than in State 1 (Figure 6H, Figure S9B), indicating that psilocybin may enhance astrocytic plasticity. When these results were compared with our CSF proteomic findings, we found a concordant upregulation of CD44 at both the transcriptomic and CSF proteomic levels (Figure S9C). In the CSF, CD44 was consistently upregulated at both 24 hours and 1-week post-dose, thus, it can serve as a cross-scale molecular link for astrocyte remodeling. *CD44* showed preferential enrichment in post-psilocybin astrocyte State 3 (Figure S9D-E), which exhibited a hybrid reactive signature co-expressing A1-like (neurotoxic) and A2-like (neuroprotective) markers, suggesting a transitional activation state (Figure S9F). In summary, these results reveal a coordinated remodeling of the deep-layer neuronal-glial landscape following psilocybin administration with attenuated NRXN-mediated neuron input to astrocytes, enhanced astrocyte-to-neuron BMP and neurotrophin signaling toward deep-layer populations, and a shift in microglial polarization toward anti-inflammatory M2 states strengthened by NCAM-mediated glial crosstalk.

Discussion

Our study identified coordinated, temporally organized plasticity induced by a single dose of psilocybin in the primate brain. Psilocybin preferentially engaged deep-layer excitatory neurons via 5-HT_{2A}-related signaling, upregulated mitochondrial and synaptic plasticity, and drove glial remodeling involving astrocytes toward ECM-related states and microglia toward M2 polarization. These cellular changes occurred alongside sustained bioenergetic and redox signatures, a temporal switch from early prostaglandin-dominated inflammatory signaling to later omega-3 fatty acid and ECM-remodeling, and persistent reorganization of CSTC circuits, fronto-parietal-occipital white matter pathways, and large-scale functional networks. These findings appear to delineate a multi-scale coordinated mechanism by which psilocybin may produce enduring therapeutic effects beyond its acute pharmacological window. Further studies are required to determine the causal relationships among neuronal bioenergetic adaptation, glial ECM remodeling, inflammatory lipid signaling, circuit reorganization, and antidepressant-related behavioral outcomes, particularly in disease-relevant primate models and clinical populations.

Psilocybin produces time- and domain-specific behavioral changes

At the behavioral level, a single dose of psilocybin produced selective effects that varied across post-dose windows. During the first three days, monkeys showed increased deep sleep, reduced light sleep and wakefulness, and greater water and sucrose intake even though sucrose preference itself remained unchanged. Given that the animals were healthy and exhibited no baseline depression-like behaviors, the sucrose preference task was used to assess reward-related consummatory behavior rather than anhedonia. The simultaneous increases in water and sucrose intake without change in sucrose preference suggest that psilocybin induced a generalized augmentation of fluid consumption rather than a disruption of hedonic valuation. In contrast, locomotor activity and most social measures examined at 3-6 days post-dose remained largely unchanged, with the exception of an observed increase in sitting time. These behavioral findings indicate that, in a non-human primate group, psilocybin induced predominant changes in sleep architecture and consummatory behaviors, without alterations in reward processing, motor function, or social interaction. Since these behaviors were assessed at different post-dose intervals, the small changes in locomotor and social activity observed at 3-6 days cannot rule out transient pharmacological effects during the acute exposure window. The time- and domain-specific behavioral profile provides a phenotype for interpreting the

following neural, molecular, and cellular adaptations induced by psilocybin.

Psilocybin induces sustained bioenergetic adaptation in deep-layer neurons

Three converging lines of evidence indicate that psilocybin causes sustained bioenergetic changes in deep-layer dlPFC neurons that outlast the acute pharmacological window. Although *HTR2A* was broadly expressed across dlPFC neuronal populations, its psilocybin-induced upregulation was largely restricted to deep-layer excitatory neurons, particularly L3-5 neurons, which also showed elevated synaptic function scores. This aligns with classical immunocytochemical evidence of high 5-HT_{2A} receptor expression in layer V pyramidal neurons of the human PFC^{28,29}. Given that psilocybin's half-life is only several hours³⁰, the days-long synaptic and circuit-level adaptations we observed indicate that psilocybin's mechanisms go beyond receptor-level persistence, possibly involving prolonged intracellular signaling via lipophilic psilocin³¹. Our transcriptomic profiling at 48 hours post-dose identified a metabolic signature that may underlie this longer-term plasticity. We found a significantly upregulated mitochondrial co-expression network centered on *ND3*, with constituent genes primarily associated with aerobic electron transport and oxidative phosphorylation. This finding supports a rodent study showing that 5-HT_{2A} activation acutely elevates mitochondrial respiration, followed by a delayed transcriptional remodeling at 48 hours³². The mitochondrial upregulation we observed may thus reflect compensatory adaptation driven by acute energy demand.

This cellular signature is further corroborated by longitudinal MRS measurements in our study. Glx and tCr in the dlPFC remained significantly elevated from 24 hours to one week post-dose. Sustained Glx elevation indicates persistent excitatory drive during the subacute phase, imposing continued energetic demand. The tCr increase reflects enhanced creatine/ATP buffering capacity to meet this demand. At the systemic level, longitudinal CSF proteomics showed sustained upregulation of central energy-metabolism and redox-buffering enzymes at both post-dose time points, with NAD(P)-dependent oxidoreductase activity as the most robust biofluid signature. The convergence of single-cell mitochondrial upregulation, regional neurochemical elevation, and systemic biofluid signatures provides cross-scale evidence that psilocybin engages a sustained bioenergetic process in deep-layer neurons that may support long-term maintenance of synaptic plasticity. Given that depressed patients exhibit impaired PFC energy metabolism³³ and that effective antidepressant treatment can reverse reduced cortical ATP levels³⁴, this bioenergetic adaptation may constitute a key substrate for psilocybin's

sustained antidepressant effects.

Psilocybin orchestrates multicellular coordination of inflammatory resolution, metaplasticity, and ECM remodeling

In addition to changes in neuronal bioenergetics, psilocybin profoundly reshaped the glial landscape in the primate dlPFC inducing distinct state transitions in both astrocytes and microglia. Astrocytes transitioned toward a State 3 enriched in acidic amino acid transport and cell-ECM adhesion pathways. One of the genes involved in this transition was *CD44*. Microglia shifted toward an M2 repair polarization with a significant decrease in the M1/M2 ratio within the M2 subset. The elevated acidic amino acid transport signature in astrocyte State 3 suggests enhanced glutamate uptake capacity, which may offset the persistent excitatory tone characterized by sustained Glx elevation, and thereby supports prolonged plasticity and eliminates excitotoxicity^{35,36}. These glial alterations, however, likely represent more than a homeostatic response to neuronal activity. An emerging metaplasticity framework may indicate that psychedelics recruit non-neuronal cells to transiently loosen ECM scaffolds and reopen critical periods of heightened neural plasticity³⁷. The concurrent astrocytic reprogramming toward a high-stemness state²⁵ with enhanced ECM-regulatory capacity as well as the microglial shift toward repair-oriented functions provides the first primate cellular evidence consistent with this framework.

A cell-cell communication analysis revealed three coordinated signaling changes that may underlie neuronal-glial coordination. First, NRXN-mediated neuron-to-astrocyte adhesion signaling was attenuated, potentially loosening pre-existing synaptic stabilization^{38,39}, while astrocyte-to-neuron BMP signaling was strengthened toward deep-layer excitatory neurons, a pathway by which astrocytes can regulate neuronal synaptic function⁴⁰. Second, neuronal neurotrophic signaling was strengthened, with L5/6 IT Car3 neurons showing the most prominent BDNF upregulation and an increase in efferent BDNF-NTRK2 signaling activity, consistent with autocrine/paracrine reinforcement, which may engage mTOR-dependent remodeling⁴¹. Third, NCAM-mediated astrocyte-microglia crosstalk was enhanced, consistent with broader glial reorganization toward protective and repair states^{42,43}. The concurrent attenuation of synaptic stabilization, upregulation of neurotrophic signaling, and strengthening of astrocyte-microglia ECM communication suggest coordinated multicellular remodeling of the extracellular environment, which may transiently loosen ECM scaffolds and create favorable conditions for synaptic rewiring.

These cellular alterations were supported by longitudinal CSF multi-omics findings. An acute prostaglandin-enriched lipid signature at 24 hours plausibly reflects an injury-like signaling cascade that coincides with the timeline of rapid dendritic spine remodeling observed *in vivo*¹⁴. At 1 week, this acute profile switches to a pro-resolving state dominated by omega-3-derived lipids and emerging cytoskeletal regulators. At the protein level, this transition was accompanied by a bidirectional ECM signature. CD44 abundance increased at both post-dose time points, whereas the ECM-associated fibulin-family proteins EFEMP1, EFEMP2, and FBLN5 decreased concordantly. Rather than indicating generalized ECM activation, this reciprocal pattern is consistent with a reorganization of the matrix composition. CD44 provides a cross-scale link to the astrocyte remodeling state identified by single-nucleus transcriptomics, whereas the persistent decrease in EFEMP1 provides a directionally complementary CSF marker of extracellular structural remodeling. Together, these findings support coordinated changes in ECM sensing⁴⁴ and composition during the post-psilocybin plasticity window⁴⁵.

Psilocybin induces structural and functional reorganization

We found that psilocybin-induced microscopical cellular and molecular changes were accompanied by macroscopic structural and functional reorganization. Psilocybin triggered sustained microstructural enhancement within the CSTC circuits, particularly in the thalamus and putamen, which may extend the thalamic gating hypothesis⁴⁶ from transient functional disruption to lasting structural reorganization. Coordinated microstructural enhancement was also observed across major association and projection pathways supporting fronto-parietal, temporo-frontal, and occipito-frontal integration. The evolutionary expansion of these higher-order association networks in primates^{47,48} may provide a neuroanatomical substrate for the cognitive flexibility observed in clinical trials⁴⁹.

At the whole-brain level, psilocybin produced a biphasic topological reconfiguration. FPN-DMN integration increased at 24 hours and remained elevated at one week. FPN-limbic integration emerged only at one week. FPN-SMN coupling transiently decreased at 24 hours before recovery, and global system segregation increased only at one week. These temporal dynamics may help reconcile two conflicting viewpoints in the field: The first is that acute psilocybin promotes global integration and flexible brain states^{13,50}, as also reported in macaques shortly after dosing²¹, and the other is that lasting clinical benefit intuitively requires a more organized brain configuration. Our data suggest that these viewpoints are not mutually exclusive but temporally separated: Psilocybin initially promotes widespread cross-network

coupling, after which the system stabilizes into a configuration exhibiting both heightened modularity and global integration. The global changes were consistent with that found in the regional functional connectivity analysis. The reduced coupling between DMN/limbic hubs and dorsal-attention or visual networks suggests a relaxation of the internally oriented and self-referential coupling reported in psychedelic effects^{11,51}. Conversely, connectivity from limbic and default-mode regions to frontoparietal control nodes was strengthened. These interwoven changes provides a circuit-level instantiation of the flexibility-enabling state predicted by the entropic brain framework^{12,50,51}. Importantly, the white matter pathways of these cortical regions showing altered functional connectivity were remodeled within the same 24-hour-to-one-week post-dose window, suggesting that the structural and functional reorganizations are coupled rather than independent processes.

There were also limitations of the current study. First, the overall sample size was modest (15 animals), with even smaller sample sizes for some modality-specific analyses, particularly the snRNA-seq analysis ($n = 3$ per group). This limited statistical power and the precision of effect estimates, reduced our ability to characterize interindividual variability and detect more subtle effects, and may constrain the generalizability of the findings. Although the longitudinal within-subject design and convergence across multiple modalities provide some support for the robustness of the observed patterns, they do not substitute for independent replication. Therefore, these findings should be considered exploratory and require confirmation in larger, independent non-human primate cohorts. Second, the snRNA-seq data were acquired at a single time point (48 hours post-dose), precluding characterization of the full temporal trajectory. Transcriptomic responses may evolve dynamically over the first 24-72 hours. For instance, a previous scRNA-seq study of mouse mPFCs reported broad suppression of mitochondrial genes at 24 hours post-psilocybin⁵². Whether the mitochondrial upregulation observed in our 48-hour primate dataset reflects a species difference, a delayed rebound phase, or methodological differences remains unclear. Future studies integrating data from multiple time points within the same experimental protocol are needed to address this question. Third, although we emphasize the role of 5-HT_{2A} signaling, psilocin binds broadly across serotonin receptor subtypes. Schuler et al. reported that the most robustly affected excitatory neurons in the mouse medial PFC (mPFC) (L5/6 NP) express high levels of *Htr2c* but low *Htr2a* and that their sensitivity likely reflects integrated signaling across multiple co-expressed serotonin receptor subtypes rather than any single receptor⁵². The relative contributions of multiple serotonin receptors to the effects observed in the primate dlPFC remain to be determined. Fourth, the

MRS and snRNA-seq datasets were acquired at partially overlapping but not identical time points. Their convergence is therefore suggestive rather than confirmatory, and temporally aligned within-subject multimodal designs are needed to strengthen the inference. Fifth, the CellChat-based communication analysis relies on computational inference from ligand-receptor co-expression and does not provide direct evidence of signaling pathway activation. Experimental perturbation studies are needed to validate the proposed neuron-astrocyte signaling mechanisms. Sixth, while we identified significant alterations in functional connectivity between specific subcortical hubs and cortical regions across hemispheres (e.g., Amyg_R–PCip_L), these contralateral functional couplings should be interpreted with caution. Given the predominant ipsilateral anatomical connectivity of subcortical structures such as the amygdala, these functional changes likely reflect indirect or shared upstream modulation rather than direct monosynaptic connections. Lastly, all subjects were healthy non-human primates. Whether these cellular and network changes are the same in disease states such as MDD and whether they causally contribute to antidepressant efficacy requires investigation using translational disease models with behavioral evaluations.

In summary, our multi-scale analysis shows that a single dose of psilocybin initiates a coordinated, temporally staged program in the primate dlPFC and its connected circuits, linking 5-HT_{2A}-biased engagement of deep-layer neurons, sustained mitochondrial and bioenergetic adaptation, glial-mediated inflammatory resolution and ECM remodeling, and coupled structural and functional network reorganization. By resolving these events across cellular, molecular, neurochemical, and network levels within a single primate framework, this work bridges rodent mechanistic studies and human neuroimaging and identifies bioenergetic and extracellular-matrix remodeling as candidate substrates for psilocybin's durable effects. Establishing whether these substrates causally support antidepressant response will require temporally aligned, within-subject multimodal designs, first in disease-relevant primate models and ultimately in patients. Our study helps define this direction.

Methods

Animals. A total of 15 healthy adult female cynomolgus monkeys (*Macaca fascicularis*; age 16-18 years; body weight 5.0-7.5 kg) were enrolled in this study. A single-sex cohort was used to reduce sex-related biological and behavioral heterogeneity and to facilitate stable group housing and dyadic social interaction testing. The animals were allocated into a psilocybin group ($n = 10$) and a control group ($n = 5$). To accommodate the experimental procedures, the 10 psilocybin-treated monkeys were divided into two cohorts of five animals and were administered psilocybin in separate dosing sessions; all five animals within each cohort received psilocybin during the same dosing session. For dyadic social interaction testing, all five monkeys in the first psilocybin cohort were paired one-to-one with the five control monkeys. In the second cohort, three psilocybin-treated monkeys and three control monkeys were designated for *in vivo* dlPFC tissue collection at 48 hours post-dose and therefore did not undergo the subsequent social interaction test. The remaining two psilocybin-treated monkeys were paired one-to-one with the remaining two control monkeys. Thus, social interaction data were obtained from seven psilocybin-treated monkeys in total. The same five control monkeys were used across the two experimental cohorts, with two control monkeys participating in both social interaction sessions. All animals were maintained under the same housing and handling conditions. This 48 hour time point was chosen to capture the delayed transcriptional remodeling that follows the acute pharmacological window, consistent with rodent evidence that 5-HT_{2A} activation drives delayed transcriptional reprogramming by approximately 48 hours³². All animals were obtained from the State Key Laboratory of Primate Biomedical Research, Institute of Primate Translational Medicine, Kunming University of Science and Technology (Kunming, China), and were housed in an AAALAC-accredited non-human primate facility. Monkeys were group-housed under controlled environmental conditions (temperature: 22 ± 1 °C; humidity: $50\% \pm 5\%$; 12h light/dark cycle). Animals were randomly assigned to experimental groups. All animal care and experimental procedures were conducted in accordance with institutional guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of Kunming University of Science and Technology (permit No. KUST202401005).

Drug administration and experimental design. Psilocybin was administered intravenously (0.44 mg/kg) via the saphenous vein; this dosage was comparable to doses previously administered to non-human primates (0.3 mg/kg intravenously and 0.4 mg/kg intramuscularly)^{18–20}. All 10 monkeys underwent a series of baseline behavioral assessments

including sucrose preference, locomotor activity, sleep monitoring, and social interaction tests. Following psilocybin administration, brain tissues from three of the experimental monkeys were collected for snRNA-seq resulting in a final cohort of seven monkeys available for longitudinal behavioral analyses before and after drug administration (Figure 1B). Behavioral evaluations were conducted using a staggered timeline to capture different phases of the functional response: Sleep monitoring and sucrose preference tests commenced immediately on the day of administration (Days 0-3 post-dose), whereas locomotor activity and social interaction tests began three days post-administration (Days 3-6 post-dose). For snRNA-seq, dlPFC tissues were obtained from three monkeys 48 hours after psilocybin injection and from three monkeys without psilocybin administration (controls). In addition, multimodal MRI scans were performed for all the 10 monkeys before and 24 hours after psilocybin administration, with seven monkeys also scanned 1-week after psilocybin administration. CSF samples were collected at the same time points: baseline ($n = 10$), 24 hours post-dose ($n = 10$), and 1-week post-dose ($n = 7$).

Locomotor activity. Locomotor activity was recorded daily at 14:00. Each subject was transferred to a dedicated recording cage for a 70 min session. Video data were analyzed using the PrimateScan system (Clever Sys Inc.) to quantify total travel distance and software-defined postural behaviors, including sitting, hang-cuddled behavior, and hang-vertically-from-hang-cuddled posture. Baseline locomotor activity was recorded for four consecutive days prior to dosing. Post-dose recordings were conducted for four consecutive days starting three days after psilocybin administration (Days 3-6 post-dose), and the average frequency and duration were used for statistical analyses.

Social interaction test. Monkeys were housed in adjacent standard cages separated by a removable opaque partition. Each day at 09:00, the partition was removed to allow a 60-minute dyadic interaction between the experimental subject and a control partner with sessions recorded for offline analysis. Social behaviors were categorized into three types: active social engagement, passive acceptance, and free activity. In this paired-interaction paradigm, an active social behavior initiated by one animal was scored as passive acceptance for the partner. Baseline social behavior was recorded for four consecutive days. Post-dose recordings were conducted for four consecutive days starting three days after administration (Days 3-6 post-dose). Both the frequency and duration of each type of behavior were quantified, and their averages were used for statistical analyses.

Sleep monitoring. Each monkey's nighttime behavior was continuously video-recorded for 12 hours, from 20:00 to 08:00, in a specialized cage. Recordings were divided into consecutive 1-min epochs, and each epoch was assigned exclusively to one of three behavioral states: deep sleep, light sleep, or wakefulness based on eye status and the number of observable body movements. An epoch was classified as deep sleep when the monkey's eyes were closed and no more than three movements occurred; as light sleep when the eyes were closed but more than three movements occurred; and as wakefulness when the eyes were open, irrespective of movement frequency. The total number of minutes assigned to each state was summed for each night. Sleep was monitored for four consecutive nights before dosing and for four consecutive nights from the day of administration through post-dose Day 3. The nightly durations of each state were then averaged within the pre-dose and post-dose periods for statistical analysis. These categories represent behaviorally defined sleep states rather than polysomnographically verified sleep stages.

Sucrose preference test. A two-bottle choice task was used to characterize reward-related consummatory behavior and relative preference for a palatable sucrose solution. Because the animals were healthy and had not been exposed to stress or depression-inducing procedures, this task was not interpreted as a direct measure of anhedonia. Each day from 12:00 to 16:00, the monkeys were provided with two bottles, one containing a 1.5% sucrose solution and the other containing pure water, positioned on opposite sides of the cage. The consumption volume of each liquid was recorded separately. Sucrose preference was calculated as the volume of sucrose solution consumed divided by the total volume of sucrose solution and water consumed, multiplied by 100. Baseline intake was recorded for four consecutive days. Post-dose intake was monitored for four consecutive days beginning on the day of psilocybin administration (Days 0-3 post-dose). Daily sucrose intake, water intake, total fluid intake, and sucrose preference were averaged separately within the pre-dose and post-dose periods for statistical analysis.

Magnetic resonance imaging (MRI) data acquisition. We performed multimodal magnetic resonance imaging scans on the monkeys at three different time points: prior to psilocybin administration, 24 hours post-dose, and 1-week post-dose. The multimodal MRI data were acquired on a 3.0 Tesla Siemens Prisma scanner (Siemens Healthineers, Erlangen, Germany) equipped with a special non-human primate head coil at Kunming University of Science and Technology. Animals were anesthetized and positioned in a stereotaxic holder to minimize head motion during scanning. Anesthesia was induced by a veterinarian via intramuscular injection

of atropine (0.05 mg/kg), Zoletil (tiletamine/zolazepam, 10 mg/kg), and xylazine hydrochloride (10 mg/kg). The anesthesia lasted approximately 1 hour to allow for MRI scanning. During and after the MRI scanning period, the experimental monkeys were cared for by a veterinarian until mobility recovery. High-resolution structural images were obtained using a three-dimensional T1-weighted magnetization-prepared rapid gradient-echo (3D T1-MPRAGE) sequence with the following parameters: repetition time (TR) = 1400 ms, echo time (TE) = 3.55 ms, inversion time (TI) = 900 ms, flip angle = 8° , field of view (FOV) = $135 \times 135 \text{ mm}^2$, matrix size = 224×224 , slice thickness = 0.6 mm, isotropic voxel size = $0.6 \times 0.6 \times 0.6 \text{ mm}^3$, 160 sections, acceleration factor = 2, and number of excitations (NEX) = 3. Resting-state functional MRI (rs-fMRI) data were acquired using a gradient-echo echo-planar imaging (EPI) sequence. Imaging parameters were as follows: TR = 1000 ms, TE = 30 ms, flip angle = 90° , 30 axial slices, slice thickness = 1.5 mm, isotropic voxel size = $1.5 \times 1.5 \times 1.5 \text{ mm}^3$, matrix size = 64×64 , FOV = $96 \times 96 \text{ mm}^2$, and 250 time points. During rs-fMRI acquisition, the animals were kept in a resting state without external stimulation. Diffusion kurtosis imaging (DKI) was performed using a spin-echo EPI sequence with 30 non-collinear diffusion-encoding directions and one non-diffusion-weighted (b0) image. Imaging parameters were: TR = 8500 ms, TE = 77 ms, b values = 0, 1000, and 2000 s/mm^2 , 60 axial slices, FOV = $128 \times 128 \text{ mm}^2$, matrix size = 128×128 , isotropic voxel size = $1 \times 1 \times 1 \text{ mm}^3$, acceleration factor = 2, and NEX = 1.

Magnetic resonance spectroscopy (MRS) data acquisition. Proton magnetic resonance spectroscopy (^1H -MRS) data were acquired on the same 3.0 T Siemens Prisma system to quantify regional changes of neurochemical profiles. A single-voxel point-resolved spectroscopy (PRESS) sequence was used, with the voxel of interest (VOI) positioned according to anatomical landmarks derived from the T1-weighted images. We mainly measured the neurochemical profiles of the left dlPFC because this region corresponds to the area used for snRNA-seq. The VOI size was fixed at $10 \times 10 \times 10 \text{ mm}^3$. Two echo-time acquisitions were performed to optimize metabolite detection. The long echo-time acquisition parameters were: TR = 1400 ms, TE = 135 ms, flip angle = 90° , and NEX = 250. The short echo-time acquisition the parameters were: TR = 1400 ms, TE = 30 ms, flip angle = 90° , and NEX = 50. Six metabolites (Asp, mI, NAA, Glx, tCho, and tCr) quantified at three time points: pre-dose, 24 hours post-dose, and 1-week post-dose, were used in subsequent mixed-effects model analyses.

Structural MRI data preprocessing. T1-weighted (T1w) images were preprocessed for subsequent structural and functional analyses. Skull stripping was first performed using the Brain Extraction Tool (BET2) implemented in FMRIB Software Library (FSL:

www.fmrib.ox.ac.uk/fsl). Then, the skull-stripped T1w images were segmented into gray matter, white matter, and cerebrospinal fluid, generating tissue probability maps for each compartment. Detailed procedures for structural MRI segmentation have been described in our previous study⁵³. Using the cross-species homogenous Regional Map (RM) atlas⁵⁴, the brains were parcellated into 92 subregions, which provided the regional framework for the subsequent functional connectivity and microstructural analyses.

DKI data preprocessing. To characterize the microstructural integrity and tissue complexity of the white matter and cortex, DKI data were processed through a multi-step pipeline. First, diffusion-weighted images were subjected to eddy current and head motion correction using affine registration to the non-diffusion-weighted ($b = 0$) volumes via the FSL (www.fmrib.ox.ac.uk/fsl). Subsequently, a diffusion tensor model was fitted using the FSL *dtifit* tool to calculate diffusion metrics including fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD). To quantify non-Gaussian water diffusion and tissue complexity, a mean kurtosis (MK) map was derived using the Diffusion Kurtosis Estimator (DKE). Finally, the MK map was spatially normalized to the INIA19 macaque template space to facilitate standardized group-level statistical analyses⁵⁵. The whole brain white matter tracts were defined using the XTRACT atlas⁵⁶, and the tract-wise mean FA and MK values, together with region-wise mean MK values based on the RM atlas⁵⁴, were included in subsequent mixed-effects model analyses.

Resting-state fMRI data preprocessing. The resting-state fMRI data were preprocessed using the following steps: The first 10 volumes of each functional run were discarded. The remaining images were realigned to the first volume to account for head motion. Subjects were excluded if head displacement exceeded a threshold of 1.5 mm in translation or 1.5° in rotation, and no subject was excluded based on this criterion. For normalization, the mean functional image was first co-registered to the corresponding T1-weighted structural image and then normalized to the INIA19 macaque template at a resampled voxel size of $1 \text{ mm} \times 1 \text{ mm} \times 1 \text{ mm}$. The normalized functional data were then spatially smoothed using a Gaussian kernel of 3 mm full-width at half maximum (FWHM). To minimize the impact of non-neural fluctuations, we performed nuisance regression by including Friston's 24 head motion parameters, as well as the WM, CSF, and global mean signals, as covariates in a general linear model (GLM) for each voxel's time series. Temporal low-pass filtering with a cutoff of 0.05 Hz was applied to the time series. To further mitigate motion-related artifacts, a scrubbing procedure using spline interpolation was conducted for frames with a frame-wise displacement (FD) exceeding 0.2

mm. To explore the psilocybin effects on longitudinal changes in functional connectivity (FC), the pairwise functional connectivity between brain regions was calculated. To calculate the FC, the BOLD time series were averaged within each region defined by the RM atlas⁵⁴. Then, the pairwise Pearson's correlations were computed between all the regional time series, and the resulting functional connectivities were used in the subsequent mixed-effects model analyses to identify significant changes in these connectivities.

Cerebrospinal fluid collection. To collect the CSF of monkeys, deep anesthesia was induced via intramuscular injection of atropine (0.05 mg/kg), Zoletil (tiletamine/zolazepam, 10 mg/kg), and xylazine hydrochloride (10 mg/kg). After anesthesia, the hair over the lumbar region was shaved, and the animal was positioned in lateral recumbency. The puncture site was disinfected with povidone-iodine (iodophor) prior to CSF collection via lumbar puncture. To remove cellular debris, the acquired CSF was immediately centrifuged at $3,500 \times g$ for 10 min at 4°C. The resulting supernatant was aliquoted, snap-frozen in liquid nitrogen, and stored at -80°C until analysis.

Preprocessing of CSF samples for proteomic analysis. For untargeted proteomic analysis, the samples were solubilized in lysis buffer containing urea (Sinopharm, #10023218) and triethylammonium bicarbonate (TEAB, Sigma, #T7408). Proteins were reduced with dithiothreitol (DTT, Sigma, #D9163) at 56°C for 1 hour and alkylated with iodoacetamide (IAM, Sigma, #I6125) in the dark at room temperature for 1 hour. Digestion was performed using mass spectrometry-grade trypsin (Promega, #V5280) at 37°C with a 4 hours pre-digestion followed by overnight incubation. The resulting peptides were desalted using C18 cartridges (Thermo Fisher) and dried under vacuum (Labogene, Scan Speed 40). Peptide separation was carried out on a PepMapTM Neo analytical column (150 $\mu\text{m} \times 15 \text{ cm}$, 2 μm , Thermo Fisher) at 50°C using a VanquishTM Neo nano-UHPLC system (Thermo Fisher) coupled online to an Orbitrap Astral mass spectrometer (Thermo Fisher). Data-independent acquisition (DIA) was performed with full MS scans (m/z 380-980) at a resolution of 240,000, followed by MS2 acquisition using 300 variable isolation windows (2-Th) at a resolution of 80,000 on the Astral analyzer with a normalized collision energy of 25%. Raw data were processed using DIA-NN (version 1.8) in direct DIA mode against the UniProt reference database (taxonomy ID 2565859; 80,184 sequences). The search parameters included precursor and fragment mass tolerances of 10 ppm and 0.02 Da, respectively; trypsin specificity with up to two missed cleavages; fixed carbamidomethylation of cysteine; and variable modifications of N-terminal acetylation, methionine loss, and methionine loss with acetylation. The resulting identifications were

filtered to retain peptide-spectrum matches (PSMs) with $\geq 99\%$ confidence, and the final peptide and protein identifications were controlled at a false discovery rate (FDR) of $< 1\%$ at the peptide and protein levels.

Preprocessing of CSF samples for metabolomic analysis. After obtaining CSF samples, the metabolites were extracted using an established liquid sample protocol: 100 μL of CSF was mixed with 400 μL of pre-cooled 80% methanol aqueous solution, vortexed, incubated on ice for 5 min, and centrifuged at $15,000 \times g$ for 20 min at 4°C . A portion of the supernatant was diluted with MS-grade water to a final methanol concentration of 53%, followed by another centrifugation under the same conditions. The resulting supernatant was collected for LC-MS analysis. For quality control, pooled quality control (QC) samples were prepared by combining equal volumes of all experimental samples to monitor system stability, and blank samples (53% methanol) were processed identically to account for background signals. Chromatographic separation was performed on a Vanquish UHPLC system (Thermo Fisher) equipped with a Hypersil Gold C18 column (100×2.1 mm, $1.9 \mu\text{m}$) maintained at 40°C . Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B was methanol, delivered at a flow rate of 0.2 mL/min. The gradient elution program was as follows: 2% B held for 1.5 min, increased to 85% B from 1.5 to 3 min, maintained at 100% B from 3 to 10 min, then returned to 2% B from 10 to 12 min for column re-equilibration. Mass spectrometry was conducted on a Q ExactiveTM HF instrument (Thermo Fisher) with an electrospray ionization source operating at a spray voltage of 3.5 kV, sheath gas flow of 35 psi, auxiliary gas flow of 10 L/min, and capillary temperature of 320°C . Full MS scans were acquired from m/z 100 to 1500 at a resolution of 120,000, followed by data-dependent MS/MS scans with a dynamic exclusion time of 5 s. Raw data files were converted to mzXML format using ProteoWizard. Peak detection, alignment, and quantification were performed with XCMS. Metabolite identification followed a multi-step approach: primary matching against an in-house standard library based on accurate mass (mass error ≤ 10 ppm) and retention time; subsequent MS spectral matching against public databases (HMDB, METLIN, MassBank) with a similarity score threshold of ≥ 0.7 ; lipid species were additionally annotated using the LIPID MAPS database. Background ions were subtracted using blank samples, and system stability was assessed via QC samples. Normalization was applied using the formula: relative peak area = (raw peak area) / (sum of metabolite peak areas in the sample / sum of metabolite peak areas in QC1). Only metabolites exhibiting a coefficient of variation (CV) $< 30\%$ across QC samples were retained to ensure data reliability and reproducibility.

Differential proteomic and metabolomic analyses. To further investigate alterations in the CSF proteomic and metabolomic profiles across pre- and post-dose time points, differential analyses were performed separately for proteins and metabolites between the pre-dose baseline and two post-dose time points (24 hours and 1-week post-dose). For the proteomic analysis, differential protein expression was assessed using the limma package (R/Bioconductor) with empirical Bayes moderation. Given the limited sample size ($n = 7$ paired samples), individual subject effects were modeled as a numeric covariate to account for inter-individual variability. Proteins with $|\log_2FC| \geq 0.5$ and unadjusted $P < 0.05$ were considered differentially expressed. For the metabolomic analysis, metabolites meeting the criteria of $P < 0.05$ and $|\log_2FC| > 1$ were defined as significantly altered.

Brain tissue collection for dlPFC. Prior to tissue collection, the animals underwent MRI scanning to precisely localize the target region of the left dlPFC. Deep anesthesia was induced via intramuscular injection of atropine (0.05 mg/kg), Zoletil (tiletamine/zolazepam, 10 mg/kg), and xylazine hydrochloride (10 mg/kg). Following the confirmation of surgical-level anesthesia, the cranial vertex was shaved and disinfected. A midline incision was made to expose the skull, followed by careful separation of the underlying fascia. The animals were then secured in a stereotaxic apparatus, and a small craniotomy was performed at predefined coordinates using a dental drill. Tissue biopsies were extracted from the left dlPFC using a microsampling needle. Samples were briefly rinsed with sterile saline, blotted to remove excess liquid, and immediately snap-frozen in liquid nitrogen for storage at -80°C . Hemostasis was meticulously achieved before suturing the surgical site. The entire procedure was completed within 60 minutes. For postoperative care, animals received antibiotics and analgesia (Tolfedine CS, 0.1 mL/kg) in accordance with ethical guidelines.

Nuclei isolation and single-nucleus RNA sequencing. The brain samples from monkeys with and without psilocybin administration were processed within the same nuclei isolation batch to minimize potential batch effects. Frozen brain tissues were maintained on dry ice prior to processing and homogenized in ice-cold RNase-free lysis buffer (0.32 M sucrose, 5 mM CaCl_2 , 3 mM MgAc_2 , 0.1 mM EDTA, 10 mM Tris-HCl, 1 mM DTT, 0.1% Triton X-100 in DEPC-treated water) using a glass Dounce homogenizer until complete tissue dissociation was achieved. Homogenates were incubated on ice to facilitate nuclear release, followed by filtration through a 30- μm cell strainer to remove debris. Nuclei were further purified by centrifugation and enriched using density gradient ultracentrifugation. Purified nuclei were resuspended in DEPC-treated PBS, incubated briefly on ice, and sequentially filtered through

30- μ m strainers to eliminate residual aggregates. Nuclear concentration and integrity were assessed by light microscopy and hemocytometer counting. Only intact, translucent nuclei with minimal debris contamination were used for downstream analysis. For snRNA-seq, nuclei were diluted to 2,000 nuclei/ μ L and loaded onto the 10x Genomics Chromium platform following the manufacturer's protocol. Sequencing libraries were pooled and sequenced on an Illumina NovaSeq system.

The snRNA-seq data preprocessing and analysis. The snRNA-seq libraries were prepared using the 10x Genomics Chromium Single Cell 3' platform (v3 chemistry) by a commercial provider according to the manufacturer's instructions. Raw sequencing data were converted to FASTQ format and processed via the Cell Ranger pipeline (10x Genomics) with default parameters. Reads were aligned to the *Macaca fascicularis* reference genome. Unique molecular identifiers (UMIs) were quantified to generate the initial gene-by-cell count matrices.

Quality control, clustering, and cell-type annotation. Downstream bioinformatic analyses were performed using the Seurat R package (v 5.1.0)⁵⁸. To ensure high-quality data, we applied stringent filtering at the sample level: Nuclei were excluded if they contained > 25,000 UMIs, > 6,000 detected genes, or > 5% mitochondrial-derived transcripts. Additionally, genes detected in fewer than three cells were removed. Following filtration, a total of 53,958 high-quality nuclei remained, with a median of 2,493 genes and 5,949 UMIs per nucleus. As all samples were sequenced in a single batch, the raw count matrices were merged and normalized. We identified the top 3,000 highly variable genes (HVGs) for principal component analysis (PCA). Clustering was performed using the Leiden algorithm on the shared nearest neighbor (SNN) graph. For visualization, two-dimensional embeddings were generated via Uniform Manifold Approximation and Projection (UMAP). Major cell types were manually annotated based on the expression of canonical marker genes.

Differential expression and functional enrichment analysis. Differentially expressed genes (DEGs) between with- and without-psilocybin conditions within each cell cluster were identified using the FindMarkers function. *P*-values (Wilcoxon rank-sum test) were adjusted for multiple comparisons using the Bonferroni correction. Genes meeting the criteria of an adjusted *P*-value (P_{adj}) < 0.05 and an average $|\log_2FC| > 0.5$ were defined as significant DEGs. Functional enrichment analysis was conducted using the clusterProfiler R package (v4.12.6)⁵⁹. To obtain comprehensive functional annotations, a gene ontology (GO) enrichment analysis, including biological process (BP), molecular function (MF), and cellular component (CC), was performed. *P*-values were adjusted using the Benjamini-Hochberg method, with $P_{adj} < 0.05$.

considered significant. To visualize the functional landscape, significantly enriched pathways were clustered into a network using the aPEAR R package (v1.0)⁶⁰, highlighting the predominant biological process.

Quantification of cell-type enrichment (RO/E). To quantify the compositional shifts in cell subpopulations following psilocybin administration, we calculated the ratio of observed to expected frequencies (RO/E). A contingency table (condition $\times$ cell subtype) was constructed, and expected counts were derived based on χ^2 statistics. The RO/E score (observed / expected) was used to represent the degree of enrichment or depletion for each subpopulation across conditions.

Gene signature and trajectory inference. Gene set activity at the single-cell level was evaluated using the AUCell R package (v1.12.0)⁶¹. This approach calculates an area under the curve (AUC) score for each cell based on the recovery of a target gene set within the ranked expression list, providing a measure of signature intensity independent of normalization methods. To assess the impact of psilocybin on neuronal synaptic function, we selected four gene sets from the GO database related to synaptic structure and functional plasticity (GO:0007268, GO:0050808, GO:0007269, and GO:0048169). Furthermore, neuroinflammation scores were quantified using a curated gene set combining GO terms (GO:0150078 and GO:0150076) and high-relevance genes from GeneCards (<https://www.genecards.org/>, Relevance score > 3). To ensure the biological specificity of the inflammation signature, we excluded highly expressed genes with low functional impact to minimize potential background noise. For an astrocyte lineage analysis, pseudotemporal ordering was performed using Monocle 2⁶². We selected 156 DEGs between conditions ($P_{adj} < 0.05$, $|\text{Log}_2\text{FC}| > 0.5$) as ordering genes. The trajectory was inferred using the DDRTree algorithm on size-factor normalized counts. Furthermore, we applied CytoTRACE (Cellular Trajectory Reconstruction Analysis using gene counts and expression) to predict the differentiation potential of astrocytes⁶³. Cells were ranked by their CytoTRACE scores, where higher values indicate a more primitive or plastic state.

Cell-cell communication and co-expression network analysis. The biomaRt R package (v2.60.1) was used to convert macaque Seurat objects into human-homolog Seurat objects by querying Ensembl orthologs. Intercellular signaling networks were modeled using the CellChat(v1.6.1) package⁶⁴. Normalized expression data were integrated with the CellChatDB.human database. Interaction probabilities were estimated using the triMean robust model, and only signaling pathways supported by at least 10 cells were retained. We

systematically examined the intercellular crosstalk between astrocyte, neuronal, and microglial subtypes. Specifically, we compared the communication strength and bidirectional signaling patterns (both outgoing and incoming) between monkeys with and without psilocybin administration. This analysis encompassed not only astrocyte-neuronal interactions but also the coordinated signaling between microglia and neurons, as well as the crosstalk between astrocytes and microglia. High-dimensional weighted gene co-expression network analysis (hdWGCNA, v0.4.00) was applied to neurons to identify psilocybin-associated co-expression modules⁶⁵. We filtered for genes detected in $\geq 5\%$ of cells and constructed “metacells” ($k = 25$, overlap ≤ 10) based on Harmony-corrected embeddings. After library-size normalization and selection of a soft-thresholding power for scale-free topology, consensus modules were identified. These modules were then correlated with experimental condition to pinpoint gene programs specifically modulated by psilocybin.

Statistical analysis of behavior. For behavioral metrics, the Wilcoxon signed-rank test was employed to assess differences between pre- and post-dose conditions. This non-parametric approach was selected due to the non-Gaussian distribution of the behavioral scores and to ensure statistical robustness given the relatively small sample size. Statistical significance was defined at a threshold of $P < 0.05$.

Global and large-scale brain functional networks segregation and integration. To characterize the global and large-scale network topological reorganization following psilocybin administration, we calculated two complementary network-level metrics derived from the functional connectivity matrix: system segregation and between-network integration. Functional connectivity between each pair of brain regions was calculated as the Pearson correlation coefficient between their respective BOLD time series, and the resulting correlation coefficients were transformed to z -values using Fisher’s z -transformation. To study large-scale functional organization induced by psilocybin, each brain region was assigned to one of seven canonical resting-state networks (visual, VIS; somatomotor, SMN; dorsal attention, DAN; ventral attention, VAN; limbic, LIM; frontoparietal, FPN; default-mode, DMN) based on the brain region’s maximal spatial overlap with the seven-network atlas. We calculated the two parameters as follows:

(i) *System segregation*⁵⁷. System segregation quantifies the balance between within- and between-network connectivity and was defined as

$$SyS = \frac{\bar{W}_{\text{within}} - \bar{W}_{\text{between}}}{\bar{W}_{\text{within}}},$$

where $\bar{W}_{\text{within}}$ and $\bar{W}_{\text{between}}$ denote the mean functional connectivity of the within- and between-networks. Higher values indicate stronger functional segregation between networks.

(ii) *System integration.* To quantify the network coupling between the seven networks, we adopted a *network-allegiance* framework adapted from Daws et al.¹³. For each subject and each session, the Louvain algorithm was run 100 times on the functional connectivity matrix, and a region-by-region allegiance matrix was constructed by counting the proportion of times each pair of regions was assigned to the same module across iterations. For each of the 21 unique pairs of the seven canonical networks, the integration value was defined as the mean allegiance across all between-network region pairs linking the two networks. To control for differences in network size and chance-level co-assignment, the allegiance values were normalized against a null distribution generated by randomly shuffling the region-to-network labels 1,000 times, yielding a z-scored integration index for every network pair, subject, and session.

Statistical analysis of network-level metrics. Session effects on system segregation and network integration were assessed using a linear mixed-effects model. For indices with significant differences, post-hoc paired *t*-tests were performed to identify differences between different time points.

Brain-behavior association analysis. To explore whether psilocybin-induced reorganization of large-scale brain networks was related to concurrent behavioral changes, we performed an exploratory brain-behavior association analysis based on within-subject change scores ($\Delta = \text{post-dose} - \text{pre-dose}$). Only those network metrics and behavioral measures showing significant changes after psilocybin were used for further analyses. The Spearman's rank correlation coefficient (ρ) was used to quantify the associations. Statistical significance was assessed by a two-sided permutation test with 10,000 permutations. To control for multiple comparisons across all pairs, permutation *P*-values were adjusted using FDR correction with $P < 0.05$.

Linear mixed-effects modeling and permutation testing. To evaluate longitudinal alterations across three time points (pre-dose baseline and two post-dose sessions), we conducted linear mixed-effects (LME) models for each region of interest (ROI). The regional metrics including MRS-derived metabolite concentrations and diffusion parameters (MK and FA), and functional connectivity values were modeled with session as a categorical fixed effect. Subject ID was included as a random intercept to account for the inherent within-subject correlation structure. The statistical significance of the session effect was determined via a permutation-based approach (1,000 iterations). To preserve the within-subject dependency, session labels were randomly shuffled within each subject. For each iteration, the LME was refitted to the permuted

data to generate a null distribution of F -statistics. The empirical P -value for each ROI was defined as the proportion of permutations where the permuted F -statistic exceeded the observed F -statistic. Statistical significance was defined at a threshold of $P_{PER} < 0.05$.

Resource availability

Lead contact. Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Jiaojian Wang (jiaojianwang@uestc.edu.cn).

Materials availability. This study did not generate new unique reagents.

Data and code availability

The multimodal neuroimaging data generated in this study have been deposited at OpenNeuro under accession number ds008738 and will be publicly available as of the date of publication. The mass spectrometry proteomics data have been deposited with the ProteomeXchange Consortium via the PRIDE partner repository under accession number PXD083381 and will be publicly available as of the date of publication. The untargeted metabolomics data have been deposited in the MetaboLights repository under accession number MTBLS15482 and will be publicly available as of the date of publication. The single-nucleus RNA-sequencing data generated in this study have been deposited in the China National GeneBank DataBase (CNGBdb) under accession number CNP0010110 and will be publicly available as of the date of publication. Reviewer access to datasets that remain private during peer review is provided through the repository-specific private links and reviewer access credentials supplied with the submission. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon reasonable request. This paper does not report original code.

Acknowledgements

This work was supported by the Yunnan Fundamental Research Projects (202501AV070005). J.W., T.Y., Y.C., J.H., X. Z. and X.W. contributed to the conception and design of the study. J.C., W.S., C.L., Z.Y., F.Y., Y.Z., X.W., Y.W., Y.M., Q.S. and Y.Z. contributed to experiment execution, data acquisition, processing and analyzing. H.W. was responsible for drug preparation. J.C., C.L., Z.Y., J.W., T.Y., X. Z., J. M., S. B. and S. C. wrote and edited the manuscript. J.C., Z.Y., C.L., Y.W., and Y.M. produced the figures. All the authors commented and discussed the results.

Compliance with Ethical Standards

All animal care and experimental procedures were conducted in accordance with institutional guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of Kunming University of Science and Technology (permit No. KUST202401005).

Conflict of Interest

The authors declare no competing interests.

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Figures

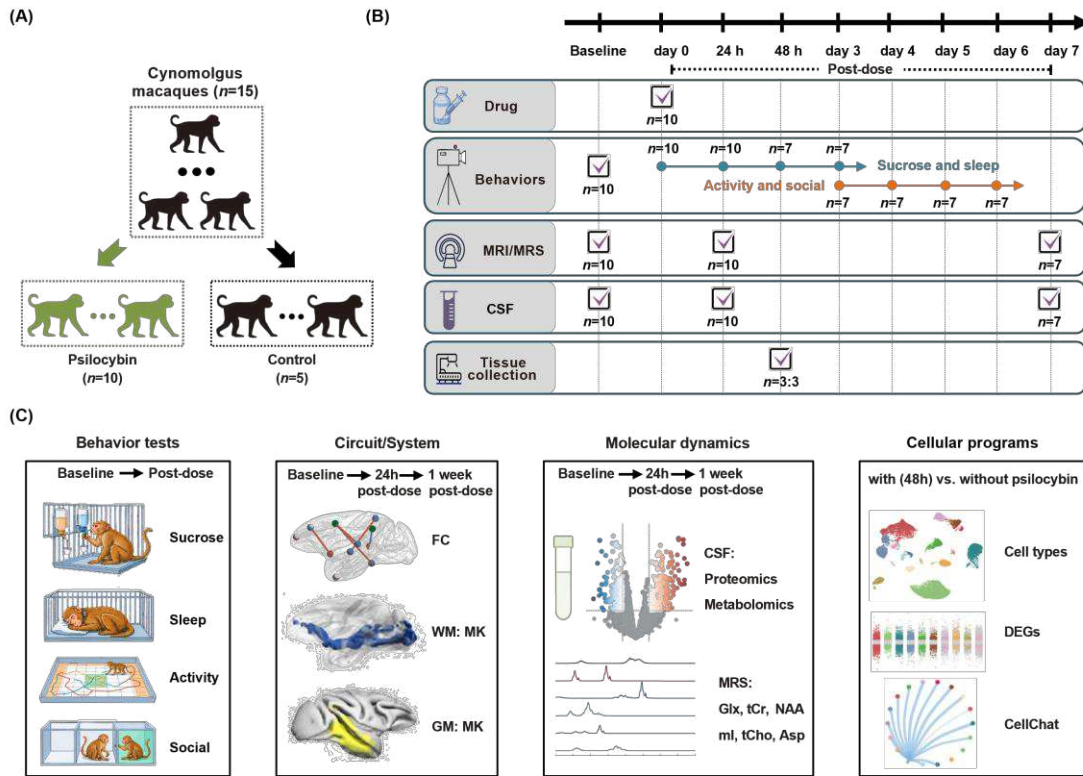


Figure 1. Experimental design for investigating multi-scale psilocybin effects in cynomolgus macaques. (A) Cohort allocation flowchart. A total of 15 female cynomolgus macaques were enrolled and allocated into a psilocybin group ($n = 10$, single intravenous dose of 0.44 mg/kg) and a control group ($n = 5$, without psilocybin administration). (B) Measurement timeline. The horizontal axis spans from the pre-dose baseline period to Day 7 (1 week post-dose). “Baseline” refers collectively to measurements obtained before psilocybin administration; drug administration was performed on Day 0. *In vivo* dlPFC brain tissue collection was performed at 48 hours post-dose for psilocybin ($n = 3$) and control ($n = 3$) animals for snRNA-seq analysis. Multimodal MRI scans, magnetic resonance spectroscopy (MRS), and cerebrospinal fluid (CSF) collections were performed at three time points: baseline ($n = 10$), 24 hours post-dose ($n = 10$), and 1 week post-dose ($n = 7$; reduced owing to 3 animals being allocated to brain tissue collection). Behavioral assessments were conducted from baseline through the post-dose period for both the control ($n = 5$ pre-dose; $n = 2$ continuing after tissue collection) and psilocybin animals ($n = 10$ pre-dose; $n = 7$ continuing after tissue collection). (C) Overview of the multimodal data collection strategy, organized across four complementary levels spanning behavioral, circuit, molecular, and cellular scales. Behavior: sucrose preference test and sleep (measured from baseline through post-dose days 0–3), together with activity testing and social behavior (measured from baseline through post-dose

days 3–6). Circuit/System: resting-state functional MRI (rs-fMRI) for functional connectivity analysis, and diffusion kurtosis imaging (DKI), yielding mean kurtosis (MK) maps for gray and white matter microstructural assessment. Molecular: cerebrospinal fluid (CSF) proteomics and metabolomics; *in vivo* magnetic resonance spectroscopy (MRS) quantification of glutamate + glutamine (Glx), total creatine (tCr), N-acetylaspartate (NAA), myo-inositol (mI), total choline (tCho), and aspartate (Asp). Cellular: single-nucleus RNA sequencing (snRNA-seq)-based cell-type identification, differentially expressed genes (DEGs), and intercellular communication analysis via CellChat.

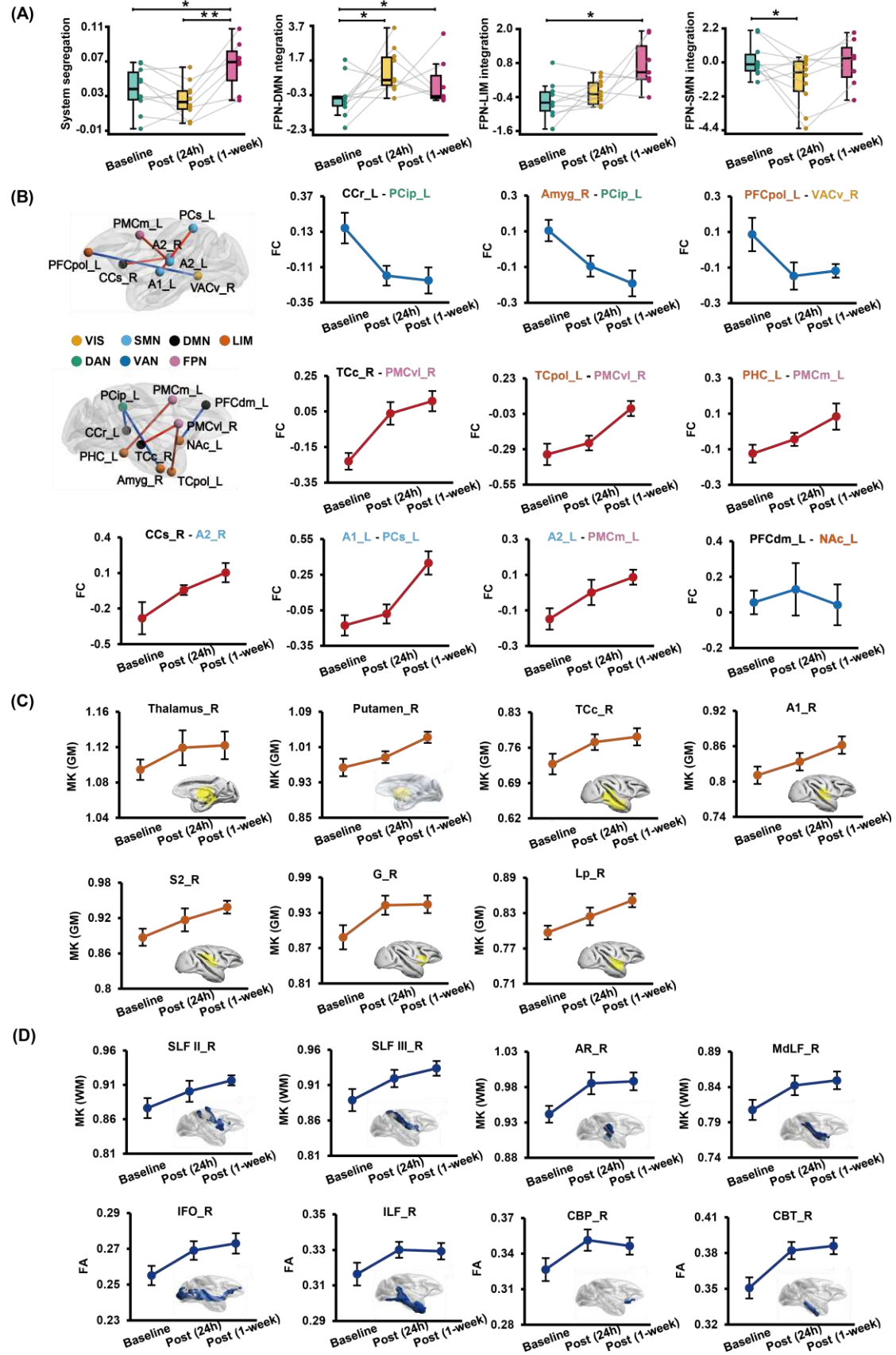


Figure 2. Mixed-effects modeling reveals multidimensional brain functional and structural alterations following psilocybin administration. (A) Longitudinal changes in four

complementary global graph-theoretical metrics derived from whole-brain functional connectivity matrices across pre-dose (Baseline), 24 hours post-dose, and 1 week post-dose. From left to right: system segregation quantifying the relative strength of within- versus between-network connectivity; FPN-DMN integration, between the frontoparietal control network and the default-mode network; FPN-LIM integration, between the frontoparietal control network and the limbic network; and FPN-SMN integration, between the frontoparietal control network and the somatomotor network. Only parameters exhibiting a significant statistical difference in linear mixed-effects models (likelihood-ratio test, $P < 0.05$) are displayed. Post-hoc paired t -tests were performed to identify differences between different time points; $*P < 0.05$, $**P < 0.01$. (B) Functional connectivity changes across different time points were identified using linear mixed-effects models with permutation testing (1,000 permutations; $P_{PER} < 0.05$). Left, brain network visualization showing significant edges mapped onto the seven canonical functional networks (visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, and default mode). Right, representative significant connections showing longitudinal trajectories across pre-dose, 24 h post-dose, and 1-week post-dose. Data are shown as mean $\pm$ SEM. (C) Longitudinal changes in gray matter mean kurtosis (MK) across subcortical and cortical regions. Values are shown for pre-dose, 24 hours post-dose, and 1 week post-dose. Only regions exhibiting significant effects in linear mixed-effects models with permutation testing (1,000 permutations; $P_{PER} < 0.05$) are displayed. (D) Longitudinal changes in white matter MK and fractional anisotropy (FA) in significant tracts identified by mixed-effects modeling with permutation testing (1,000 permutations; $P_{PER} < 0.05$).

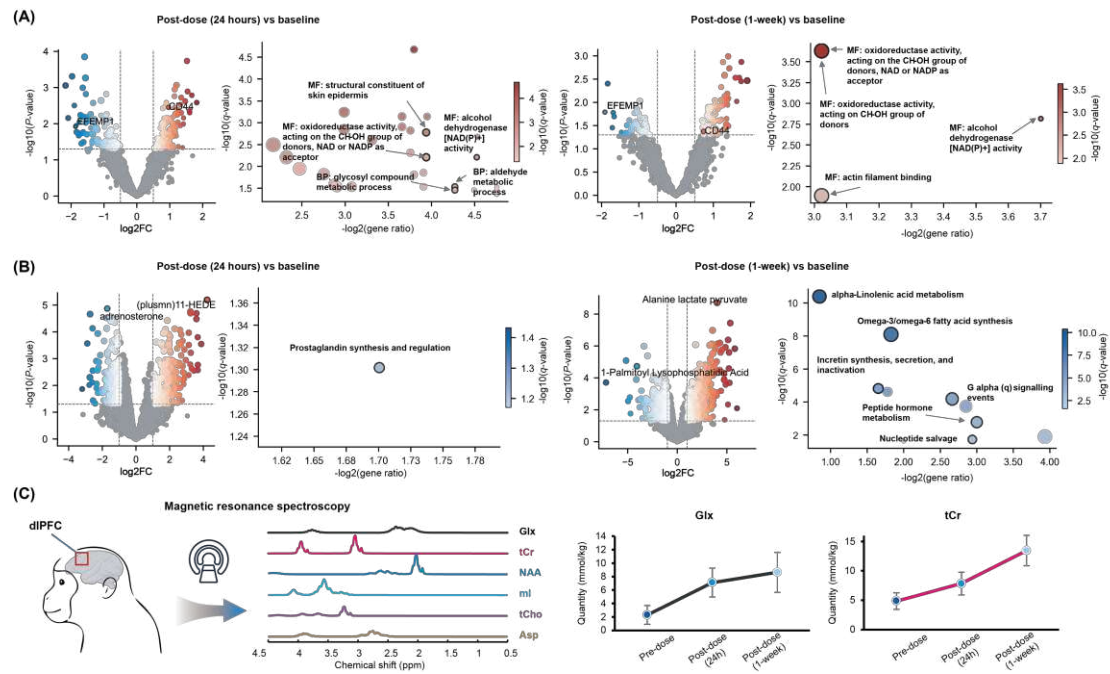


Figure 3. Psilocybin induces time-dependent remodeling of the cerebrospinal fluid proteome and metabolome, corroborated by *in vivo* cortical neurochemistry. (A) Differential protein expression in cerebrospinal fluid (CSF) following psilocybin administration. The left two subpanels show data at 24 hours post-dose versus baseline, and the right two subpanels show data at 1 week post-dose versus baseline. At each time point, the left subpanel is a volcano plot showing log₂ fold change versus -log₁₀(*P*-value), with representative proteins annotated; the right subpanel shows a Gene Ontology (GO) enrichment analysis of significantly dysregulated proteins ($P_{FDR} < 0.05$), in which dot position along the x-axis indicates gene ratio (-log₂), the y-axis shows *q*-values (-log₁₀), and the dot color represents -log₁₀(*q*). MF, molecular function; BP, biological process. (B) Differential metabolite abundance in CSF following psilocybin administration. The left two subpanels show data at 24 hours post-dose versus baseline, and the right two subpanels show data at 1 week post-dose versus baseline. Within each time point, the left subpanel is a volcano plot showing log₂ fold change versus -log₁₀(*P*-value), with representative metabolites annotated; the right subpanel shows the pathway enrichment analysis of significantly altered metabolites, with the x-axis indicating enrichment ratio (-log₂), the y-axis indicating *q*-values (-log₁₀), and dot color representing -log₁₀(*q*). (C) *In vivo* longitudinal ¹H magnetic resonance spectroscopy (MRS) of the left dorsolateral prefrontal cortex (dlPFC). Left: schematic illustrating voxel placement and a representative MRS spectrum with major metabolite peaks labeled (Glx, glutamate-glutamine complex; tCr, total creatine; NAA, N-acetylaspartate; mI, myo-inositol; tCho, total choline; Asp, aspartate). Middle: Glx concentrations across pre-dose, 24-hour post-dose, and 1 week

post-dose time points. Right: tCr concentrations across the same time points. Among all quantified metabolites, only Glx and tCr exhibited significant time effects in linear mixed-effects models with permutation testing (1,000 permutations; $P_{PER} < 0.05$) and are presented here.

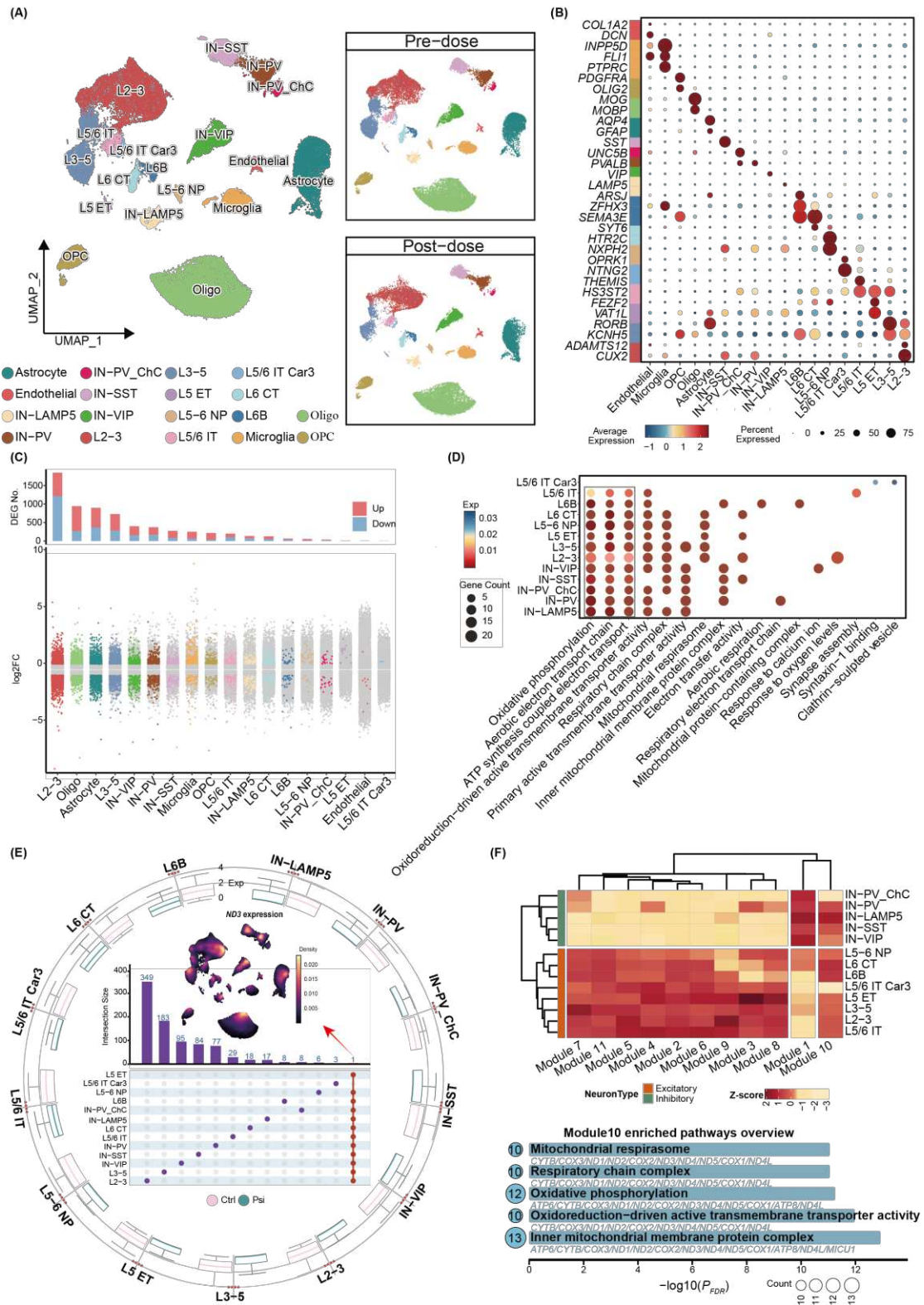


Figure 4. Psilocybin induces cell-type-specific transcriptional remodeling in the dlPFC.

(A) UMAP projection of snRNA-seq profiles from the macaque dorsolateral prefrontal cortex (dlPFC), colored by major cell types. Right panels show cell-type distributions with (Psi) and without (Ctrl) psilocybin administration. (B) Dot plot showing expression of canonical marker genes across major cell types. (C) Top: number of differentially expressed genes (DEGs)

identified in each cell type following psilocybin administration. Bottom: mean log₂ fold change of DEGs per cell type; gray indicates non-significant changes. (D) Gene Ontology (GO) biological process enrichment analysis of up-regulated genes in neuronal subclusters in the psilocybin group. (E) Circular plot illustrating *ND3* expression changes across neuronal subclusters following psilocybin administration. The inner ring indicates overlap of up-regulated genes across neuronal subtypes, and the top panel shows the distribution of *ND3* expression. Statistical significance was assessed using the Wilcoxon rank-sum test (**** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant). (F) Hierarchical heatmap of 11 neuronal-specific co-expression modules identified by hdWGCNA. Representative GO terms enriched in Module 10 are shown at the bottom.

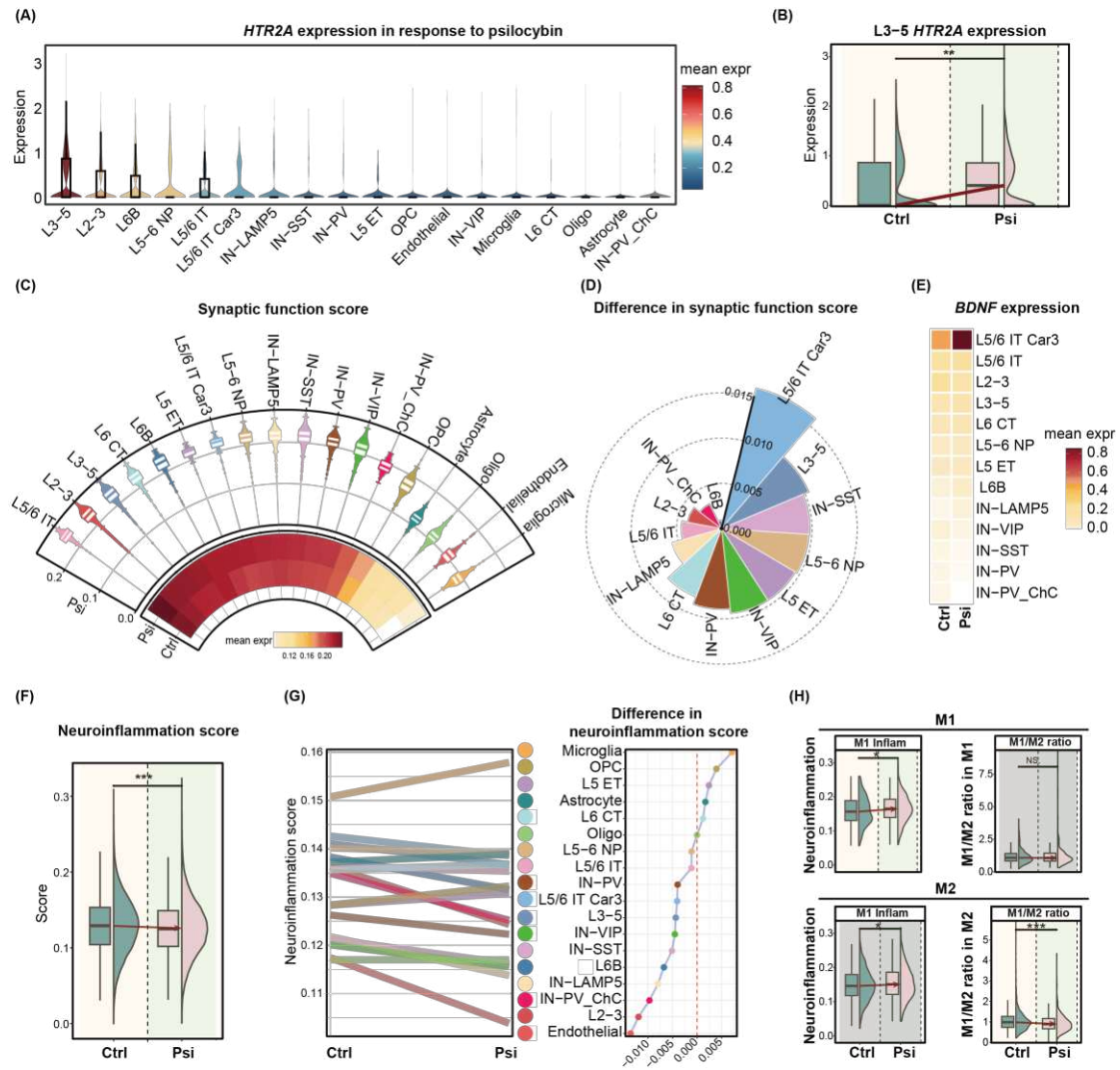


Figure 5. Psilocybin promotes dynamic changes in receptor expression, synaptic-related signatures, and inflammation-related programs across cell types. (A) Cell-type-resolved mean expression of *HTR2A* following psilocybin administration. (B) Comparison of *HTR2A* expression in L3-5 intratelencehalic (IT) excitatory neurons with (Psi) and without (Ctrl) psilocybin administration (Wilcoxon test, ** $P < 0.01$). (C) Synaptic-function scores across major cell types following psilocybin administration (upper), with the corresponding differences between the with- and without-psilocybin conditions shown in the lower heat map. (D) Differences in synaptic-function signatures across cell types between with- and without-psilocybin conditions (Δ scores). (E) Cell-type-specific *BDNF* expression in major neuronal subtypes under with- and without-psilocybin conditions. (F) Global neuroinflammation score comparison between with- and without-psilocybin groups (Wilcoxon test, *** $P < 0.001$). (G) Mean trajectories of neuroinflammation scores between with- and without-psilocybin conditions across individual cell types. (H) Left, shifts in neuroinflammation scores in M1 versus M2 microglia following psilocybin administration compared to control; right,

corresponding changes in the M1/M2 inflammation score ratio within M1 and M2 microglial subsets (Wilcoxon test, **** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, not significant).

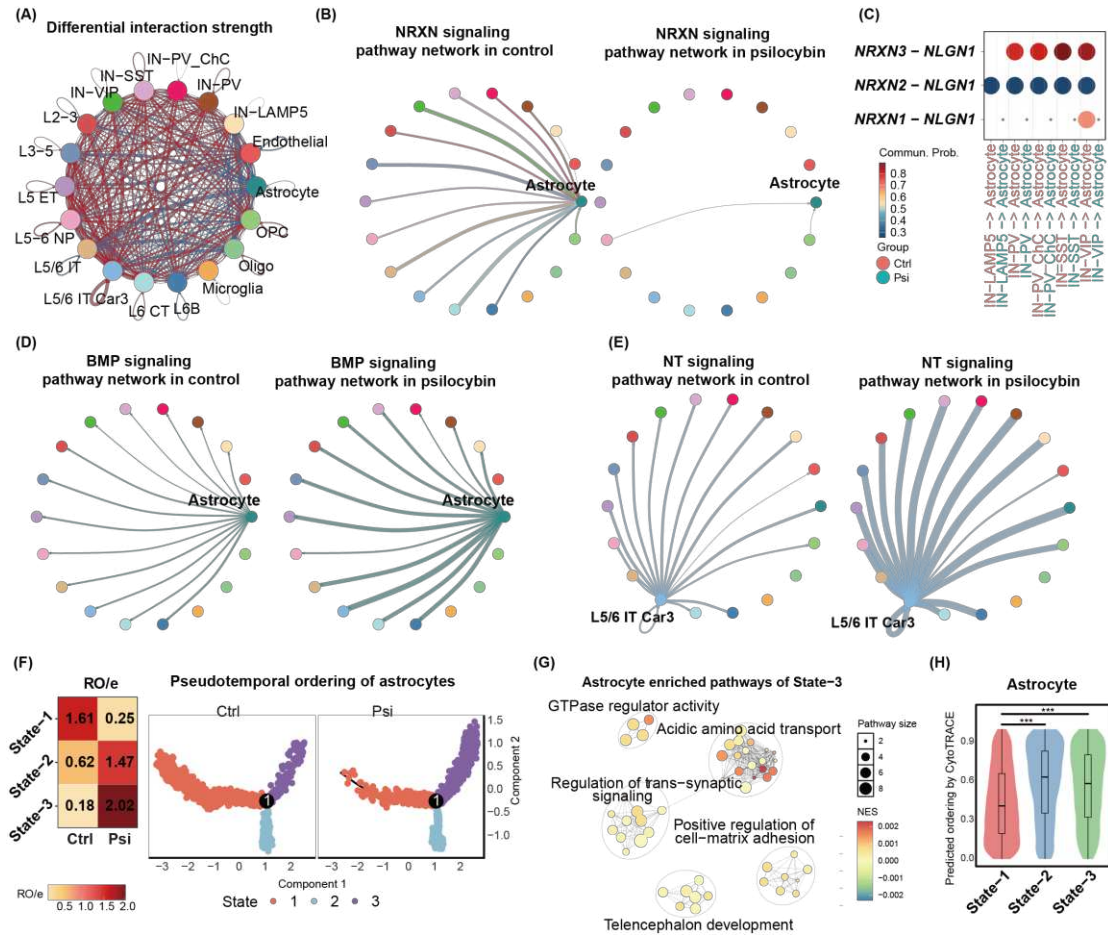


Figure 6. Psilocybin remodels neuron-microglia-astrocyte communication networks and astrocyte state transitions. (A) Psilocybin-induced shifts in overall communication strength across the dlPFC cellular landscape (with-psilocybin minus without-psilocybin scores). (B) Neuron-to-astrocyte NRXN signaling networks without (left) and with (right) psilocybin administration (node colors as in Fig. 4A). (C) Bubble heat map showing NRXN subclass-specific communication strength between inhibitory neurons and astrocytes under with- and without-psilocybin conditions. (D) Astrocyte-to-neuron BMP signaling networks without (left) and with (right) psilocybin administration. (E) Neurotrophin (NT) signaling networks originating from the L5/6 IT Car3 excitatory neuron subtype without (left) and with (right) psilocybin administration. (F) Pseudotemporal trajectory of astrocytes and distribution of states under without- and with-psilocybin conditions. (G) Network representation of pathways enriched among genes upregulated in astrocyte state 3 following psilocybin administration. (H) Distribution of CytoTRACE-inferred stemness scores across the astrocyte trajectory clusters (Wilcoxon test, *** $P < 0.001$).

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