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Review

Human pluripotent stem cells as a translational toolkit in psychedelic research *in vitro*

José Alexandre Salerno[1,2,3] and Stevens Rehen[1,4,5,6,*]

SUMMARY

Psychedelics, recognized for their impact on perception, are resurging as promising treatments with rapid onset for mood and substance use disorders. Despite increasing evidence from clinical trials, questions persist about the cellular and molecular mechanisms and their precise correlation with treatment outcomes. Murine neurons and immortalized non-neural cell lines harboring overexpressed constructs have shed light on neuroplastic changes mediated by the serotonin 2A receptor (5-HT2AR) as the primary mechanism. However, limitations exist in capturing human- and disease-specific traits. Here, we discuss current accomplishments and prospects for incorporating human pluripotent stem cells (PSCs) to complement these models. PSCs can differentiate into various brain cell types, mirroring endogenous expression patterns and cell identities to recreate disease phenotypes. Brain organoids derived from PSCs resemble cell diversity and patterning, while region-specific organoids simulate circuit-level phenotypes. PSC-based models hold significant promise to illuminate the cellular and molecular substrates of psychedelic-induced phenotypic recovery in neuropsychiatric disorders.

INTRODUCTION

Medical promises of psychedelics revisited

Psychedelics are compounds that affect perception, mood, consciousness, and cognition.[1] For centuries, they have been used as entheogens in ceremonial settings.[1] It is not clear when the scientific interest in these substances began, but the discovery of the psychoactive properties of the lysergic acid diethylamide (LSD) by the Swiss chemist Albert Hofmann in 1943 is recognized as the birth of the psychedelic era in the modern sciences.[2] This discovery was followed by a surge in psychedelic research aimed at understanding how LSD and other related compounds with mind-altering effects could be applied to treat psychiatric conditions. Social stigma and other constraints impaired progress in the field, but in the late 1990s, a renewed interest in their therapeutic potential re-emerged.[3] Since then, psychedelics have been shown promising in treating mood and substance use disorders (SUDs).[4]

Psychedelics, also known as serotoninergic hallucinogens, consist of a wide range of structurally diverse compounds, including simple indolamine tryptamines, ergotamine tryptamines, and phenylalkylamines. Despite their chemical diversity, these substances collectively act as partial or full agonists that target the 5-HT2A subclass of serotonin receptors (5-HT2AR).[1] Simple tryptamines feature an indole ring coupled with an amine group, resembling the endogenous tryptamine neurotransmitter serotonin (5-hydroxytryptamine; 5-HT). Psilocybin and its primary active metabolite, psilocin, occur naturally in *Psilocybe* sp. mushrooms.[1] Other tryptamines, such as *N,N*-dimethyltryptamine (*N,N*-DMT), and *5-methoxy-N,N*-dimethyltryptamine (*5-MeO-DMT*), are primarily recognized as the psychoactive compounds in ayahuasca brew and the venom of *Bufo alvarius* toads, respectively.[1] These agents function as agonists of several 5-HT receptors (5-HTRs) and generally demonstrate lower affinity for the 5-HT2AR compared to the hallucinogenic phenylalkylamines discussed later.

The ergotamine tryptamines are ergoline alkaloids that resemble tryptamines but possess more rigid molecular structures, featuring an indole system and a tetracyclic ring.[5] LSD, a synthetic derivative, serves as the archetype of this class of psychedelics. Despite being the most potent psychedelic agent in humans, LSD binds relatively nonselectively to various 5-HTRs alongside some dopamine and adrenaline receptors.[5] In contrast, phenylalkylamine hallucinogens like 2,5-dimethoxy-4-iodoamphetamine (DOI) and the naturally occurring mescaline found within several *Cactaceae* species exhibit considerably higher selectivity, binding to the orthosteric sites of fewer 5-HTRs, mostly 5-HT2Rs, with moderate to high affinity.[5]

[1]D'Or Institute for Research and Education (IDOR), Rio de Janeiro, Brazil
[2]Graduate Program in Morphological Sciences, Institute of Biomedical Sciences, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
[3]Department of Morphological Sciences, Biomedical Institute, Federal University of the State of Rio de Janeiro (UNIRIO), Rio de Janeiro, Brazil
[4]Department of Genetics, Institute of Biology, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
[5]Usona Institute, Fitchburg, WI, USA
[6]Promega Corporation, Madison, WI, USA
*Correspondence: stevens.rehen@idor.org
https://doi.org/10.1016/j.isci.2024.109631

Other psychoactive compounds such as dissociative anesthetics (e.g., ketamine), opioids (e.g., salvinorin A), cannabinoids (e.g., Δ(9)-tetrahydrocannabinol), and entactogen stimulants (e.g., 3,4-methylenedioxymethamphetamine), while possessing hallucinogenic-like properties, operate primarily through other mechanisms and are not referred to as *psychedelics* hereafter.

In this review, we discuss the methods most frequently employed to study psychedelics at the cellular level and show how human pluripotent stem cells (PSCs) and derived three-dimensional (3D) cellular aggregates can be used as powerful translational toolkits in psychedelic *in vitro* research. In our review, both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are referred to as PSCs.

5-HT2AR as the primary target for psychedelic-induced synaptic plasticity

In a pioneering clinical study, Vollenweider et al. showed that when administered together with the 5-HT2Rs' antagonist ketanserin, psilocybin's psychoactive effects were prevented.[6] Although ketanserin is not an exclusive 5-HT2AR blocker, 5-HT2AR knockout mice and additional clinical studies further confirmed the pivotal role of 5-HT2AR in mediating psychedelic behavioral effects.[7,8] The high level of correlation among the subjective experience, psilocin blood levels, and 5-HT2AR occupancy at cortical sites after psilocybin intake supports the role of 5-HT2AR as the primary receptor underlying psychedelic pharmacological action.[9] It has been shown that the 5-HT2AR is also essential for synaptic formation, increased dendritic complexity, and boosted functional connectivity that follow exposure to psychedelic compounds in murine neurons.[10]

Cortical neuron dendrite atrophy and loss of dendritic spines are implicated in neuropsychiatric disorders, such as mood disorders, which psychedelics target. Psychedelic-induced neuroplastic changes at cellular and circuit levels likely drive the rapid antidepressant effects observed clinically. Furthermore, neural plasticity extends beyond synaptic modifications, encompassing adaptive responses to stimuli like hypoxia. This suggests psychedelics as promising pharmacological interventions aiding recovery from dysfunctions extending beyond the synaptic level.

5-HT2AR, a member of the G-protein-coupled receptor (GPCR) superfamily that classically signals through the G_q subunit, triggers the phospholipase C (PLC) β-mediated downstream cascade upon its activation on the plasma membrane. Psychedelics have also been shown to cross the plasma membrane and bind to intracellular 5-HT2ARs, particularly within the Golgi apparatus, to induce plasticity.[11]

Psychedelics are biased agonists at 5-HT2AR, as they engage with specific structural domains and trigger certain downstream pathways over others.[12,13] For instance, crystal structure analysis of bound 5-HT2AR has shown that psychedelics are more likely to recruit β-arrestin-2 than the G_q subunit.[14] In cortical sections from rats, it has been observed that β-arrestin-2 binds with 5-HT2AR within the intracellular vesicles of pyramidal neurons.[12] Following 5-HT2AR activation, β-arrestin-2 is required for the phosphorylation of the extracellular regulated kinase (ERK), also contributing to the behavioral effects observed in rodents exposed to LSD but not to DOI or *5-MeO*-DMT.[12,15,16] Lastly, the transcriptional changes result from the phosphorylation of cyclic AMP (cAMP) response element-binding protein (CREB) via the mitogen-activated protein kinase and calcium/calmodulin-dependent kinase II pathways.[17] The identities of the signaling pathways responsible for psychedelics' hallucinogenic and therapeutic properties and whether these are distinct or overlapping pathways are some of the questions still under debate.

The G_q or β-arrestin-2 recruitment paradigm represents a glimpse into the extensive network of pathways regulated by 5-HT2AR activation via psychedelics. For example, distinct psychedelics activate phospholipase A2 and phospholipase D via ADP-ribosylation factor 1.[18–20] The activation of 5-HT2AR, triggered by various agonists including DOI and LSD, can lead to the heterodimerization of the receptor with metabotropic glutamate receptor 2 and dopamine D2 receptor, both of which are G_i-coupled receptors.[21,22] This transactivation blocks the cAMP's synthesis by adenylate cyclase and prompts heterotrimeric $G_{i/o}$ proteins to trigger Src-mediated downstream events.

A burst of glutamate also follows psychedelic administration, mainly in the cortical layer V of the neocortex, which is a 5-HT2AR-enriched area.[8,23] High glutamate levels activate the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), boosting the secretion of brain-derived neurotrophic factor (BDNF), which in turn signals through the tyrosine receptor kinase B (TrkB) and mammalian target of rapamycin (mTOR) pathways, sustaining both the AMPAR activation and BDNF secretion in a positive feedback loop of neural plasticity.[10,24]

Furthermore, the hallucinogenic properties of different psychedelics are also proposed to be influenced by other 5-HTRs, and not all 5-HT2AR agonists have hallucinogenic properties.[25] For instance, human studies also provide evidence supporting the involvement of the 5-HT1AR in the effects of psilocybin.[26] Competition binding studies on rodent brains and primary cells revealed a lack of pronounced selectivity of psilocin for 5-HT2AR over 5-HT1AR.[27] Nevertheless, the role of the 5-HT2AR remains the subject of more extensive study and investigation. Ketanserin, a selective antagonist of the 5-HT2Rs, effectively eliminates head-twitch behavioral responses in mice; however, it does not attenuate psilocybin-induced structural modifications in the prefrontal cortex.[28] As a result, uncertainties remain about whether and how the neuroplastic effects on rescuing disease phenotypes relate to behavioral responses, especially in humans. Moreover, human studies investigating the molecular mechanisms of psychedelic-induced plasticity rely on global parameters such as peripheral BDNF levels, which may not correlate with the molecular mechanisms occurring in brain cells.[29] For in-depth cellular and molecular insights into the role of 5-HT2AR, *in vitro* investigations are required.

Shortcomings of the current models used to probe psychedelic-induced cellular plasticity in human cells

Pharmacological approaches to studying psychedelics *in vitro* commonly employ commercially available cell lines stably transfected to express the human 5-HT2AR.[30] On the other hand, the psychoplastogenic properties can be probed *in vitro* using primary cortical cultures from rodents, which are consistent with *in vivo* findings and *ex vivo* slice recordings.[10,28] Typical morphological outcomes of drug-induced

plasticity are monitored by imaging dendrite markers to measure their length and the number of crossings and synaptic density markers to assay spine morphology by super-resolution microscopy.[10,11,31] Both the target- and phenotype-based approaches can be combined, as fluorescent biosensors of 5-HT2AR conformational states are also available and have already been successfully applied to distinguish psychedelics from non-hallucinogenic psychoplastogens in live murine neurons.[32] Predominantly, phenotypic screening is constrained to non-human cells, potentially overlooking interspecies differences, while mechanistic investigations heavily lean on immortalized non-neural cell lines.

Although primary neural cells from rodents have been vital in investigating the impact of psychedelics on cellular plasticity, there are known differences between the amino acid sequences of human and rodent 5-HT2ARs.[33] These differences, albeit minor, are sufficient to slow down the human 5-HT2AR recycling rate compared to its rodent counterpart.[33] Rodent cells demonstrate disparate sensitivity compared to human neurons toward psychoactive substances like the ketamine analog methoxetamine.[34] Similar differences become evident in assays evaluating drug-induced suppression of neurite outgrowth, which is particularly relevant for phenotype-based psychedelic studies.[35]

As for target-based pharmacological studies of psychedelics, a detailed examination of the assays employed is reviewed elsewhere.[30,36] Briefly, the functional assays to measure 5-HT2AR activity in these systems usually take advantage of inositol phosphates' (IP) accumulation, Ca^{2+} levels, cAMP production, or reporter gene expression. Resonance energy transfer-based assays that measure protein-protein interactions are also frequently employed to monitor G_q or β-arrestin-2 recruitment to the 5-HT2AR site.[37] Transfected human embryonic kidney cells, known as HEK293 cells, are highly transfectable and endogenously express multiple GPCRs and β-arrestins, making them an attractive *in vitro* model for studying GPCR signaling, including psychedelic studies featuring overexpressed constructs of human 5-HT2AR. Although overexpressing a single target remains of great value as a pharmacological tool to avoid confounding effects between targets and off-targets, quantitatively manipulating one target can impact its downstream functional selectivity and may modify some phenotypic responses to psychedelics.

GPCR quantitative variations, or variations in β-arrestins and G-protein-coupled receptor kinases, shift receptors' functional selectivity patterns in overexpressed systems.[38] For instance, it has been shown that the amount of GPCR angiotensin II type 1A receptors affects their desensitization dynamics and triggers alternative and atypical PLC activation.[39] Differences in agonist activities and potencies were also shown to be influenced by the expression levels of GPCR A1 adenosine receptor (A1Rs) in two distinct Chinese hamster ovary (CHO-K1) cell lines.[40] These cells were stably transfected to express recombinant human A1Rs, with expression levels that differed by over 15-fold.[40] This substantial variation in receptor expression per unit of protein in the cell membrane directly impacted cAMP production or IPs' accumulation following agonistic stimulation.[40]

In fact, not only 5-HT2AR and its direct downstream transducers account for a biomimetic model to recreate the brain's molecular environment reliably. Following exposure to psychedelics or several other compounds with antidepressant properties across different classes, such as selective 5-HT reuptake inhibitors (SSRIs) and ketamine, BDNF is upregulated and, thus, the TrkB signaling pathway is activated.[10,31,41] Recent research has revealed that psychedelics can directly bind to TrkB, allosterically enhancing the BDNF signaling pathway.[31] Failing to preserve the general native expression patterns in single target investigations, usually limited to 5-HT2AR overexpression, might overlook important features for phenotype-based analyses. The model choice in these cases should also be careful since some neuroblastoma cell lines may already naturally express elevated levels of alternative targets, such as TrkB,[42] which would still limit the translational validity depending on the experimental question despite exhibiting a neuronal phenotype.

The transfected systems are typically built immortalizing non-neural cell lines, which hampers simultaneous phenotypic examinations of tissue-specific events relevant to psychedelic medical use, such as neuronal atrophy recovery. GPCR ligands, including psychedelics, may display varying patterns of functional selectivity depending on the tissue and cell type. In C6 glioma cells, a truncated β-arrestin-2 mutation enhances 5-HT2AR desensitization by affecting internalization dynamics, a process intricately linked to agonist-induced phosphorylation and certain transcriptional changes.[43] However, the popular HEK293 cells carrying this same β-arrestin-2 mutation do not exhibit changes in the internalization dynamics of 5-HT2AR.[33]

Lastly, immortalized cell lines might fail to capture the potential contribution of receptor genetic variants and sex- or tissue-specific phenotypic traits.[43,44] This is because these immortalized cell lines have a limited capacity to mimic genetic diversity and phenotypic variability since they are produced from a single cell type with a homogenous genetic background. Genetic factors, including variations in the 5-HT2AR coding region, shape the response to SSRIs in substance-related and mood disorders targeted in psychedelic clinical trials.[45–47] Recent research has demonstrated that single-nucleotide polymorphisms (SNPs) in the 5HT2AR gene sequence can impact psychedelic potency in recruiting G_q or β-arrestin-2.[48]

WHAT DO PSC-DERIVED BRAIN CELLS BRING TO PSYCHEDELIC STUDIES?

Neural cells from PSCs for probing psychedelic modulation of plasticity

Modeling drug-induced transcriptional changes

Psychedelic-induced phenotypic changes result from a wave of transcriptional activation of elements downstream of the receptor. In the murine somatosensory cortex, psychedelics selectively stimulate immediate-early genes (IEGs) such as early growth response (*egr*)-1, *egr-2*, and *period-1*, thereby providing clues into the distinct events triggered solely by psychedelics but not all 5-HT2AR agonists.[7] This selective regulation may elucidate whether perceivable experiences are inherent to the neurobiological mechanisms alleviating disease symptoms. However, in humans exposed to LSD, this differential regulation of the same IEGs was not observed in whole-blood samples, suggesting that it might be specific to certain tissues, particular species, or both.[49] In light of these variables, PSC-derived neurons may offer a valuable opportunity for early validation of findings across mixed species model systems of brain cells.

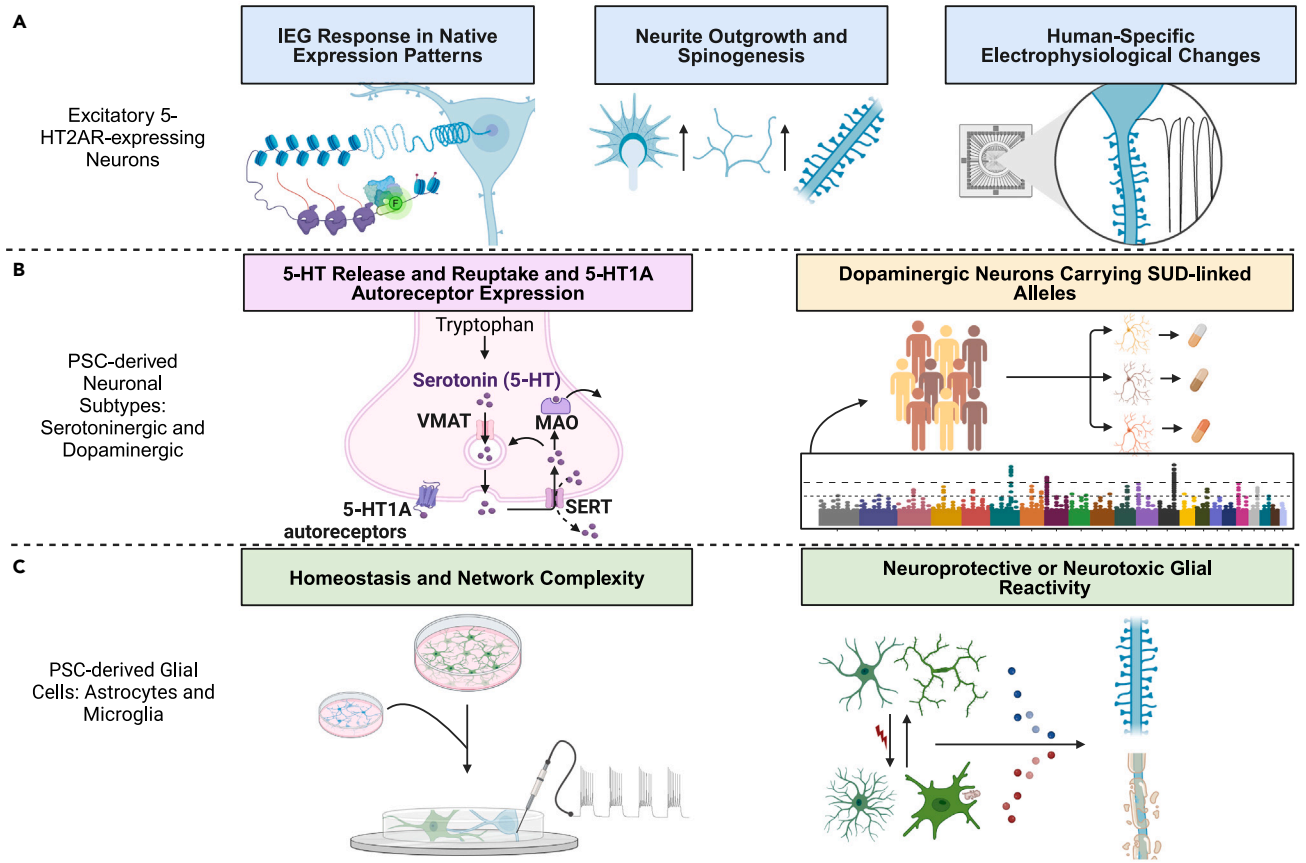

Figure 1. Disease models and patient-derived PSCs for phenotypic interrogation of psychedelics

(A) Excitatory 5-HT2AR-expressing neurons. PSCs may be developed into functional 5-HT2AR-expressing neurons and show a fast, temporary rise in the immediate-early gene (IEG) expression profile following stimulation, mirroring adult human brain patterns. These neurons allow for high-throughput dendritic spine shape and neurite outgrowth monitoring with consistent dose-response curve measurements. Furthermore, in microelectrode array (MEA) setups, PSC-derived excitatory neurons display Ca^{2+}-dependent vesicular glutamate release and voltage-gated channels with human-specific electrophysiological characteristics. MEA setups enable the evaluation of spontaneous and induced spike-like activity as functional plasticity measures while overcoming species bias.

(B) PSC-derived neuronal subtypes. PSC-derived serotoninergic neurons express specific markers and have firing patterns aligned with baseline tonic and burst-firing synaptic neurotransmission. Disease-related characteristics, replicated in depressed patient-derived neurons, shed light on the molecular mechanisms controlling serotonin production, reuptake, and presynaptic release. Similarly, dopaminergic neurons derived from patient-derived PSCs show action potentials and spontaneous synaptic activity and replicate substance use disorder-specific transcriptional regulation, demonstrating increased postsynaptic activity after exposure to addictive drugs such as alcohol, nicotine, and opioids. VMAT, vesicular monoamine transporter; MAO, monoamine oxidase; SERT, 5-HT transporter; SUD, substance use disorder.

(C) PSC-derived glial cells. When co-cultured with neurons on MEAs, PSC-derived astrocytes generate synchronous network bursts and enhanced postsynaptic currents. PSC-derived astrocytes show spontaneous calcium spikes, indicating intercellular communication, as well as reactive gliosis responses to tumor necrosis factor-α (TNF-α). PSC-derived microglia behave similarly to adult human microglia, responding to extracellular stimuli by migration, calcium influx, and cytokine production. These glial cells enable the study of polarized responsive profiles that might be induced or suppressed by psychedelics. Created with BioRender.com.

Pruunsild et al. conducted a study that generated synaptically connected neurons from PSCs characterized by spontaneous postsynaptic currents.[50] Comparisons were made with mouse primary hippocampal neurons cultured under identical conditions, focusing on classifying genes based on temporal expression patterns.[50] Their findings revealed a generic synaptic activity-responsive IEG program, yet distinct between mouse and human models, as the latter includes genes lacking orthologs in the murine genome. Notably, several human IEGs exhibited faster transient upregulation than their mouse counterparts.[50] Human neurons from PSC exhibit a rapid and transient increase of cellular oncogene Fos (*c-Fos*) and activity-regulated cytoskeleton-associated protein mRNA levels upon stimulation, which is apparent only in fully mature neurons but not in their isogenic PSC source (Figure 1A).[51]

Prior research has successfully used PSC-derived neurons to study TrkB-mediated plasticity and provided new insights into the mechanism underlying the effectiveness of neurotrophins at limited concentrations.[52] By working with PSC-derived midbrain dopaminergic and cortical neurons cultured on a multielectrode array (MEA), Bang and colleagues investigated the dependence of long-lasting network

Table 1. *In vitro* testing of psychedelics on PSC-based neural models

Compound	Source for PSC generation	PSC differentiation	Analyzed parameters	Publication
N,N-DMT	Peripheral blood mononuclear cells from healthy donors	Neurons	Hypoxic cell viability; gene expression	Szabo et al., 2016[71]
DOI	Urine cells and skin fibroblasts from healthy donors	Neurons	Inhibitory postsynaptic currents	Wang et al., 2016[183]
5-MeO-DMT	Human embryonic stem cell lineage	Forebrain organoids	Mass spectrometry-based proteomic changes	Dakic et al., 2017[160]
LSD	Skin fibroblasts from healthy donor	Forebrain organoids	Mass spectrometry-based proteomic changes	Ornelas et al., 2022[159]

potentiation on BDNF/TrkB signaling.[53] They could proxy CREB phosphorylation and human-specific IEG expression patterns.[53] TrkB and downstream signaling transducers' expression levels are comparable between PSC-derived organoids and the adult human cortex.[54] These findings support the use of PSC-derived neurons in studies aimed at recapitulating endogenous modulators of psychedelic efficacy for plasticity.

Noteworthy, neurons from PSCs have somatic and dendritic Golgi structures and replicate the intrinsic Golgi dynamics within the dendritic compartments[55] and thus would provide an adequate environment for modeling intracellular 5-HT2ARs involved in psychedelic-induced plasticity.[11] Moreover, genome editing for endogenously regulated tagging is a promising approach that does not compromise PSCs' pluripotency or differentiation potential, in which 5-HT2AR could be tagged and tracked within the neuronal progeny.[56] Fluorescent tags into endogenous neuron-specific genes have been previously employed in PSC-derived neurons and enable the detection of the signaling underlying survival and morphological changes.[57]

In summary, evidence indicates that neurons derived from PSC are suitable for studying how psychedelics affect gene expression. These models also hold promise for inclusion in target-oriented investigations, considering the recently identified location bias behind psychedelic-induced plasticity. However, utilizing PSCs presents certain obstacles, notably the complexity of achieving a sufficiently mature phenotype depending on the protocol employed, a matter later discussed. Also, the examination of psychedelics' effects on PSC-derived neural models remains limited, as delineated in Table 1.

Modeling structural and functional plasticity events

The delayed phenotypic responses triggered by IEGs vastly diverge among cell types. For example, IEG-induced actin remodeling underlies spine morphogenesis in neuronal cells, whereas in cancer cell lines, it relates to migration and invasiveness. PSC-derived neurons offer tissue-specific insights by allowing the monitoring of changes in membrane ruffling and actin reorganization.[50] Dendritic spine morphology and neurite outgrowth, the most employed readouts used to investigate psychoplastogens *in vitro*, are feasible with PSC products in high-throughput setups and provide consistent dose-response curves for other neuropsychiatric drugs while overcoming any potential species bias regarding sensitivity to dendritic outgrowth modulators[35,58] (Figure 1A).

The lack of standardized protocols poses a notable constraint in PSC-derived cells, often resulting in inadequate phenotypic maturity of the derived neurons.[59] It is imperative to acknowledge that such investigations require experimental setups featuring synaptically connected human neurons. Upon achieving these critical functional attributes, PSC-derived neurons can reveal human-specific electrophysiological signatures (Figure 1A).[34]

Improved protocols make it feasible to generate excitatory neurons from PSC that release glutamate in a Ca^{2+}-dependent vesicular manner and express voltage-gated channels with membrane capacitances, resistances, and potentials similar to those observed in native neurons.[60,61] Published protocols utilizing both H9 hESCs and hiPSCs have demonstrated the expression and functionality of N-methyl-D-aspartate receptors (NMDARs) and AMPAR subunits.[62,63] These cells exhibit glutamate-induced Ca^{2+} influx, intracellular Ca^{2+} responses to N-methyl-D-aspartate (NMDA), and NMDAR-mediated postsynaptic currents, as confirmed by calcium imaging and whole-cell voltage-clamp recordings.[62,63] Therefore, spontaneous and evoked spike-like activity can be assessed as functional plasticity parameters, as shown for other therapeutically related compounds such as ketamine.

Ketamine has been catalyzing research into fast-acting antidepressants and paving the way for the clinical application of consciousness-altering compounds.[64] Both psychedelics and ketamine promote mTOR activation, BDNF synthesis, and dendritic spine growth, with psychedelics primarily acting as 5-HT2AR agonists and ketamine via NMDAR antagonism.[23] Ketamine was tested on PSC-derived dopaminergic neurons that reproduced AMPAR-mediated BDNF and mTOR signaling activation alongside increased dendritic arborization.[65] PSC-derived neurons proved also reliable to show that ketamine boosts the expression levels of the AMPAR subunits GluR-1 and GluR-2.[66]

Furthermore, PSC-derived neurons can help explore mechanisms unrelated to 5-HT2AR and provide insights on whether designed ligands can induce other plasticity events underlying the clinical benefits seen for psychedelics. *N,N*-DMT, for instance, has a moderate affinity for the non-opioid sigma 1 receptor (Sig-1R). At high concentrations, *N,N*-DMT positively affects the plasticity of primary murine neurons structurally and functionally.[10] Based on a thorough examination of the dose-response curves, it can be inferred that the effect of this particular psychedelic is probably due to the activation of supplementary receptors, which differentiates it from either LSD or psilocin.[10] Polymorphisms at the

Table 2. Selected publications featuring disease models and patient-derived PSCs for phenotypic interrogation of psychedelics

Disorder	Source for PSC generation	PSC differentiation	Analyzed parameters	Publication
Depression	Skin fibroblasts from patients diagnosed with treatment-resistant depression	Ventral hindbrain serotoninergic neurons	Neurite branches and neural complexity; 5-HT biosynthesis and release; gene expression	Vadodaria et al., 2019[97]
SUD	Human primary lymphocytes from patients carrying a genetic variant linked to susceptibility to opioid addiction	GABAergic inhibitory interneurons	Inhibitory postsynaptic currents; synaptic density; gene expression; evoked action potentials	Halikere et al., 2020[103]
Depression	Skin fibroblasts from patients diagnosed with depression	Astrocytes	Transcriptome	Heard et al., 2021[184]
PTSD	Peripheral blood mononuclear cells or skin fibroblasts from combat veterans diagnosed with PTSD	Glutamatergic neurons	Gene expression	Seah et al., 2022[76]
Depression	Skin fibroblasts from patients diagnosed with depression	Neurons	ATP content; oxygen consumption; mitochondrial membrane potential; spontaneous action potentials	Triebelhorn et al., 2022[78]
SUD	Postmortem fibroblasts from patients deceased with opioid overdose death	Neurons	Gene expression	Mendez et al., 2023[77]
Depression	Peripheral blood mononuclear cells from patients diagnosed with major depressive disorder and exhibiting suicidal behavior	GABAergic inhibitory interneurons	Neurite branches and neural complexity; calcium signaling; evoked action potentials; transcriptome	Lu et al., 2023[91]
Depression	Peripheral blood mononuclear cells from patients diagnosed with major depressive disorder and exhibiting suicidal behavior	Ventral forebrain organoids	Calcium signaling; transcriptome	Lu et al., 2023[91]

Sig-1R locus have been linked to depression, and the approved antidepressant fluvoxamine binds to Sig-1R, indicating that Sig-1R might be involved in the antidepressant mechanism of other compounds.[67,68]

Sig-1Rs participate in the stress signaling pathway by chaperoning inositol-requiring enzyme 1 from the endoplasmic reticulum to the nucleus. Thus, Sig-1R agonists may mediate an adaptive neuroprotection mechanism under stress conditions as an alternative neuroplastic event distinct, albeit synergic, from those described at the synaptic level. This hypothesis has been raised in clinical trials of a Sig-1R agonist for the treatment of stroke.[69] Accordingly, it has been shown that *N*,*N*-DMT mitigates spreading depolarization during ischemic conditions, thereby reducing infarct size, and enhancing functional recovery, primarily through its interaction with Sig-1Rs, rather than 5-HT2ARs.[70]

Employing PSC-derived human neurons as a model system to assess the efficacy of *N*,*N*-DMT in mitigating hypoxic stress, a notable resilience to severe hypoxia (0.5% O_2) upon *N*,*N*-DMT treatment is observed. The dynamic expression profile of Sig-1R during the differentiation process of PSCs into cortical neurons illustrates the suitability of these neurons for elucidating molecular intricacies of *N*,*N*-DMT-mediated neuroprotection that may not rely on 5-HT2AR.[71] *N*,*N*-DMT prevented the upregulation of the α subunit of hypoxia-inducible factor-1 and attenuated hypoxia-induced cell death, whereas Sig-1R antagonists abolished these effects. Subsequent Sig-1R knockdown experiments further confirmed the pivotal role of this receptor in mediating *N*,*N*-DMT's modulatory effects on these human neurons.[71]

Disease models and patient-derived PSCs for phenotypic interrogation of psychedelics

The translation of drug efficacy from animal models to human therapeutics often encounters challenges, as illustrated by instances such as the lack of effectiveness of NXY-059 in ischemic stroke and minocycline in amyotrophic lateral sclerosis (ALS), despite their initial success in rodent models.[72,73] Late-stage clinical trials of drug candidates identified solely on mouse models for Rett syndrome have also faced setbacks. Patient-derived PSCs might have improved the translational validity of an insulin-like growth factor-1 analog, which was shown to rescue disease-linked neuronal phenotypes[74] and reveal the underlying molecular mechanisms[75] in these cells and is now progressing in clinical trials with promising results (NCT04181723 and NCT04279314).

At least partially motivated also by regulatory pressures to reduce animal experimentation by exploring *in vitro* alternatives, the scope of diseases modeled with PSCs has expanded to include psychedelic-targeted disorders such as post-traumatic stress disorder (PTSD),[76] SUD,[77] and depression[78] (Table 2). Despite the increasing popularity of these studies, which have predominantly emerged in the past decade, clinical

trials of compounds identified or repurposed via PSC-based models remain constrained and in their nascent stages. A notable example is the small molecule ezogabine initially identified through extensive drug screening using human PSCs, subsequently undergoing clinical trials for ALS treatment (NCT02450552).[79]

The pioneering reprogramming techniques to induce pluripotency in somatic cells utilized retroviral or lentiviral vectors to transiently express the Yamanaka factors OCT4, KLF4, SOX2, and C-MYC.[80] Currently, non-integrative methods have been developed to avoid undesired genomic insertions while maintaining high efficiency, such as Sendai virus, adenovirus, episomal plasmids, or synthetic mRNAs.[81] PSCs derived from healthy donors can have disease-associated mutations inserted into relevant genetic loci, providing a reliable model for studying specific transducers' impact on resulting phenotypic responses.[82]

Alternatively, adult somatic cells from patients with known genetic mutations can be reprogrammed into PSCs that are differentiated into functional 5-HT2AR-expressing neurons, generating repetitive action potentials and inward currents in response to a 5-HT2AR agonist.[83] PSC lines derived from donors carrying a human-specific non-synonymous SNP in the BDNF gene, which is believed to be associated with heightened susceptibility to depression, have also been documented.[84] Furthermore, genome-wide significant variants associated with the risk factors or symptomatic features of psychiatric disorders such as depression, SUDs, and PTSD are continually being identified, offering unprecedented insights for identifying novel therapeutic targets, repurposing existing drugs, and establishing target-based systems that also model relevant phenotype rescues.[85–87] Analyzing editing revertants in PSCs from donors with disease-linked variations allows for scrutinizing their genotype-phenotype relationship, whose functional implications are still unknown and could influence the clinical response to psychedelics.

Initially, PSC research focused on obvious genetic determinants, such as models of familial and monogenic traits. Now, there are significant endeavors to generate PSC lines from clinically diagnosed patients, spanning diverse profiles like recurrent depression, treatment-resistant depression, and depression with suicidal behavior (Table 2).[88–90] Notably, the differentiation of PSCs from a cohort of depression with suicidal behavior into GABAergic interneurons and ventral forebrain organoids has revealed defective expression of 5-HT2CR at both RNA and protein levels.[91] Treatment with the antidepressant trazodone rescues this phenotype, similarly to lentivirus-mediated gene insertion, to reinstate calcium signaling peaks in these models.[91] In a study assessing the effects of morphine treatment as a proxy for chronic opioid exposure, the expression patterns of neuron-related genes such as *egr-1* were found to be similar between PSC-derived neurons and the dorsolateral prefrontal cortex of individuals deceased with opioid use disorder.[77] Also, direct neuronal transdifferentiation has emerged as a means to bypass intermediate embryonic-like pluripotent stages, which may erase or modify disease-associated epigenetic traits.

The suitability of PSC-derived cells to replicate drug response variations that align with common haplotypes within the population has also been reported.[92] Screening for psychiatric drugs in these cells has proven helpful to assay phenotype-based responses that relate to the pharmacological response of patients. To mention some, PSCs from individuals with schizophrenia have allowed the identification of cholesterol biosynthesis as a trait linked to the clozapine response.[93] The properties of lithium as a mood stabilizer have also been investigated using neurons from reprogrammed cells from responders and non-responders diagnosed with bipolar disorder.[94] These cells replicated circadian electrophysiological changes, which are known to be complex traits associated with drug response.[94]

PSC-derived serotoninergic neurons to study presynaptic targets in mood disorders

In the human brain, presynaptic serotoninergic terminals expressing 5-HT1AR project to cortical layers of pyramidal neurons enriched with 5-HT2AR, which can co-express postsynaptic 5-HT1ARs.[95] In these postsynaptic neurons, 5-HT2AR activation significantly attenuates the inhibitory effect of 5-HT1AR on NMDAR currents and microtubule depolymerization.[16] However, the role that 5-HT1AR plays in psychedelic efficacy remains underexplored in preclinical research. Intriguingly, a 5-HT1AR antagonist reversed the drop in body temperature observed in mice after administration of psilocybin, whereas an agonist inhibited some psilocybin-induced effects in humans.[26,96] It is crucial to accurately differentiate between pre- and postsynaptic receptors, particularly 5-HT1AR, to capture the cellular and molecular mechanisms governing their efficacy in alleviating disease-related phenotypes associated with presynaptic 5-HT synthesis, reuptake, and release or post-synaptic structural plasticity changes related to the disease.

Neurons derived from PSC from patients diagnosed with major depressive disorder have revealed 5-HT-induced hyperactivity when compared with cells from healthy counterparts. It has been shown that a 5-HT2AR/5-HT7R antagonist may partially alleviate this phenomenon.[97] Other disease-linked phenotypes, such as impaired neurite growth and abnormal morphology, have been replicated in PSC-derived neurons, which were also helpful in identifying changes in protocadherin-α gene expression as the underlying mechanism associated with the observed phenotypes.[97]

A chemically defined protocol that guides PSCs through a stepwise ventral hindbrain differentiation process has developed, resulting in a robust 5-HT fate specification (Figure 1B).[98] These serotoninergic neurons express tryptophan hydroxylase 2 and other characteristic markers, including monoamine oxidase, 5-HT transporter, and vesicular monoamine transporter, with electrophysiological firing patterns consistent with basal tonic activity and burst-firing synaptic neurotransmission.[98] Accordingly, these neurons spontaneously release 5-HT and respond to SSRIs.[98] Future preclinical investigations concerning psychedelics as potential antidepressants should consider using these neurons to ascertain 5-HT1AR expression and to monitor their impact on 5-HT dynamics, which may influence their therapeutic efficacy in the clinical setting.

PSC-derived dopaminergic neurons to model SUDs

SUDs are characterized by presynaptic inhibition of gamma-aminobutyric acid (GABA) release and the resulting potentiation of cortical excitatory afferents to dopaminergic neurons in the midbrain ventral tegmental area (VTA). This activation stimulates the nucleus accumbens,

initiating the reward response. On the other hand, it has been shown in rats that at high doses, LSD suppresses VTA dopamine firing by binding to 5-HT1AR, D2R, and trace amine-associated receptor 1.[99] Preclinical models lacking phenotypic specialization of neuronal subtypes, or which are based on a cortical-centered background due to 5-HT2AR-enrichment, may not elucidate psychedelics' fundamental neurobiological mechanisms potentially useful for the treatment of SUD.

Maladaptive plasticity induced by chronic exposure to addictive drugs, such as alcohol, interferes with long-term potentiation/long-term depression (LTD) and BDNF signaling. Although psychedelics regulate the same molecular processes, they are not addictive.[100] In fact, psychedelics have been proposed for treating SUDs as their 5-HT2AR-mediated psychoplastogenic properties are believed to ameliorate the cognitive deficits often associated with addiction.

PSC-derived dopaminergic neurons can generate action potentials, display spontaneous synaptic activity, and express functional GABA A receptor (GABAAR), AMPAR, and NMDAR. Following chronic alcohol exposure, patient-derived cells display a SUD-specific transcriptional upregulation of NMDAR and downregulation of GABAAR subunit-encoding genes (Figure 1B).[101,102] Notably, the expression of the NMDAR subunit *GRIN1* in neurons from PSC remains significantly elevated after drug withdrawal.[102] In contrast, no changes in expression levels were noted in cells derived from non-alcohol-dependent subjects after seven days of alcohol exposure.[101] As for nicotine addiction, PSC-derived dopaminergic neurons from individuals carrying specific SNPs in the cholinergic receptor nicotinic α 5 subunit gene replicated the increased postsynaptic activity in response to nicotine and differentially expressed genes associated with ligand-receptor interaction and synaptic function.[103]

PSC-derived astrocytes to study neuron-glia crosstalk and psychedelics

The idea that glial cells are involved in psychedelic response was proposed 20 years ago, but empirical research on this topic is still scarce despite the recognition that glial cells are promising targets for mood disorder interventions.[104] Astrocytes are one of the most abundant cell populations in the brain and play supportive roles in functions spanning from synapse development and plasticity to metabolic homeostasis and neuroinflammation.[105] A body of evidence has progressively implicated structural and functional dysfunctions in astrocytes as fundamental mechanisms underlying neuropsychiatric disorders.[105] Also, the expression of various receptors and transporters, including 5-HTRs, renders astrocytes promising targets for both traditional antidepressants and psychedelic compounds.[106] Remarkably, following DOI exposure, astrocytes are stimulated to a degree comparable to excitatory 5-HT2AR-expressing neurons in the mice cortex.[107]

The contact of neurons with astroglial cells within synapse compartments, where psychedelics induce significant cellular changes, might contribute to the enhancement of neuronal transmission observed following psychedelic exposure. Astrocyte-secreted synapse-modifying factors, such as glypicans that recruit additional AMPARs, are also expected to play a role in this process.[108] Examples from both murine and human PSCs successfully enabled co-culture models where the astrocyte genotype determined the neuronal phenotypes, including survival and action potential firing (Figure 1C).[109,110] Here, we outline some of the protocol achievements of astrocytes derived from human PSCs and their potential for similar contributions to elucidating biological mechanisms underlying the therapeutic effects of psychedelics.

PSC-derived astrocytes have morphological traits and express markers of functional maturity that provide an appropriate *in vitro* model for investigating psychedelics in these cells.[111] On MEAs, co-cultures of astrocytes and neurons from PSC generate intense synchronous network bursts and an increased frequency of postsynaptic currents compared to neuronal monocultures and mixed-species co-cultures.[112] Overall, co-cultures, whether mixed-species or fully human, have been shown to exhibit sustained network burst frequency and complex oscillatory bursting, with activity increasing gradually across all frequencies over time. However, the PSC-derived co-cultures displayed greater magnitudes of change in response to an NMDA antagonist, resulting in reduced bursting at delta and theta frequencies compared to the mixed-species cultures.[113]

Human astrocytes are also distinguished by a broader functional repertoire than their murine counterparts.[114] Comparative analysis of gene enrichment highlights significant differences in molecular profiles between human and mouse astrocytes, with the former displaying enrichment in genes related to inflammatory responses while the latter exhibiting metabolic enrichment.[114]

Human PSC-derived astrocytes display spontaneous calcium spikes, suggesting the occurrence of intercellular signaling among interconnected cells. Adding glutamate or ATP results in calcium wave responses, glucose uptake, and lactate shuttling.[115–117] The excitatory signature and the corresponding increased oxygenated blood supply left by psychedelics in some areas of the human cortex[118,119] probably stimulate astrocytes to uptake excessive glutamate through high-affinity transporters and then have it converted into glutamine by glutamine synthetase.[120] Astroglial scavenging prevents neuronal excitotoxicity caused by excess glutamate, a function that is impaired in depressed patients' cortical regions and amygdala.[121] Astrocytes are also expected to release glutathione to protect neurons from the oxidative stress of glutamatergic stimulation.[122]

Interestingly, mitochondrial function has been postulated as an astrocyte-specific mechanism underlying the potential efficacy of a novel candidate for a faster-onset antidepressant, hypidone hydrochloride (YL-0919).[123] Notably, distinct variations in mitochondrial physiology further differentiate human and mouse astrocytes.[124] Human astrocytes are more vulnerable to oxidative stress and, therefore, should be the first choice when studying the molecular underpinnings of these metabolic changes in humans at the cellular level. Zandonadi et al. investigated human primary astrocytes subjected to ayahuasca preparations with different *N,N*-DMT-based concentrations.[125] Untargeted metabolomics revealed notable and distinct changes in both intracellular and secreted metabolites associated with depression.[125]

In summary, the unique set of functions, molecular characteristics, and vulnerability to oxidative stress shown in human astrocytes highlight the utility of PSC-based cultures as a valuable resource to uncover the impact of psychedelics on cellular phenotypes that are important for therapeutic outcomes but extend beyond neuronal cells.

PSC-derived brain cells to assay psychedelics immunomodulatory events

A few clinical investigations have explored inflammatory biomarkers after exposure to psychedelic substances through the analysis of peripheral fluids, yielding heterogeneous findings.[126–130] To date, there is not enough clinical evidence to support the intrinsic role of psychedelics as anti-inflammatory agents. By contrast, animal models of inflammatory diseases and cultured rat aortic smooth muscle cells have shown that tumor necrosis factor-α (TNF-α)-induced secretion of pro-inflammatory cytokines, adhesion molecules, and the nuclear translocation of the nuclear factor κB (NF-κB) are all prevented by 5-HT2AR activation following DOI exposure.[131,132] Another study using human primary monocyte-derived dendritic cells suggests that *N,N*-DMT and *5-MeO*-DMT prevent pro-inflammatory cytokine release while increasing the secretion of the anti-inflammatory interleukin (IL)-10 after inflammatory challenges, but by Sig-1R activation, rather than 5-HT2AR.[133]

In addition to the limited strength of evidence regarding psychedelics' effects on immune cells, investigating these anti-inflammatory changes in local reactive brain cells, particularly astrocytes and microglia, is still an emerging topic.[134,135]

The simultaneous involvement of microglia and astrocytes in synaptic activity and immune response in the brain has been recognized. Notably, individuals with depression often exhibit elevated levels of inflammation markers, which have been shown to impair astrocytic glutamate uptake.[136] Since the late 1990s, research has shown that 5-HT2AR is upregulated in reactive astrocytes,[137] suggesting that these inflammation-primed cells might be particularly affected by drugs that target these receptors. Fluoxetine, a first-line SSRI antidepressant, inhibits pro-inflammatory reactivity in astrocytes via 5-HT2BR and β-arrestin-2 signaling.[138] This functional selectivity has also been observed with LSD and its precursor ergotamine at human 5-HT2BR.[139]

Astrocytes respond to insults by synthesizing pro-inflammatory and anti-inflammatory cytokines, such as IL-1β and TNF-α. *N,N*-DMT has been shown to mitigate the pro-inflammatory effects of intracerebroventricular injection of $A\beta_{1–42}$ on astrocyte reactivity and TNF-α production in mice's subgranular zone.[134] Astrocytes can amplify complex neuroinflammatory cascades or trigger repair processes depending on neurotransmitter and ion regulation, gap junction function, and phagocytic activity. Species-specific differences may also play a role, as shown in a study comparing mouse and human astrocytes.[124] We have previously demonstrated that human PSC-derived astrocytes exhibit reactive gliosis in response to TNF-α, mirroring the nuclear translocation of NF-κB, cytokine release, morphological changes, and impaired uptake by glutamate high-affinity transporters.[140]

While technical challenges to isolate microglia from humans exist, PSC-derived microglia are similar to cultured adult human microglia.[141,142] Also, working with PSC-derived microglia allows high-throughput assessments, mainly recapitulating the basal transcriptional profile of *ex vivo* microglia.[142] Quantitative data show that PSC-derived microglia engulf human synaptosomes as expected, revealing the underlying regulatory molecular pathway involved.[141] PSC-derived microglia sense extracellular stimuli, migrate, exhibit calcium influx, and secrete various cytokines in response to insults.[141]

Exposing PSC-derived microglia to brain-related substrates recapitulates the transcriptional states identified in the human brain.[143] PSC-derived microglia exhibit intrinsically distinct transcriptional states *in vitro*, even without stimulation. Importantly, these states do not converge to a single transcriptional signature upon stimulation.[143] This behavior is like the one observed *in vivo*, in which microglia may adopt distinct transcriptional states depending on the location. These findings suggest that the heterogeneous response to substrates typical in microglia can be replicated in PSC-derived cells. Therefore, studies exploring the potential properties of psychedelics or related compounds for preventing neuroinflammation can benefit from these scalable and translational *in vitro* options.

Studying the effects of psychedelics on neurogenesis with brain organoids

PSCs can also be used to re-create cell diversity, thus allowing multicellular phenotypic changes to be assayed. PSC-derived neural stem cells can be cultured as cell aggregates under non-adherent conditions and guided to develop in neurospheres containing neurons and glia in a 3D environment (Figure 2A).[144] Cell-cell interactions are possible in this environment, resulting in greater network complexity than in monolayered neural cells. We have previously reported that neurosphere's maturation occurs by downregulating proliferation and upregulating axonal guidance pathways, which correlates with the neurite outgrowth phenotype.[144] Brain organoids represent a significant advancement in modeling tissue complexity and heterogeneity beyond neurospheres. Both neurospheres and organoids hold value depending on the experimental question at hand. Serving as more "sophisticated" constructs, brain organoids crafted from PSC are adept at recapitulating the developmental trajectory, cell diversity, and spatial morphology of the human brain.[145] However, their inherent variability and heterogeneity present substantial challenges to the field, particularly for their application in quantitative drug screening assays.

Animal studies have produced mixed results on the potential benefit of psychedelics on neurogenesis by activating 5-HT2AR. Some studies in rats have shown that LSD and DOI do not affect hippocampal neurogenesis, whereas others performed in mice have shown that psilocybin negatively regulates it.[146–148] In contrast, *N,N*-DMT and *5-MeO*-DMT at relatively high doses enhanced cell proliferation in the murine hippocampus by activating Sig-1Rs.[149,150] However, the influence of *N,N*-DMT on neurogenesis is still controversial since others have found that *N,N*-DMT-treated animals significantly lower the densities of progenitors and mature neurons in the subgranular zone neurogenic niche, possibly due to *N,N*-DMT's higher affinity for 5-HTRs.[134] The question of whether specific or all psychedelics trigger neurogenic plasticity in human cells and the extent to which these discoveries hold any translational significance for the adult human brain is still an open question.

These potential effects lack comprehensive exploration in the cellular milieu of human brain cells. The neurogenic niche in humans has notably expanded, driven by primate-specific outer zone progenitors,[151] which may impart distinctions of drug-induced neurogenesis in human cellular contexts. Single-cell RNA sequencing identified significant differences in proportions of cell types, laminar distributions, gene expression, and morphology between human and murine cortex, notably in serotonin receptor genes such as 5-HT2AR, ranking among the

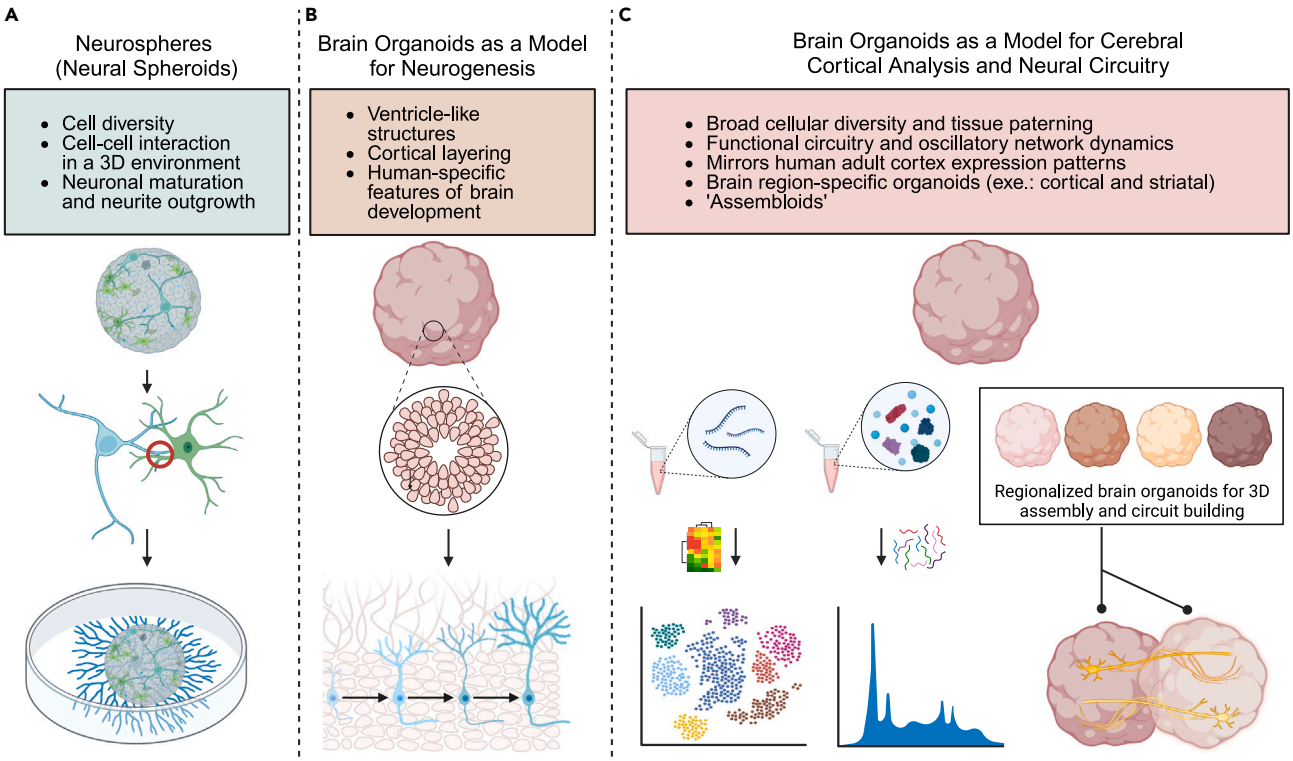

Figure 2. Studying the effects of psychedelics on PSC-derived three-dimensional systems

(A) Neurospheres or neural spheroids. Neural stem cells originating from PSCs can be grown in 3D clusters called neurospheres. These clusters can be further developed to produce neurons and glia, enabling cell-cell interaction and increasing network complexity. This technique is useful for examining phenotypic changes, including neurite outgrowth, and can provide valuable insights into phenotypes that require diverse cells.

(B) Brain organoids as a model for neurogenesis. Brain organoids are self-assembled structures that give a detailed *in vitro* representation of human brain cells, their diversity, developmental progress, and receptor expression. These organoids contain most brain cell types and have similar transcriptomic profiles to those found in the developing human brain. By mimicking the formation of ventricles, the migration of neurons, and the layering of the cortex, they can model tissue complexity and heterogeneity to assay whether psychedelics can induce neurogenesis as an alternative neuroplastic mechanism.

(C) Brain organoids as a model for cerebral cortical analysis and neural circuitry. Brain organoids from PSC can be cultured for an extended period to generate various specialized cell types when they shift spontaneous neural activity to network bursting. These organoids are suitable for screening the effects of psychedelics on a transcriptomic and proteomic level with physiological relevance to the human adult brain. Additionally, organoids resembling specific brain regions can be physically integrated to allow axonal projections across regions and synaptic assembly, recreating circuitry-level features *in vitro*. Created with BioRender.com.

most divergent gene families between the two species.[152] Brain organoids provide a nuanced portrayal of human brain cell diversity, developmental trajectories, and receptor expression, where PSCs self-assemble into cellular aggregates that mimic crucial aspects of brain tissue organization, including ventricle-like structures and cortical layering (Figure 2B).[153] As brain organoids contain most brain cell types with similar transcriptomic profiles to those in the developing human brain,[151,153] they provide a highly attractive and feasible alternative for testing findings where animal models are inconclusive among different drugs, aiding in compound selection for enhanced translational validity of neurogenesis as an additional level of psychedelic-induced plasticity.

Indeed, PSC-derived organoids have previously modeled polygenic neurodevelopmental features that are specific to humans, which were otherwise inaccessible due to idiopathic genetic backgrounds affecting brain development.[154,155] Hence, they are poised to elucidate whether neurogenesis is triggered selectively by certain psychedelics or universally by all, in addition to promoting synaptic plasticity in the existing neurons.

Studying the effect of psychedelics on neural networks with brain organoids and assembloids

Although developmental aspects of the brain have been more intensely studied in organoids, research on neural circuits and functional networks in these models has just recently begun. Organoids can be cultured long-term for further maturation, enabling the detection of circuit-level phenotypes in patient-derived cells (Figure 2C).[156] In these cases, they exhibit more pronounced gliogenesis and gradually form mature network bursting.[54,156] Accordingly, they exhibit synchronized neural activity and oscillatory networks that resemble human preterm electroencephalography patterns.[157] Their utility extends beyond developmental processes as they activate essential synaptic biochemical

machinery to generate self-organized activity patterns. This capability may effectively replicate critical aspects of how psychedelics influence neuronal phenotypes, rendering them also a suitable biomimetic model of the adult human brain *in vitro* at both molecular and functional levels. We have recently shown that these organoids recapitulate proteomic signatures found in *postmortem* tissues from patients.[158] In PSC-derived cerebral organoids, the expression levels of molecular transducers of the psychedelic signaling pathway, such as ERK and *c-Fos*, *egr-1*, and *egr-2* IEGs, are comparable to those observed in the adult human brain cortex.[54]

Using liquid-chromatography mass spectrometry, our group analyzed the proteome of PSC-derived forebrain organoids exposed to psychedelics.[159,160] We found that LSD upregulates mTOR and other plasticity-related pathways, such as DNA replication, LTD, axon guidance, and synaptic vesicle cycle.[159] The detection of differentially expressed synaptic proteins, particularly the synaptic vesicle glycoprotein 2A (SV2A), in brain organoids post-LSD exposure underscores its potential translational relevance. SV2A reduction has been documented in the brains of depressed patients,[161] and studies in pigs have demonstrated an increase in SV2A levels following psilocybin administration,[162] an effect later also shown in humans following the fast antidepressant ketamine.[163] The unbiased approach used for proteomics analysis on organoids also provided evidence of the inflammatory NF-κB pathway downregulated by *5-MeO*-DMT in human brain tissue.[160] Given that such modulation was not seen after LSD exposure and considering the resemblance between brain organoids and adult human brain expression patterns, organoids could aid in distinguishing pharmacological applications among psychedelic compounds.

As brain organoid cells aggregate, mature, and establish networks among cell clusters, they activate key signaling pathways like TrkB and develop more robust electrical properties. Retinal circuits have been modeled with guided organoids, and output projection tracts from others have been shown to evoke muscle contraction.[164,165] The electrophysiological correlates of the oscillatory power during the psychedelic state in humans can be studied on a microscale with brain organoids that demonstrate synchronous and stereotypical nested oscillatory network events, which develop as cell diversity becomes more complex, especially due to the emergence of inhibitory neuronal and glial populations.[166,167] Also, a significant debate persists in the field regarding whether the behavior effects induced by psychedelics are essential for their therapeutic benefits. Nonetheless, modeling these phenomena *in vitro* has been poorly explored, since it requires even more tailored models due to their strong reliance on interconnected brain regions.

Prominent models like the cortico-striatal thalamocortical loop (CSTC) and the relaxed beliefs under psychedelics (REBUS) suggest that psychedelics induce alterations in whole-network dynamics, extending beyond the well-examined 5-HT2AR-enriched cortical regions. CSTC proposes heightened thalamic hyperconnectivity, facilitating increased sensory information flow within cortical-subcortical circuits. Conversely, REBUS suggests that psychedelics attenuate higher level cortical network control, which is consistent with heightened cortical entropy and increased bottom-up processing. The recent advancements that have introduced region-specific human brain organoids can now be employed to model subcortical regions impacted by psychedelics in terms of both behavioral responses and plasticity outcomes, such as the thalamus.[23]

These regionalized organoids can be used to study the functions of striatal and thalamic regions of the brain and later combined with cortical organoids to create complex "assembloids,"[162] which allow for modeling *in vitro* the activity within and between the cortical and subcortical cells during circuit building.[168,169] Striatal organoids resemble the developing human striatum and include electrically active medium spiny neurons from distinct striatal cell lineages.[169] These neurons mature electrophysiologically following the assembly of the organoids and display calcium activity after optogenetic stimulation.[169] Next, specific imaging techniques for epifluorescence can monitor the formation of cortico-striatal and cortico-thalamic axonal projections and synaptic connections.

Revah et al. achieved a significant milestone by transplanting PSC-derived cortical organoids into early-postnatal rat somatosensory areas, demonstrating robust axonal projections, network formation, and maturation of human cortical neurons.[170] Remarkably, their study also highlighted integration with host thalamic connections with impact on host behavior.[170] Significant strides are still necessary before establishing connections between the electrical patterns, circuit architecture, and gene expression alterations observed in microscale organoid physiology and the biological correlates of altered consciousness states in humans, primarily due to our limited understanding of whether such correlates exist.

Bioengineering is rapidly evolving to supplement brain organoids with cerebrospinal fluid production, blood vessels, blood-brain barrier, and microglial cells, further improving the model for testing drug interventions that better reflect adult human brains.[171–174] These innovations are expected to be incorporated into a single system in the following years. Future studies focusing on these approaches hold great potential to uncover the impact of psychedelic mind-altering properties on the biological correlates of disease-linked phenotype recovery.

LIMITATIONS AND FUTURE PERSPECTIVES ON THE USE OF PSC-DERIVED CELLS AND ORGANOIDS IN PSYCHEDELIC RESEARCH

It is essential to acknowledge that PSCs do not aim to replace conventional models but rather complement them. PSC-derived models offer unique insights into cellular behavior and drug responses by mirroring human tissue, but cells expressing all receptors of the brain fail to discern target from off-target effects accurately. Thus, integrating both approaches promises comprehensive target identification and phenotypic validation, with initial screening in single receptor-overexpressing cells followed by phenotypic investigations using PSC-derived models to elucidate complex cellular interactions and responses. Integrative approaches will counterbalance the major caveats of PSC-based models, such as reproducibility, incomplete reprogramming, and insufficient maturation.

Variations in donor characteristics, genetic integrity, and experimental factors contribute to the diversity observed in PSC models, influencing their differentiation potential, cellular composition, morphological features, and levels of transcripts and proteins. Reminiscent epigenetic features from source cells and incomplete reprogramming are potential shortcomings to be addressed when using PSC-derived cells in

disease modeling and psychedelic drug testing. Genetic or epigenetic variations may influence the ease of differentiation of certain PSC lines into specific cell types. Stringent protocols and assessment of whether gene expression patterns are neuron specific and thus not found in either their isogenic PSC counterparts or cancer cell lines are good practices that may help to circumvent this shortcoming and ensure the quality of PSC products.[56,57]

Depending on the adopted protocol for guided differentiation, certain PSC-derived cells may remain immature. Most available characterization of PSC-derived cultures is compared to fetal expression patterns. Protocols adopting aging-inducing cocktails and prolonged culture periods have successfully simulated mature and age-related cellular features while fading away residual epigenetic signatures.[175] Taking Alzheimer's disease research as an example, PSC-derived neurons from patients recapitulate the elevated Aβ production and tau hyperphosphorylation without manipulating expression levels, indicating that given the proper conditions, these models can achieve sufficient maturity to recreate late cellular hallmarks.[176]

The use of 3D culture models has been also recognized as a strategy to improve the maturity of products from PSCs. However, discrepancies in PSC genetic backgrounds and culturing methods lead to morphology variations and batch inconsistencies, raising concerns about the comparability of developmental trajectories and cell identities across methodologies. Standardizing brain organoid culture protocols has become a critical endeavor for advancing the utility and reliability of these models in neuroscientific research. It should be noted that under stringent protocols, the variation observed among individual forebrain organoids is comparable to the natural variability seen in human cerebral structures.[177,178]

With the increasing popularity of genome editing tools, isogenic PSC lines submitted to gene editing—instead of those derived from family- and gender-matched healthy control subjects—may minimize PSC product variation and provide appropriate controls for neuropsychiatric disease modeling and psychedelic drug testing.[179] Genome editing tools can be applied to editing a mutation site in a wild-type PSC line or to correct disease-linked mutations in patient-derived cells for phenotype confirmation.

It is well accepted that the possibility of pairing an *in vitro* model with an individual exposed to the same psychedelics is one of the upsides of patient-derived PSCs over other systems. However, PSCs are still expensive and labor intensive to be maintained for large cohorts. Similar to what has happened with other technologies, such as DNA sequencing, more cost-effective methods are already being introduced to enhance the yield of PSCs.[180] As an alternative, there is a growing trend of establishing biobanks and repositories that contain disease-specific PSC lines, including cells from underrepresented populations.[181,182] This development is anticipated to enhance the accessibility and popularity of PSC technology.

ACKNOWLEDGMENTS

This work was supported by intramural grants from the D'Or Institute for Research and Education (IDOR). J.A.S. was supported by a PhD scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The funders had no role in the preparation of the manuscript.

AUTHOR CONTRIBUTIONS

Conceptualization, J.A.S. and S.R.; writing – original draft, J.A.S.; writing – review and editing, J.A.S. and S.R.; supervision, S.R.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT to improve language and readability. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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