CellPress
OPEN ACCESS

Trends in
Pharmacological Sciences

Review

The ABCs of psychedelics: a preclinical roadmap for drug discovery

Alex C. Kwan [1,*], John R. Mantsch[2,*], and John D. McCorvy[2,3,*]

There is growing interest in developing psychedelic-inspired drugs for treating psychiatric disorders. However, identifying next-generation psychedelic analogs with ideal receptor selectivity and true therapeutic efficacy remains a major challenge. Recent progress has been driven by advances in determining agonist-induced biased signal transduction, high-content behavioral phenotyping via automated video analysis, drug-evoked structural neural remodeling, and activity-dependent gene expression. In this review, we outline a framework for evaluating psychedelics and non-hallucinogenic serotonin 2A (5-HT_{2A}) receptor agonists. We critically examine current methods for assessing (A) agonism, (B) behavior, and (C) cellular plasticity. We highlight emerging techniques that may improve translation to humans. We contend that an effective discovery pipeline must align with specific experimental goals and incorporate multiple approaches to be successful for psychedelic drug development.

Highlights

Psychedelics can be evaluated using preclinical assays organized into three key domains: agonism, behavior, and cellular plasticity. This organization provides a conceptual framework for developing next-generation, psychedelic-inspired therapeutics.

A focus of the approaches is the characterization of non-hallucinogenic serotonin 2A (5-HT_{2A}) receptor agonists, which are gaining traction for their potential therapeutic effects without inducing acute subjective experiences.

Recent advances in tools such as biased signaling assays, structural plasticity imaging, high-content behavioral phenotyping, and transcriptomic profiling offer powerful methods of probing psychedelic drug action.

Because no single assay captures the full spectrum of psychedelic effects, an integrated, multipronged strategy across the three ABC domains (agonism, behavior, and cellular plasticity) is essential to link receptor pharmacology to both acute and enduring functional outcomes.

Psychedelics as potential therapeutics

Psychedelics (see Glossary) have emerged as potential therapeutics. Positive results have been demonstrated for psilocybin with psychological support for treatment-resistant depression [1] and major depressive disorder [2], psilocybin-assisted psychotherapy for alcohol use disorder [3], and lysergic acid diethylamide (LSD)-assisted therapy for anxiety [4]. Psilocybin and LSD likely represent the first generation of such treatments. There are ongoing efforts to develop the next generation of compounds based on similar **mechanisms of action**. It is essential to develop psychedelic-inspired novel chemical entities that will improve upon efficacy or safety, or both.

Assays for psychedelic drug discovery have evolved. A historical example from the 1950s is the rat fundus strip assay [5], which assesses compounds based on their ability to induce smooth muscle contraction. Although this assay predates the discovery and cloning of serotonin (5-HT) receptors, we know now that psychedelics were preferentially detected due to the high density of 5-HT_2 receptors in the stomach fundus. Since then, the field of psychedelic research has gained more sophisticated and quantitative tools. In this article, we survey approaches that interrogate the ABCs of psychedelic drug action: (A) agonism, (B) behavior, and (C) cellular plasticity. We discuss the strengths and limitations of the different approaches. We propose that an integrated framework incorporating readouts from all three domains is crucial for the development of the next generation of psychedelic-inspired therapeutics.

Defining psychedelics and non-hallucinogenic 5-HT_{2A} receptor agonists

There is no universally accepted definition of psychedelics. The term was originally coined to describe compounds such as LSD and mescaline as 'mind-manifesting' [6]. However, this framing does not fully capture the complexity of psychoactive substances, which produce a mix of distinct and overlapping behavioral effects [7–9]. The later discovery of 5-HT receptors (Figure 1) provided a more specific framework: psychedelics can be more precisely defined as agonists

[1]Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY 14850, USA
[2]Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226, USA
[3]Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226, USA

*Correspondence:
alex.kwan@cornell.edu (A.C. Kwan), jomantsch@mcw.edu (J.R. Mantsch), and jmccorvy@mcw.edu (J.D. McCorvy).

 https://doi.org/10.1016/j.tips.2025.07.017

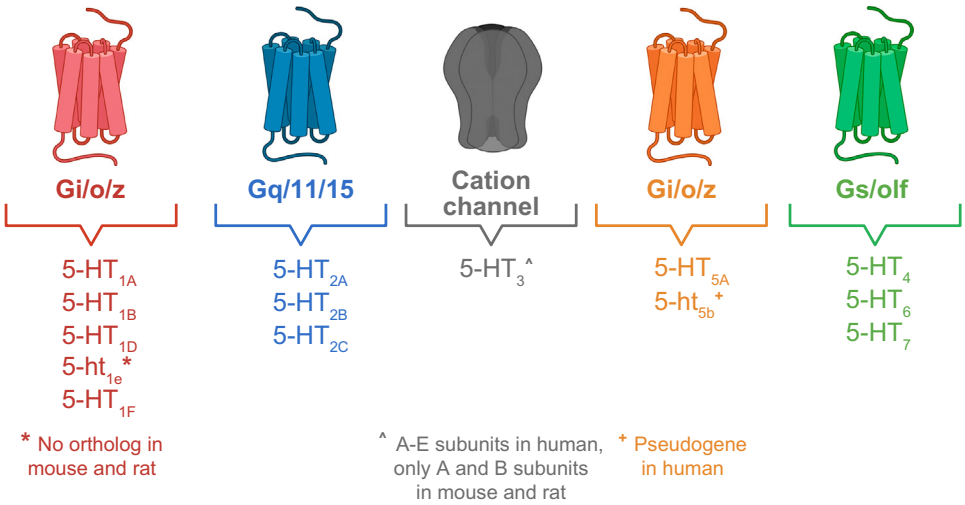


Figure 1. Serotonin receptor subtypes. There are 14 subtypes of serotonin receptors. Most are G protein-coupled receptors, except the serotonin 3 (5-HT_3) receptor that is a ligand-gated ion channel. There are notable species differences. The 5-ht_{1e} receptor is present in humans, but there is no ortholog in mice or rats. The 5-HT_3 receptor is composed of subunits, for which there are five types in humans and two types in mice and rats. The gene for the 5-ht_{5b} receptor does not produce a functional protein in humans. The lowercase appellation for 5-ht_{1e} and 5-ht_{5b} follows the current conventions set by the International Union of Basic and Clinical Pharmacology. Created with BioRender (created by author J.D. McCorvy, 2025).

at the 5-HT_{2A} receptor that produce subjective effects in humans [10]. According to this receptor-based classification, psychedelics include tryptamines such as psilocybin, *N*,*N*-dimethyltryptamine (DMT), and 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT), ergolines such as LSD, and phenethylamines such as mescaline and 2,5-dimethoxy-4-iodoamphetamine (DOI). This definition excludes substances such as ketamine, 3,4-methylenedioxymethamphetamine (MDMA), and salvinorin A, which are other examples of hallucinogens with different pharmacological mechanisms of action.

Increasing attention has turned to **non-hallucinogenic 5-HT_{2A} receptor agonists** that do not induce the 'trip' yet may still confer therapeutic benefits [11]. Some have proposed alternative terms such as neuroplastogens or psychoplastogens [12], emphasizing their putative mechanism in enhancing neural plasticity. Several examples, including 6-fluoro-*N*,*N*-diethyltryptamine (6-F-DET), Ariadne, lisuride, and 2-Br-LSD, have been tested in humans and found to lack acute perceptual effects (e.g., [13]). More recently, tabernanthalog (TBG), IHCH-7079, IHCH-7086, (*R*)-69, (*R*)-70, 25N-N1-Nap, and 25N-NBPh have been developed [14–18], but the absence of acute behavioral effects for these agents has so far only been inferred from animal models. It must be cautioned that non-hallucinogenic 5-HT_{2A} receptor agonists may not be necessarily devoid of all psychopharmacological activity and may possess activities at other targets. For instance, lisuride shows partial agonism at the 5-HT_{2A} receptor [19], but has dopamine receptor agonism to stimulate movement. Moreover, whether non-hallucinogenic 5-HT_{2A} receptor agonists have therapeutic potential like classic psychedelics remains to be determined in clinical trials.

Advances in approaches for determining agonism

A key to determining whether a compound is a psychedelic is to determine whether compounds have sufficient 5-HT_{2A} receptor agonism to induce acute subjective effects [20,21]. Non-hallucinogenic 5-HT_{2A} agonists, such as 2-Br-LSD and lisuride, often demonstrate weak partial

agonism at the 5-HT_{2A} receptor [18,22]. **Target engagement**, therefore, must be thoroughly assessed and is shaped by several pharmacological properties, including **affinity**, **ligand residence time**, **intrinsic activity**, and **biased agonism** (Figure 2). For psychedelics, **specificity** and **selectivity** are key characteristics because most psychedelics interact with other 5-HT receptor subtypes or targets [23], thus knowing the full receptor pharmacological profile is important for shaping their psychopharmacological and therapeutic effects. For example, serotonergic drugs such as fenfluramine and ergotamine have been linked to cardiac valvulopathy due to their off-target activation of the 5-HT_{2B} receptor [24]. Therefore, achieving selectivity for 5-HT_{2A} over 5-HT_{2B} receptor is highly desirable to reduce potential risks. Other receptor subtypes such as 5-HT_{1A} can shape the behavioral effects of psychedelics [25,26]. Later we will discuss methods for assessing target engagement, specificity, and selectivity.

Methods for quantifying binding affinity and ligand kinetics

Affinity is commonly measured using competitive binding assays. The biological target (e.g., human 5-HT_{2A} receptor) is recombinantly expressed in a cell line (e.g.,HEK-293). A tracer is a labeled molecule (e.g., a radioligand, fluorescent ligand, or bioluminescent ligand) that binds to the target with an equilibrium dissociation constant (Kd). The test compound competes with the tracer for the same binding site at the target. By varying the concentration of the test compound, the inhibitory constant (Ki) can be calculated to estimate binding affinity. A goal of affinity measurement is to predict **receptor occupancy**: the degree to which a test compound engages its target in native tissues (e.g., 5-HT_{2A} receptor in the human brain). Ultimately linking 5-HT_{2A} receptor binding affinity with *in vivo* receptor occupancy has value for psychedelic drug development because it is associated with the dose that may be psychoactive in humans [27]. However, affinity values derived from different tracers are not always comparable. This is particularly relevant for 5-HT receptors, which can adopt multiple active and inactive conformational states [28]. Because tracers preferentially bind to specific conformations, binding assays are subject to **probe dependence** (Box 1). Binding assays provide limited insight into intrinsic activity. For example, LSD and the non-hallucinogenic 5-HT_{2A} receptor agonist 2-Br-LSD have similar binding affinities at the 5-HT_{2A} receptor, but different efficacies in activating G_q protein pathways [22].

The kinetics of ligand binding and unbinding affect receptor occupancy and ultimately behavioral effects. This principle is exemplified by other G protein-coupled receptor (GPCR)-targeting drugs, where therapeutic efficacy (e.g., tiotropium for asthma) and side effects (e.g., haloperidol producing involuntary movement) are shaped not only by binding affinity, but also by their slow dissociation rates. For psychedelics, there is evidence that LSD has a prolonged residence time at the human 5-HT_{2A} receptor [29], which may contribute to its plasma elimination half-life of ~4 h [30] and extended psychotropic effects. Association (k_{on}) and dissociation (k_{off}) rates can be determined using traditional binding assays. Surface plasmon resonance is a more sensitive, label-free method, but requires purified targets, making it impractical for screening compound libraries or studying large complexes such as G protein-bound 5-HT receptors.

Novel techniques for high-throughput, functional assays

Functional assays report the activation of intracellular signaling pathways, including G protein engagement or second messenger production such as calcium flux. These types of intrinsic activity are usually measured relative to a reference (e.g., 5-HT or LSD), allowing for direct comparisons between compounds in terms of **potency** (EC_{50}) and efficacy (E_{max}). Measurement of second messengers is easy to perform, but one must consider confounds from signal amplification and temporal kinetics (Box 2). As a better alternative, G protein activity can be measured more proximal to the receptor–transducer interaction, providing a quantification of ligand potency and efficacy.

Glossary

Affinity: a measure of the strength of the interaction of a drug with a receptor target (Ki or Kd).
Biased agonism: the ability of a drug to induce differential responses in a receptor target's various signaling pathways, often leading to a distinct biological effect from a reference compound (e.g., 5-HT).
Dendritic spines: protrusions in the dendrite of pyramidal cells in the neocortex and hippocampus. Most dendritic spines have apposing axonal terminals, and together they constitute a functional excitatory synapse.
Functional plasticity: a process where an existing synapse alters its strength.
Intrinsic activity: a measure of the maximal response a compound can induce at a biological target (E_{max}).
Ligand residence time: a measure of how long a drug remains bound to a receptor target (reciprocal of k_{off}).
Mechanism of action: specific effect (s) (e.g., agonist, antagonist, or biased agonist) on receptor target(s) that a drug is intended to produce.
Metaplasticity: changes to the propensity for a synapse to alter its strength. For example, the presence of extracellular glutamate may sometimes induce a new synapse to form. After the administration of a drug, the chance of glutamate-evoked spinogenesis increases.
Non-hallucinogenic 5-HT_{2A} receptor agonist: a compound that is a 5-HT_{2A} receptor agonist but does not elicit acute perceptual effects in humans.
Orthogonal assays: multiple methods designed to measure the same biological effect (e.g., G_q activation) based on different principles and empirical tools.
Potency: a measure of the ability of a drug to induce a biological effect *in vitro* or *in vivo* (EC_{50} or ED_{50}, respectively).
Probe dependence: a phenomenon where the use of a probe, which is needed for a measurement, influences the result. Specifically for affinity or functional assays, the tracers can change an allosteric aspect (e.g., conformation or protein–protein interaction) of the biological target.
Psychedelic: originally defined as a compound that produces 'mind-manifesting' effects in humans. In this article, we consider psychedelic as a compound that is a 5-HT_{2A} receptor

The GTPγS assay uses a labeled, non-hydrolyzable analog of GTP such as [^{35}S]GTPγS to detect G protein activation. Pertinent to psychedelics, the method has been used to assess 5-HT$_{2A}$ receptor activity *ex vivo* in post-mortem human tissue samples [31]. However, GTPγS assays suffer from weak signal-to-noise ratios and rely on membrane preparations, limiting their ability to capture GPCR signaling in a native cellular context. Mini-G proteins, which are engineered G$_\alpha$ subunits devoid of GTP-binding capability, have been developed to stabilize specific conformational states [32], including psychedelic-bound 5-HT receptors for cryo-electron microscopy studies [33]. However, mini-G$_q$ proteins include only the α5 helical tail that inserts into the receptor's intracellular recognition site and cannot assemble into functional G protein complexes, which may compromise the translational fidelity of potency and efficacy measurements. A versatile approach is bioluminescence resonance energy transfer (BRET)-based assays, including methods that monitor G protein dissociation via G$_\alpha$–G$_{\beta\gamma}$ dissociation (e.g., TRUPATH) or free G$_{\beta\gamma}$ dimers, and effector membrane translocation assays (EMTA), including enhanced bystander BRET-based versions [34–37]. A limitation is that the engineered expression systems may not capture the same ratios or specific β/γ pairs of G protein heterotrimers present in neuronal tissues [38]. The requirement for coexpression of G protein chaperones and GTPase-activating proteins will affect heterotrimer formation and the rate of dissociation, and thus should be carefully considered when interpreting results. Moreover, although available data indicate that the use of fluorescent or luminescent tags minimally affects receptor–G protein function under typical experimental conditions [34], in principle the use of large labels conceivably could perturb signaling at other effectors downstream. Notwithstanding the few caveats, BRET-based assays offer a sensitive and physiologically relevant approach to characterize psychedelic-induced signaling across 5-HT receptor subtypes.

For psychedelics, β-arrestin recruitment is suggested to associate with short-term tachyphylaxis and long-term tolerance, possibly due to desensitization and internalization of 5-HT$_{2A}$ receptors. Moreover, differential recruitment of G protein versus β-arrestin at the 5-HT$_{2A}$ receptor may be responsible for distinct behavioral effects in animals [16,18], suggesting that engineering for biased agonism may be a strategic way to refine mechanisms of action for next-generation drugs. Assays have been developed to detect β-arrestin recruitment at GPCRs, including transcription-based methods (e.g., Tango), luminescence-based systems (e.g., NanoBiT or DiscoverX), and BRET-based measurements [39–41]. Each method has its caveat. Tango assays are typically amplified and rely on transcriptional responses that take hours to detect. DiscoverX utilizes stable cell lines and may therefore overlook the contributions of coexpressed GPCR kinases or other effectors needed for high-affinity GPCR-β-arrestin interactions, resulting in a narrower dynamic range of detectable activity. Other BRET- or luminescence-based approaches require the coexpression of multiple signaling components (receptor, either β-arrestin1 or β-arrestin2, and GPCR kinases), which may not reflect stoichiometric GPCR complexes in native tissues. Any approach should include complementary assays that measure receptor desensitization or internalization, which can provide additional validation of β-arrestin recruitment and downstream receptor downregulation induced by psychedelic compounds [18].

Regardless of the chosen assay, parameters such as log (τ/K_A) or log (E_{max}/EC_{50}) can be determined for a test compound relative to a reference (e.g., 5-HT) or a bias plot can be constructed to illustrate G protein versus β-arrestin pathway preference [42]. However, these simple metrics mask the complexity in biased agonism [43]. G protein signaling and β-arrestin recruitment can influence each other; therefore, independent measurements of each pathway in isolation are unlikely to reflect the actual bias in native tissue. Moreover, biased signaling is inherently dynamic [44], influenced by the kinetics of ligand association, active-like conformations, and transducer signaling and competition, meaning preference for G protein or arrestin may shift over time.

agonist that produces subjective effects in humans.

Receptor occupancy: a measure of the extent a drug interacts with the receptor target in tissue or a whole organ (e.g., the brain).

Receptor reserve: a phenomenon where there is an excess of receptors, such that the maximal response can be achieved by occupying only a fraction of the receptors.

Selectivity: a measure of the preference of a drug for a receptor target in comparison to other receptor target (s).

Specificity: a measure of how well a drug interacts with a receptor target, usually in a defined manner (e.g., affinity or potency). For example, DMT demonstrates high specificity for the 5-HT$_{2A}$ receptor in terms of on-target efficacy but lacks selectivity because it also binds other 5-HT receptor subtypes.

Structural plasticity: a process where a new synapse is formed. This can be a new synapse for pairs of neurons that were previously unconnected, or an additional synapse for pairs of neurons that were already connected.

Target engagement: the physical interaction between a drug and a receptor target.

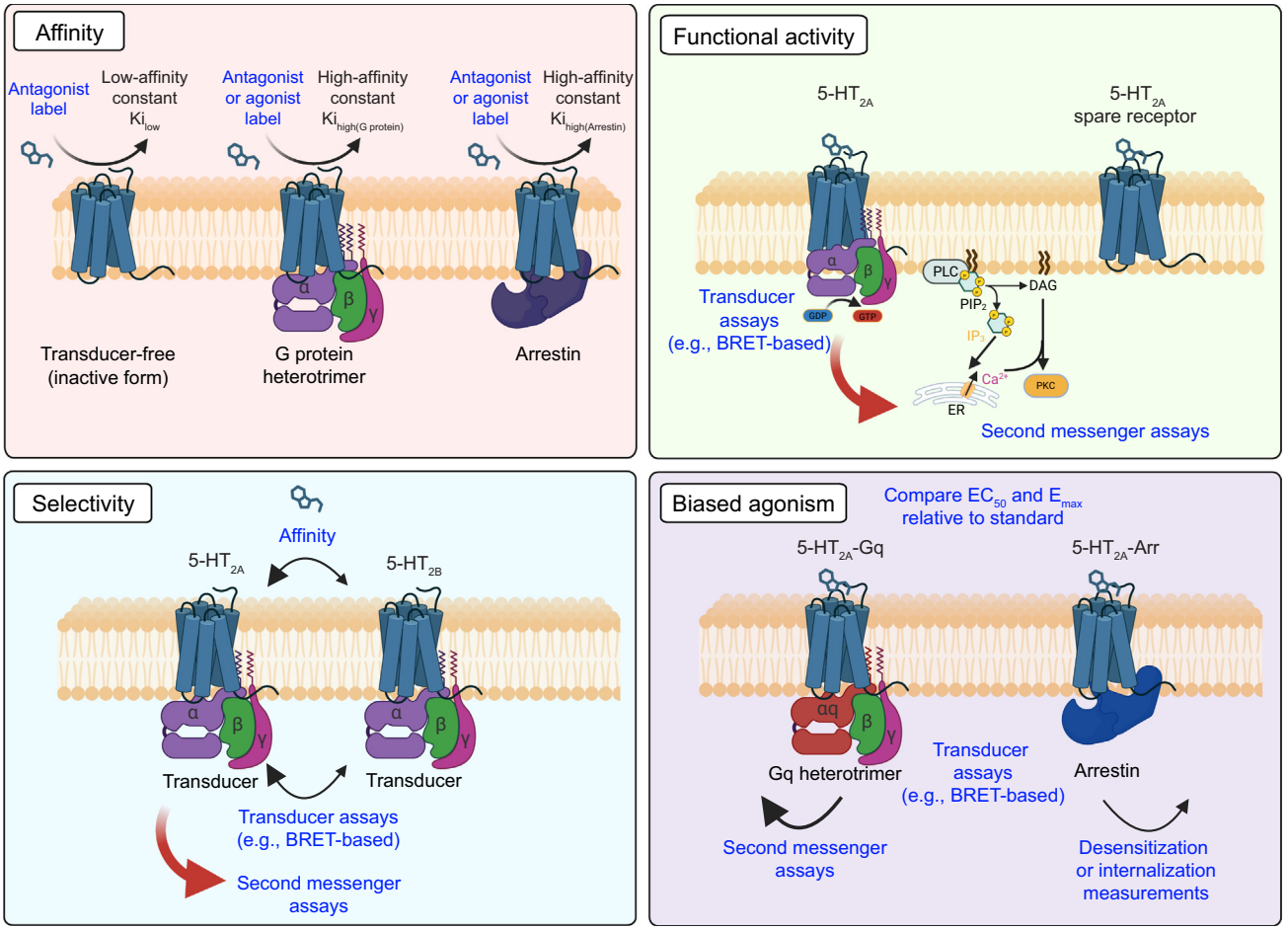


Figure 2. Determination of the mechanism of action. The cartoons illustrate four main concepts: affinity, functional activity, selectivity, and biased agonism. Affinity can be determined at the binding site of a target using either an antagonist or agonist label, resulting in various Ki estimates. Functional activity refers to the activation of G protein or other transducers, which can be measured either at the level of the transducer or second messengers. Second messengers represent an amplified signal (red arrow), because one activated molecule may recruit multiple downstream molecules. Spare receptors would have constitutive activity, which will also be amplified to affect the detection ceiling. Selectivity and biased agonism can be quantified using a variety of affinity and functional assays. Each assay or measurement (highlighted in blue) has caveats and so should be validated via secondary orthogonal assays to validate the results. Abbreviations: 5-HT_{2A}, serotonin 2A; BRET, bioluminescence resonance energy transfer; Ca^{+2}, calcium; ER, endoplasmic reticulum; IP_3, inositol 1,4,5-trisphosphate; Ki, inhibitory constant; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C. Created with BioRender, (created by author J.D. McCorvy, 2025).

Bias can be context-dependent, varying across cell types, subcellular compartments, and physiological states. Importantly, signaling at 5-HT receptors likely extends beyond just two pathways. For instance, 5-HT_1 receptors couple to all 6 Gi/o/z subtypes, and non-canonical Gi/o/z protein coupling has been observed at the 5-HT_{2A} receptor [18,45]. Future investigations should move beyond binary designations (agonist/antagonist) to capture the full repertoire of signaling possibilities that a psychedelic compound may have at 5-HT receptor targets.

Curating assays into a discovery pipeline

Selecting appropriate assays is crucial and should be guided by the experimental objectives. If the goal is to screen candidate compounds for putative non-hallucinogenic effects, often associated with partial agonism at the 5-HT_{2A} receptor, assays with high sensitivity to graded responses

such as BRET-based G protein dissociation or G protein effector membrane translocation assay (GEMTA) platforms are particularly useful. These assays can resolve subtle differences in efficacy to help make early decisions to triage compounds. If the goal is to detect all potential 5-HT_{2A} receptor agonists, including weaker partial agonists that might otherwise be missed, then high-expression systems with robust readouts such as inositol-1-phosphate (IP1) accumulation or calcium flux may be good options. If the goal is to confirm activity in native tissues, GTPγS assays may be informative, although they are low throughput and lack specificity for receptor–transducer pairs and therefore should be applied at later stages in the discovery pipeline as proof of target engagement. By contrast, mini-G proteins should not be used initially for predicting signaling or determining biased agonism and should be reserved for structural approaches such as cryo-electron microscopy. It is worth noting that given the inherent limitations of affinity and functional assays, overreliance on any single method may result in overlooking context-dependent effects or misrepresenting the underlying mechanism of action. A rigorous pipeline that considers the discovery objectives, integrates multiple **orthogonal assays**, and includes various comparator compounds should therefore be at the core of any psychedelic drug discovery effort.

Advances in approaches for determining behavior

Psychedelics induce intense perceptual and emotional changes during the acute phase, with onset and duration differing between drugs and influenced by dose and pharmacokinetics, including the route of administration [8,9]. For oral psilocybin or LSD, the subjective effects typically begin within 0.5 h and reach maximal effects at 2 h [46,47]. The typical effect durations are 5 and 8 h for psilocybin and LSD, respectively [47]. The durations are consistent with LSD's longer elimination half-life of ~4 h [30], relative to psilocybin's half-life of ~2 h [30,48]. The long-term therapeutic benefits reported for these psychedelics outlast the acute effects and persist to weeks and months [1,2,4].

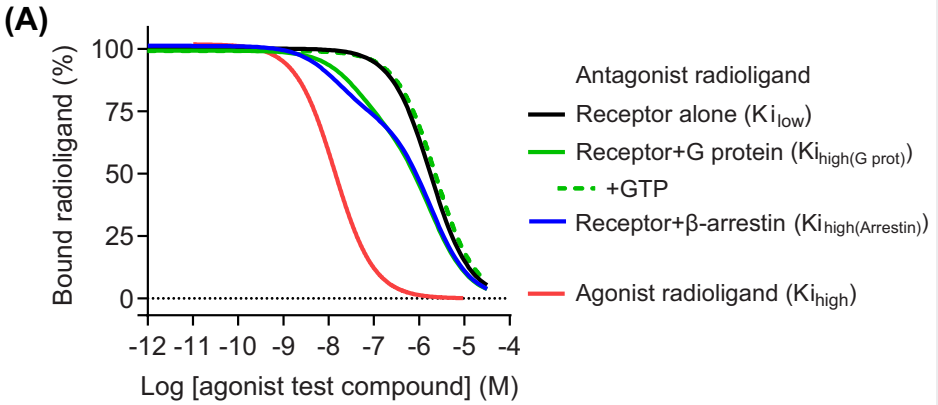
It is unknown if the long-term therapeutic benefits depend on the acute behavioral effects. Clinical studies have found that certain aspects of the subjective experience are correlated with long-term therapeutic outcomes, for example, for psilocybin and depression or smoking

Box 1. Probe dependence from antagonist and agonist tracers

Tracers are labeled ligands that bind to the target. A major challenge in measuring the affinity of a test compound to 5-HT receptors is controlling for probe dependence. Typically, antagonist tracers (i.e., tracers made by labeling ligands that are antagonists at the target) bind to the inactive forms of the receptor. When using an antagonist tracer to evaluate an agonist test compound, the displacement curve can reveal binding with either a low-affinity constant ($Ki_{(low)}$) or high-affinity constant ($Ki_{(high)}$) (Figure I). The high-affinity constant $Ki_{(high)}$ may provide a better estimate of receptor occupancy of an agonist *in vivo*. GTP or related analogs may be added to the assay to uncouple G proteins from the receptors, and if the displacement curve shifts rightward, then this would indicate binding initially occurred with $Ki_{(high)}$, validating that the test compound is an agonist that prefers the active, G protein-coupled form of the receptor.

However, $Ki_{(high)}$ may not always be revealed with antagonist tracers, due to the assay conditions (e.g., the receptor may not be coupling to G proteins in the cell line, or the G proteins of interest are not expressed). Furthermore, β-arrestin-bound receptor may interact with the test compound via a high-affinity constant ($Ki_{(high,\ arrestin)}$) [105], which can be difficult to tell apart from the high-affinity constant of G protein-bound receptors ($Ki_{(high,\ G\ protein)}$). Using cell lines with either G protein or arrestin knockouts can help clarify which $Ki_{(high)}$ affinity state is measured, which is important if the goal is to develop a biased agonist.

A reasonable alternative is to use an agonist tracer (i.e., tracers made by labeling ligands that are agonists at the target), as it can bind specifically to the receptor's high-affinity states. However, agonist radiotracers are not available for every 5-HT receptor of interest. In particular, they are either poorly developed (e.g., 5-HT_{1F}, 5-HT_4, and 5-HT_{5A}) or no longer commercially available, such as [^{125}I]-DOI that was used for 5-$HT_{2A/2B/2C}$ receptors. Although [^{3}H]-LSD is available and acts as an agonist at most 5-HT receptors except 5-HT_7, it has extremely slow kinetics at 5-HT_2 receptors. Moreover, its high-affinity binding appears to be independent of G proteins and arrestins [29]. These factors limit the utility of [^{3}H]-LSD as a radiotracer for determining the $Ki_{(high)}$ values of interest.



(B)

Receptor	Antagonist radiotracer	Agonist radiotracer
5-HT_{1A}	[^{3}H]Way100635	[^{3}H]8-OH DPAT
5-HT_{1B}	[^{3}H]GR125743	[^{3}H]5-CT
5-HT_{1D}	[^{3}H]GR125743	[^{3}H]5-CT
5-ht_{1e}		[^{3}H]5-HT
5-HT_{1F}		
5-HT_{2A}	[^{3}H]Ketanserin [^{3}H]N-methylspiperone	[^{125}I]DOI[a] [^{3}H]LSD[b] [^{3}H] 25I-NBOMe
5-HT_{2B}	[^{3}H]Rauwolscine	[^{125}I]DOI[a] [^{3}H] LSD[b]
5-HT_{2C}	[^{3}H]Mesulergine	[^{125}I]DOI[a] [^{3}H]LSD[b]
5-HT_3	[^{3}H]GR65630	
5-HT_4	[^{3}H]GR113808	
5-HT_{5A}		[^{3}H]LSD
5-HT_6		[^{3}H]LSD
5-HT_7		[^{3}H]5-CT [^{3}H]LSD[b]

Trends in Pharmacological Sciences

Figure I. Probe dependence and radiotracers for 5-HT receptors. (A) Schematic illustrating the displacement curves for the same receptor obtained for different conformational states and via an antagonist or agonist tracer. (B) List of common antagonist and agonist radiotracers used to target 5-HT receptors for binding affinity assays. Abbreviations: 5-CT, 5-carboxamidotryptamine; 5-HT, serotonin; 25I-NBOMe, 4-iodo-2,5-dimethoxy-*N*-(2-methoxybenzyl) phenethylamine; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tatralin; DOI, 4-iodo-2,5-dimethoxyamphetamine; $Ki_{(high)}$, high affinity inhibition constant; $Ki_{(low)}$, low affinity inhibition constant; LSD, lysergic acid diethylamide. [a]No longer commercially available. [b]High-affinity binding may not be dependent on G protein or arrestin.

cessation [49] or for LSD and anxiety [4]. However, counter arguments have been made that the acute subjective effects may not be a prerequisite for long-term positive changes [50], suggesting a path for developing psychedelic-inspired compounds that preserve the therapeutic efficacy while eliminating the subjective effects, like the non-hallucinogenic 5-HT_{2A} receptor

agonists, or at least shorten the acute experience, like 5-MeO-DMT, bretisilocin, or RE104 [51]. While this remains a debated and unresolved topic, details of the acute and long-term behavioral effects of a test compound can be evaluated using various animal models for drug discovery.

Standard and emerging methods for measuring acute effects

Psychedelics evoke rapid side-to-side head movements in mice, which can be recorded using a high-speed camera or a magnet attached to the head or ear. This head-twitch response is a proxy for a compound's potency for 5-HT_{2A} receptor activation [52], particularly via the G_q-coupled pathway [18]. Notably, non-hallucinogenic 5-HT_{2A} receptor agonists such as 2-Br-LSD, 6-F-DET, and Ariadne have markedly attenuated head-twitch responses [13,22,53]. The assay is straightforward, requires no animal training, and shows if a test compound reaches centrally to the brain. However, the head-twitch response is exhibited only by certain species such as mice, rats, rabbits, and mole-like shrews [54] (Box 3), and bears little resemblance to the human psychedelic experience. Thus, while useful for assessing target engagement, head-twitch response should not be regarded as a definitive predictor of hallucinogenic potential in humans.

In a drug discrimination assay, animals are trained to press one lever after receiving a drug (e.g., a psychedelic) and another lever after receiving vehicle. During testing, an unknown compound is administered, and the animal's lever preference indicates the degree to which the test compound produces interoceptive effects that can substitute for the training drug. This method allows for testing of various drugs at different concentrations, provided that a cohort of trained animals is maintained. The assay is typically performed using rats, but can also be developed for non-human primates [55]. As the animal responds if the test compound 'feels' like a psychedelic, drug discrimination assays have face validity for estimating the potential subjective effects of a test compound, particularly if this link is further tested by co-administering with 5-HT_{2A} receptor blockers. However, the exact neural basis leading to the animal's interoceptive response is unknown, and likely differs across various drug classes [56].

Box 2. Second messenger assays

Second messenger assays can use either biosensors or analyte detection methods, such as luminescence, homogeneous time-resolved fluorescence, or other techniques [106]. Common second messenger assays measure the signaling pathways activated by the different types of GPCRs: inositol phosphate accumulation or calcium flux for $G_{q/11}$-mediated signaling at the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors, cAMP inhibition for $G_{i/o/z}$-mediated signaling at the 5-HT_1 and 5-HT_5 receptors; cAMP accumulation for $G_{s/olf}$-mediated signaling at the 5-HT_4, 5-HT_6, and 5-HT_7 receptors, are just a few examples of second messenger measurements.

It is important to consider the kinetics of the second messenger responses when interpreting assay results. Calcium flux assays, such as those performed with fluorescence imaging plate reader (FLIPR) systems, are fast and compatible with high-throughput screening. However, the transient nature of the calcium signals, which last only a few minutes, may not fully capture the extent of receptor activation due to the issue of hemi-equilibria [107]. This limitation is relevant for psychedelics with slow binding kinetics, such as LSD [29], because ligand potency and efficacy may be severely underestimated by calcium flux assays. Instead, measuring the longer-term accumulation of inositol phosphates may provide a more complete view of $G_{q/11}$-mediated signaling [108]. Moreover, differences in receptor expression levels, ligand binding rates, and second messenger amplification across receptor subtypes can complicate interpretation of selectivity (Figure I). It is prudent to calibrate first with a known full agonist (e.g., 5-HT) to confirm that comparable signal levels can be achieved across receptor subtypes in an assay.

Second messengers are amplified signals, which enhance sensitivity but also introduce complications. When using systems with low receptor expression, there is a risk of classifying a test compound as inactive or an antagonist lacking agonist activity, while it may show agonism in systems with higher receptor expression. Moreover, the amount of amplification can vary across different receptors and cell lines, and therefore should be calibrated. Overexpression of a receptor can lead to **receptor reserve**, where even if not all receptors are occupied by the drug, amplification and maximum second messenger production can still be achieved (Figure I). Effectively, this lowers the detection ceiling of the assay, making partial agonists appear indistinguishable from full agonists and ultimately limiting an assay's usefulness as a screen. This is especially problematic for 5-HT_{2A} receptor agonists, which display varying degrees of partial agonism in signaling pathways [18]. Discrepancies may arise. For example, a drug may appear as potent in molecular assays due to receptor reserve, yet fail to produce behavioral effects *in vivo*, where receptors are expressed at endogenous levels.

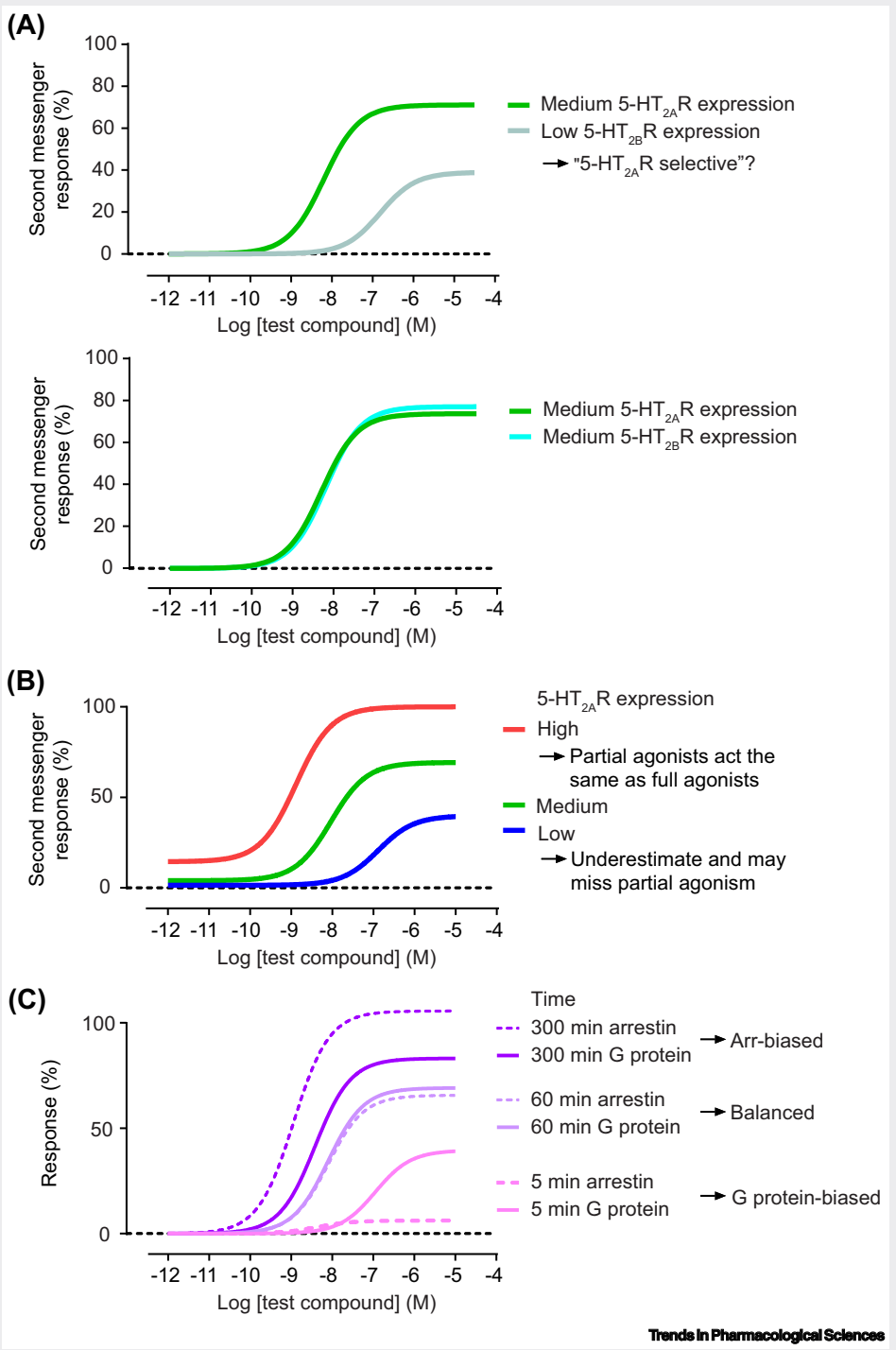


Figure I. Common pitfalls for the determination of selectivity, intrinsic activity, and biased agonism. (A) Second messenger response is sensitive to receptor reserve (e.g., using a cell line that expresses 5-HT_{2A} receptor at a medium level but 5-HT_{2B} receptor at a low level) and cell types (e.g., CHO vs. HEK). Certain responses like calcium flux are susceptible to time-dependent artifacts due to hemi-equilbria. These factors skew the determination of receptor selectivity. (B) Receptor overexpression creates a receptor reserve, where amplification enables maximal signaling even without full receptor occupancy. This lowers the assay's detection ceiling, making partial agonists appear similar to full agonists. Low receptor expression can lead to false negatives by misclassifying compounds as inactive or lacking agonist activity. (C) Bias can shift over time. The balance between G protein and β-arrestin signaling is a dynamic process shaped by kinetics of signaling and other factors. Abbreviations: 5-HT_{2A}, 5-hydroxytryptamine-2A; CHO, Chinese hamster ovary; HEK, human embryonic kidney.

Prepulse inhibition (PPI) is a phenomenon in which a weak sensory stimulus, typically acoustic, is presented 30–500 milliseconds before a strong startle-inducing stimulus, resulting in reduced startle response. In rodents, this innate startle reflex can be measured by quantifying an animal's

Box 3. Species difference and considerations

There are notable differences in the neuropharmacology of 5-HT_{2A} receptors across different species. Publicly available data (UniProt) [109] provide a percent identity matrix, which quantifies the amount of identical amino acid residues across species (Figure I). The sequence for *HTR2A* is highly conserved between humans and non-human primates (96–97% identical), but the similarity decreases to 92% between humans and mice. Of note, the serine residue at position 5.46 in transmembrane 5 in the human 5-HT_{2A} receptor is important for ergoline and tryptamine ligand recognition [110]. A serine to alanine substitution (S242A) at this position decreases the rate of dissociation for LSD by several fold [33] (Figure I). Therefore, tests with mouse or rat 5-HT_{2A} receptor may report a much weaker effect for a psychedelic than tests with human 5-HT_{2A} receptors. Ketanserin is a 5-HT_{2A} receptor antagonist used for blocking psychedelic effects in human studies [20]. However, ketanserin's ability to cross the blood–brain barrier varies across species: penetrance is higher in humans, monkeys, and minipigs than mice and rats, likely due to the compound being a P-glycoprotein substrate and species-related differences in P-glycoprotein-mediated removal of substances at the blood–brain barrier [111].

Beyond 5-HT_{2A} receptors, there may be species-specific differences at the 5-HT_{2B} subtype, as evidenced by an example of recognition of an adenosine-based antagonist [112]. Another example is the 5-HT_{1B} receptor, where a threonine at position 7.39 in the human receptor is an asparagine in rodent isoforms, which leads to weaker affinity for select tryptamine and ergoline compounds [113,114]. An underexplored topic is the rodent's lack of the 5-ht_{1e} isoform that is present in humans. The 5-ht_{1e} receptor can be activated by select multicyclic antipsychotics [115], but little is known about potential activation by psychedelics. Conversely, rodents possess a functional 5-ht_{5b} subtype in the brain, which is a non-functional pseudogene with a premature stop codon in humans.

Dosing is an important consideration when performing translating across species. The approximate dose can be estimated using allometric scaling formulas that account for metabolic rate and surface area differences between animals [116]. For psilocybin, the standard dose of 25 mg in a 60 kg human translates to 5.1 mg/kg in mice. These numbers provide a rough guideline dosage but are not determined specifically with psychedelics in mind. Ideally, it should be empirically determined that the chosen dosages produce similar levels of receptor occupancy in animals and humans.

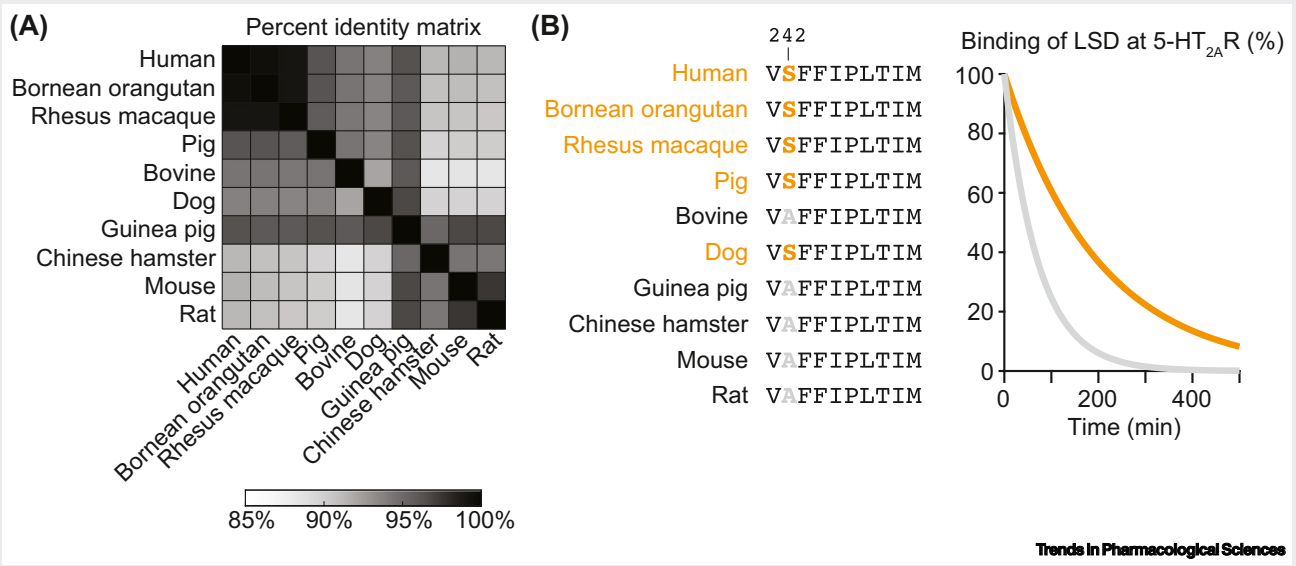


Figure I. Species differences in 5-HT_{2A} receptor. (A) The percent identity matrix shows the amount of identical amino acid residues across species. (B) The human 5-HT_{2A} receptor has a serine residue at position 5.46 in transmembrane 5. In other species such as mice and rats, there is a serine to alanine substitution in their 5-HT_{2A} receptors. Different dissociation kinetics for LSD at the human 5-HT_{2A} receptor with $S242^{5.46}$ or $S242A^{5.46}$ mutation, based on Kim *et al.* [33]. Abbreviations: 5-HT_{2A}, serotonin 2A; LSD, lysergic acid diethylamide.

movement. PPI is acutely suppressed in rats and mice following the administration of LSD, an effect that is dependent on activation of the 5-HT_{2A} receptor [16,57]. Interestingly, non-hallucinogenic 5-HT_{2A} receptor agonists like (*R*)-69 and (*R*)-70 do not affect PPI [16]. PPI has merit as a cross-species measure, because similar startle responses can be assessed in humans. Consistent with animal data, psilocybin and LSD reduce short-interval PPI in human subjects [58,59]. One caveat is that PPI can be influenced by different neuropsychiatric conditions and other classes of drugs. Specifically, PPI is also bidirectionally modulated by dopaminergic agents and attenuated by ketamine [60]. Relatedly, psychedelics cause other acute behavioral changes in rodents, such as reducing body temperature [61] and disrupting

ultrasonic vocalizations [62], but these effects can likewise be caused by other drugs and are not exclusive to psychedelics.

Instead of implementing specific predefined tasks, an emerging approach is high-content behavioral phenotyping via analyzing a large number of video recordings of spontaneous movements [63]. The videos are processed with machine learning algorithms to identify stereotyped actions (e.g., [64,65]), allowing for unsupervised classification of behavioral patterns, such as turning, rearing, or tail flicking of mice. Due to the simplicity of the measurement, videos can be obtained for hundreds of conditions to classify drugs with high accuracy [66,67]. The approach is unbiased because it does not rely on assumptions about the behavioral effects of a drug. However, therapeutic effects may not always be apparent from characterizing spontaneous movements alone. For example, psilocybin's propensity to improve behavioral flexibility might be expected to reveal its impact in tasks requiring decision-making [68,69], rather than in a mouse freely moving in an empty arena. In general, acute behavioral responses to psychedelics may engage neural circuits that are distinct from those that underlie the long-term effects of psychedelics in animals [70,71], and should not be presumed to be predictive of therapeutic potential.

Challenges in assessing long-term behavioral effects of psychedelics

A wide range of animal models and behavioral paradigms have been used to study the effects of psychedelics in the context of neuropsychiatric indications. For psilocybin alone, a recent meta-analysis identified over 70 preclinical studies examining its behavioral effects [72]. However, animal models vary greatly in their construct, face, and predictive validity, such that the outcome of an experiment testing the effects of a psychedelic may depend on the specific animal models chosen (e.g., different acute and chronic stress paradigms for modeling depression) [73]. The reported effect sizes tend to be small, raising concerns about the reproducibility of studying long-term effects of psychedelics using tasks such as the forced swim test, elevated plus maze, and three-chamber social interaction test [74]. Rather than revisiting these issues, we focus the discussion here on key methodological considerations for evaluating the behavioral impact of psychedelic compounds.

One important factor is context. For example, psilocybin promotes the extinction of conditioned fear, but it is effective only when administered immediately before extinction training, whereas administering the drug after extinction training failed to produce long-term changes [75]. Potential context-dependent effects of the non-hallucinogenic 5-HT_{2A} receptor agonist TBG on cue-induced heroin seeking have also been reported [14]. The context can be manipulated, for example, by applying restraint stress immediately following psilocybin exposure, which can blunt the subsequent positive behavioral effects of the drug [76]. One possible explanation for context dependence is that psychedelics enhance neural plasticity, but concurrent behavioral experience is required to activate the relevant circuits for modification. The context dependence in animal models has implications for evaluating the effectiveness of psychedelic-based therapies with psychological support.

Dose is another important factor. In humans, while a 10 mg dose of psilocybin starts to sufficiently occupy brain 5-HT_{2A} receptors to elicit acute subjective effects [46], a higher dose (25 mg) may be required to achieve therapeutic effects in treatment-resistant depression [1]. This mismatch may reflect dose-dependent recruitment of different brain regions, each contributing to distinct behavioral effects [70,71]. Indeed, studies have demonstrated region-specific profile of psychedelic-evoked transcriptional responses as a function of dose in rats [77]. Some behavioral outcomes produced by psychedelics may follow an inverted U-shaped response curve [75]. Dose considerations must also account for pharmacokinetics; while psychedelic drugs are typically taken orally in humans, animal studies often rely on parenteral routes.

Psychedelics may act differently in disease states, underscoring the importance of using animal models that mimic aspects of the underlying pathophysiology. A difficulty is ensuring a stable and persistent behavioral phenotype. For instance, learned helplessness is a model for depressive-like behavior. While most mice have altered escape behavior 1 week after the initial induction, this percentage declines to ~20% by 2 weeks [78]. The spontaneous recovery from stress restricts the window for assessing the long-term effects of psychedelics, as the behavioral maladaptations resolve over time independent of drug treatment. One promising avenue is the use of selectively bred rats [79] or genetic mouse models [16], which may provide more consistent phenotypes for investigating the effects of psychedelics. Regardless of the exact model used, translating findings from animals to humans remains a challenge, due to the heterogeneity of human biology and the varied presentations of mental illnesses. It may be that psychedelics are beneficial for specific behavioral domains, such as cognitive flexibility or reward sensitivity, which cut across traditional diagnostic categories and can differ from person to person.

Advances in approaches for determining cellular plasticity

In the brain, 5-HT receptors are expressed on neurons. When psychedelics activate these receptors, they initiate molecular signaling cascades that alter neuronal physiology. A consistently observed effect across multiple studies is that psychedelics and other rapid-acting psychiatric interventions promote **structural plasticity** [80]. Increases in the number of **dendritic spines** indicate more synaptic connections between excitatory neurons, while increases in the size of dendritic spines reflect the strengthening of excitatory connections. By facilitating the growth of excitatory connections, psychedelics may help reverse the synapse loss associated with neuropsychiatric disorders such as major depressive disorder [80,81]. Given the potential relevance of neural plasticity in the therapeutic process, a key objective in psychedelic drug discovery is to identify compounds that reliably and robustly enhance dendritic spine growth or other forms of neural plasticity, preferably in disorder-affected circuits in native tissue.

Measuring structural plasticity *in vitro* and *in vivo*

In cultured neurons, psychedelics promote dendritic branch elongation and synapse formation [82], which can be quantified using Scholl analysis and immunostaining of synaptic proteins, respectively. A major advantage of *in vitro* systems is that the response to psychedelics can be studied in neurons derived from induced pluripotent stem cells (iPSC) [83], which contain human 5-HT receptors and may be sourced from patients to study disease-specific effects. The approach is well suited toward efficient testing of many compounds. However, neuronal cultures lack key components of the nervous system such as serotonergic neuromodulation and neurovascular interactions, which likely contribute to the overall therapeutic action of psychedelics. Some structural changes observed *in vitro*, such as the outgrowth of a new dendritic branch after psychedelic exposure, are unlikely to occur under physiological conditions *in vivo*. Many drugs beyond psychedelics can induce structural neural plasticity *in vitro* [84]. Another potential issue is that many culture media contain serum with micromolar levels of serotonin [85], which could desensitize 5-HT receptors and confound experimental results.

The formation of new dendritic spines has been detected after administering psychedelics to live animals, including psilocybin [70,86], 5-MeO-DMT [62], DOI [14,87], and non-hallucinogenic 5-HT_{2A} receptor agonists [14,88]. Longitudinal two-photon imaging enables the same set of dendritic spines to be monitored across a time course of weeks and in different excitatory cell types [70]. The repeated measurements allow for comparisons of dendritic spines before versus after a drug or before versus after a disease-related manipulation. The within-subject design for *in vivo* imaging is powerful because spine density on a dendritic segment can vary widely, so *ex vivo* comparisons between different cohorts of animals require a large sample size to achieve

the same statistical power (Cohen's d was 3.5 larger for *in vivo* relative to *ex vivo* measurements in [86]). A limitation of two-photon microscopy is the spatial resolution, which cannot clearly resolve the size and shape of dendritic spines (i.e., mushroom vs. thin spines or filipodia). Another limitation is throughput, because chronic imaging and spine counting analyses are time consuming, although advances in machine learning may soon automate the image analysis step. Despite these challenges, *in vivo* imaging of dendritic spines is a valuable tool for assessing the plasticity potential of a new compound in native tissues.

Additional readouts of psychedelic-evoked neural plasticity

Besides structural remodeling, synapses can undergo other forms of neural plasticity. For instance, psilocybin can induce lasting potentiation of synaptic strength in the hippocampus [89]. In other brain regions, psychedelics such as LSD and psilocybin may not modify synaptic strength directly, but instead evoke **metaplasticity** [90]. These effects of psychedelics on **functional plasticity** and metaplasticity can be demonstrated using patch-clamp electrophysiology in brain slices. While the approach provides mechanistic insights, its low throughput makes it less suitable for large-scale drug screening. In the future, tools such as genetically encoded fluorescent probes, including 5-HT_{2A} receptor-specific sensors [17], may enable cell type- and circuit-specific characterization of psychedelic-evoked neural plasticity mechanisms.

Gene expression analysis provides another window into neural plasticity. Synaptic modifications require the synthesis of new proteins, which usually involve immediate early genes. One widely studied immediate early gene is c-Fos, whose expression can be mapping at cellular resolution across the entire mouse brain using whole-tissue clearing and light sheet fluorescence microscopy. This approach has been applied to study ketamine and psilocybin, revealing select brain regions activated by both or either of the drugs [91,92]. These whole-brain datasets are useful for exploratory analyses, as they can be compared with other publicly available transcriptional atlases to ask whether the expression level of any gene in the mouse genome predicts drug-evoked c-Fos activation [91]. Brain-wide c-Fos expression maps may be used to compare an unknown test compound to known psychedelics and related psychoactive drugs [53]. However, c-Fos expression is influenced by many factors beyond direct drug action [93], and the various immediate early genes may respond differently to psychedelics [94]. Tagging neural ensembles based on intracellular calcium elevation may be a better approach and has been applied to identify psilocybin-activated neurons [95]. A more comprehensive and unbiased approach to quantify gene expression after psychedelic administration is single-cell transcriptomics [71,96]. Currently, these studies of psychedelics have demonstrated sample sizes of ~50 brains [53,96], with cost as a limiting factor. As technologies advance, whole-brain mapping and sequencing approaches are expected to become increasingly scalable and feasible for use in psychedelic drug discovery.

Emerging techniques to improve translation

Translating findings from animals to humans has historically had a low success rate, but emerging tools offer new readouts that may improve the odds for psychedelic drug discovery. Preclinical evidence implicates the 5-HT_{2A} receptor as a key target mediating the therapeutic effects of psychedelics [70,87,97] (but see, e.g., [89]). If the 5-HT_{2A} receptor is indeed the critical target, it would be sensible to obtain early proof-of-concept data for target engagement in humans before committing to large clinical trials. Positron emission tomography (PET) imaging offers a powerful approach to assess *in vivo* 5-HT_{2A} receptor occupancy, which can apply to both humans [46] and rodents [98]. Relatedly, synaptic density can now be quantified in humans using synaptic vesicle glycoprotein 2A (SV2A) radioligands [81]. These measurements provide translational counterparts to the preclinical binding affinity and structural plasticity assays.

In vivo electrophysiology is poised to play a larger role in preclinical drug discovery. Recent advances, such as high-density Neuropixels electrodes, have made it possible to record the neural ensemble activity in the mouse brain during psychedelic exposure [70,99]. Such results may lead to more translational biomarkers like field potential signatures [100], which can be applied to humans to distinguish drug-evoked altered states [101], potentially as part of clinical trials. Another area of opportunity is in assessing the hallucinogenic potential of psychedelic compounds. Large-scale recordings in the visual cortex may be used to detect DOI-induced disruptions in perceptual processing [102]. A non-hallucinogenic analog would be expected to lack such neural activity changes, whether in animals passively viewing visual stimuli or actively performing tasks designed to probe perceptual distortions [103]. These electrophysiological and behavioral approaches offer a more translationally relevant method for identifying non-hallucinogenic 5-HT_{2A} receptor agonists.

Concluding remarks and future perspectives

Although individual assays have their own methodological limitations, together the approaches hold promise for linking receptor pharmacology to functional outcomes. Psilocybin and LSD currently lead clinical efforts as the prototypical psychedelics [1,2,4], with extensive data spanning the three domains discussed: (A) agonism, (B) behavior, and (C) cellular plasticity. These classic psychedelics exhibit a polypharmacological profile, engage both Gq and β-arrestin signaling, promote structural neural plasticity, and cause various acute and long-term behavioral effects in animal models. For the next generation of psychedelic-inspired compounds, an expanding palette has emerged, with some candidates progressing through early-stage clinical trials and several entering Phase 2 studies where their therapeutic efficacy will be determined. These developments mark an exciting era for drug discovery and underscore the value of an integrated preclinical approach. To advance preclinical assays, an important next step will be to bridge the domains. Indeed, studies have begun to systematically delineate the relationship between a compound's molecular mechanism of action and its neural and behavioral effects, showing how biased agonism relates to head-twitch response [18] and how ligand lipophilicity relates to structural plasticity [104]. These efforts exemplify how pharmacological profiling may help identify new psychedelic-inspired compounds with improved safety and efficacy.

In summary, we outline a framework for psychedelic drug discovery organized around three domains: agonism, behavior, and cellular plasticity. Success will hinge on integrating diverse assays across these domains, because no single method can capture the full complexity of psychedelic drug effects. Instead, the strengths and limitations of each assay highlight the value of using multiple orthogonal methods to ensure rigor, reproducibility, and translational relevance (see Outstanding questions). There is optimism that with ongoing advances in high-throughput pharmacological profiling, behavioral phenotyping, cellular-resolution and brain-wide optical imaging, and transcriptomic technologies, the field will be well positioned to discover novel psychedelic-inspired compounds with therapeutic potential.

Outstanding questions

What is the desired selectivity profile for an ideal psychedelic-based or non-hallucinogenic therapeutic?

What biological and genetic factors underlie individual variability in response to psychedelic drugs?

What molecular signaling pathways connect receptor activation to neural plasticity and therapeutic outcomes?

Is structural neural plasticity necessary for the long-term behavioral effects of psychedelics?

What are the cell types and circuits involved that distinguish a psychedelic's neural plasticity from maladaptive remodeling?

To what extent do downstream gene expression changes sustain the behavioral effects of psychedelics?

Can non-hallucinogenic 5-HT_{2A} receptor agonists, designed to avoid the acute 'trip', still deliver durable benefits for mental health conditions such as anxiety and depression?

Do psychedelics and related compounds (e.g., ketamine and MDMA) share common mechanisms of action for producing long-term behavioral effects?

Which behavioral assays in animals will provide the best model for the acute and long-term behavioral effects observed in humans?

Acknowledgments

This work was supported by National Institutes of Health (NIH) grants R01MH121848 (A.C.K.), R01MH128217 (A.C.K.), R01MH137047 (A.C.K.), R01DA059194 (J.R.M.), R01MH136708 (J.R.M.), R01DA052169 (J.R.M.), R35GM133421 (J.D.M), R01MH133849 (J.D.M), and R01DA061433 (J.D.M), and a One Mind – COMPASS Rising Star Award (A.C.K.).

Declaration of interests

A.C.K. has been a scientific advisor or consultant for Boehringer Ingelheim, Eli Lilly, Empyrean Neuroscience, Freedom Biosciences, and Xylo Bio. A.C.K. has received research support from Intra-Cellular Therapies. J.R.M. is a co-founder of Promentis Pharmaceuticals. The other authors report no financial relationships with commercial interests.

References

1. Goodwin, G.M. *et al.* (2022) Single-dose psilocybin for a treatment-resistant episode of major depression. *N. Engl. J. Med.* 387, 1637–1648
2. Raison, C.L. *et al.* (2023) Single-dose psilocybin treatment for major depressive disorder: a randomized clinical trial. *JAMA* 330, 843–853
3. Bogenschutz, M.P. *et al.* (2022) Percentage of heavy drinking days following psilocybin-assisted psychotherapy vs placebo in the treatment of adult patients with alcohol use disorder: a randomized clinical trial. *JAMA Psychiatry* 79, 953–962
4. Holze, F. *et al.* (2023) Lysergic acid diethylamide-assisted therapy in patients with anxiety with and without a life-threatening illness: a randomized, double-blind, placebo-controlled Phase II study. *Biol. Psychiatry* 93, 215–223
5. Vane, J.R. (1959) The relative activities of some tryptamine analogues on the isolated rat stomach strip preparation. *Br. J. Pharmacol. Chemother.* 14, 87–98
6. Osmond, H. (1957) A review of the clinical effects of psychotomimetic agents. *Ann. N. Y. Acad. Sci.* 66, 418–434
7. Studerus, E. *et al.* (2010) Psychometric evaluation of the altered states of consciousness rating scale (OAV). *PLoS One* 5, e12412
8. Shulgin, A. and Shulgin, A. (1990) *PIHKAL: A Chemical Love Story*, Transform Press
9. Shulgin, A. and Shulgin, A. (2002) *TiHKAL: The Continuation*, Transform Press
10. Kwan, A.C. *et al.* (2022) The neural basis of psychedelic action. *Nat. Neurosci.* 25, 1407–1419
11. McClure-Begley, T.D. and Roth, B.L. (2022) The promises and perils of psychedelic pharmacology for psychiatry. *Nat. Rev. Drug Discov.* 21, 463–473
12. Olson, D.E. (2018) Psychoplastogens: a promising class of plasticity-promoting neurotherapeutics. *J. Exp. Neurosci.* 12, 1179069518800508
13. Cunningham, M.J. *et al.* (2023) Pharmacological mechanism of the non-hallucinogenic 5-HT(2A) agonist Ariadne and analogs. *ACS Chem. Neurosci.* 14, 119–135
14. Cameron, L.P. *et al.* (2021) A non-hallucinogenic psychedelic analogue with therapeutic potential. *Nature* 589, 474–479
15. Cao, D. *et al.* (2022) Structure-based discovery of nonhallucinogenic psychedelic analogs. *Science* 375, 403–411
16. Kaplan, A.L. *et al.* (2022) Bespoke library docking for 5-HT(2A) receptor agonists with antidepressant activity. *Nature* 610, 582–591
17. Dong, C. *et al.* (2021) Psychedelic-inspired drug discovery using an engineered biosensor. *Cell* 184, 2779–2792.e2718
18. Wallach, J. *et al.* (2023) Identification of 5-HT(2A) receptor signaling pathways associated with psychedelic potential. *Nat. Commun.* 14, 8221
19. Glatfelter, G.C. *et al.* (2024) Comparative pharmacological effects of lisuride and lysergic acid diethylamide revisited. *ACS Pharmacol. Transl. Sci.* 7, 641–653
20. Vollenweider, F.X. *et al.* (1998) Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport* 9, 3897–3902
21. Becker, A.M. *et al.* (2023) Ketanserin reverses the acute response to LSD in a randomized, double-blind, placebo-controlled, crossover study in healthy participants. *Int. J. Neuropsychopharmacol.* 26, 97–106
22. Lewis, V. *et al.* (2023) A non-hallucinogenic LSD analog with therapeutic potential for mood disorders. *Cell Rep.* 42, 112203
23. Cameron, L.P. *et al.* (2023) Beyond the 5-HT(2A) receptor: classic and nonclassic targets in psychedelic drug action. *J. Neurosci.* 43, 7472–7482
24. McIntyre, R.S. (2023) Serotonin 5-HT(2B) receptor agonism and valvular heart disease: implications for the development of psilocybin and related agents. *Expert Opin. Drug Saf.* 22, 881–883
25. Glatfelter, G.C. *et al.* (2024) Serotonin 1A receptors modulate serotonin 2A receptor-mediated behavioral effects of 5-methoxy-N,N-dimethyltryptamine analogs in mice. *ACS Chem. Neurosci.* 15, 4458–4477
26. Warren, A.L. *et al.* (2024) Structural pharmacology and therapeutic potential of 5-methoxytryptamines. *Nature* 630, 237–246
27. Luethi, D. and Liechti, M.E. (2018) Monoamine transporter and receptor interaction profiles *in vitro* predict reported human doses of novel psychoactive stimulants and psychedelics. *Int. J. Neuropsychopharmacol.* 21, 926–931
28. Nussinov, R. *et al.* (2023) Protein conformational ensembles in function: roles and mechanisms. *RSC Chem. Biol.* 4, 850–864
29. Wacker, D. *et al.* (2017) Crystal structure of an LSD-bound human serotonin receptor. *Cell* 168, 377–389.e312
30. Holze, F. *et al.* (2024) Pharmacokinetics, pharmacodynamics and urinary recovery of oral lysergic acid diethylamide administration in healthy participants. *Br. J. Clin. Pharmacol.* 90, 200–208
31. Odagaki, Y. *et al.* (2017) Functional activation of Galpha (q) coupled to 5-HT(2A) receptor and M(1) muscarinic acetylcholine receptor in postmortem human cortical membranes. *J. Neural Transm. (Vienna)* 124, 1123–1133
32. Nehme, R. *et al.* (2017) Mini-G proteins: novel tools for studying GPCRs in their active conformation. *PLoS One* 12, e0175642
33. Kim, K. *et al.* (2020) Structure of a hallucinogen-activated Gq-coupled 5-HT2A serotonin receptor. *Cell* 182, 1574–1588.e1519
34. Olsen, R.H.J. *et al.* (2020) TRUPATH, an open-source biosensor platform for interrogating the GPCR transducerome. *Nat. Chem. Biol.* 16, 841–849
35. Avet, C. *et al.* (2022) Effector membrane translocation biosensors reveal G protein and betaarrestin coupling profiles of 100 therapeutically relevant GPCRs. *eLife* 11, e74101
36. Namkung, Y. *et al.* (2016) Monitoring G protein-coupled receptor and beta-arrestin trafficking in live cells using enhanced bystander BRET. *Nat. Commun.* 7, 12178
37. Masuho, I. *et al.* (2015) Distinct profiles of functional discrimination among G proteins determine the actions of G protein-coupled receptors. *Sci. Signal.* 8, ra123
38. Olsen, R.H.J. and English, J.G. (2023) Advancements in G protein-coupled receptor biosensors to study GPCR-G protein coupling. *Br. J. Pharmacol.* 180, 1433–1443
39. Kroeze, W.K. *et al.* (2015) PRESTO-Tango as an open-source resource for interrogation of the druggable human GPCRome. *Nat. Struct. Mol. Biol.* 22, 362–369
40. Pottie, E. *et al.* (2020) Identification of psychedelic new psychoactive substances (NPS) showing biased agonism at the 5-HT (2A)R through simultaneous use of beta-arrestin 2 and miniGalpha(q) bioassays. *Biochem. Pharmacol.* 182, 114251
41. Blough, B.E. *et al.* (2014) Interaction of psychoactive tryptamines with biogenic amine transporters and serotonin receptor subtypes. *Psychopharmacology* 231, 4135–4144
42. Kenakin, T. (2019) Biased receptor signaling in drug discovery. *Pharmacol. Rev.* 71, 267–315
43. Kolb, P. *et al.* (2022) Community guidelines for GPCR ligand bias: IUPHAR review 32. *Br. J. Pharmacol.* 179, 3651–3674
44. Klein Herenbrink, C. *et al.* (2016) The role of kinetic context in apparent biased agonism at GPCRs. *Nat. Commun.* 7, 10842
45. Wright, S.C. *et al.* (2024) Conformation- and activation-based BRET sensors differentially report on GPCR-G protein coupling. *Sci. Signal.* 17, eadi4747
46. Madsen, M.K. *et al.* (2019) Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels. *Neuropsychopharmacology* 44, 1328–1334
47. Ley, L. *et al.* (2023) Comparative acute effects of mescaline, lysergic acid diethylamide, and psilocybin in a randomized, double-blind, placebo-controlled cross-over study in healthy participants. *Neuropsychopharmacology* 48, 1659–1667
48. Holze, F. *et al.* (2023) Pharmacokinetics and pharmacodynamics of oral psilocybin administration in healthy participants. *Clin. Pharmacol. Ther.* 113, 822–831
49. Yaden, D.B. and Griffiths, R.R. (2021) The subjective effects of psychedelics are necessary for their enduring therapeutic effects. *ACS Pharmacol. Transl. Sci.* 4, 568–572
50. Olson, D.E. (2021) The subjective effects of psychedelics may not be necessary for their enduring therapeutic effects. *ACS Pharmacol. Transl. Sci.* 4, 563–567
51. Bryson, N. *et al.* (2024) RE104: synthesis and activity of a novel serotonergic psychedelic prodrug of 4-hydroxy-N,N-diisopropyltryptamine. *ACS Chem. Neurosci.* 15, 2386–2395

52. Halberstadt, A.L. *et al.* (2020) Correlation between the potency of hallucinogens in the mouse head-twitch response assay and their behavioral and subjective effects in other species. *Neuropharmacology* 167, 107933
53. Aboharb, F. *et al.* (2025) Classification of psychedelics and psychoactive drugs based on brain-wide imaging of cellular c-Fos expression. *Nat. Commun.* 16, 1590
54. Canal, C.E. and Morgan, D. (2012) Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: a comprehensive history, a re-evaluation of mechanisms, and its utility as a model. *Drug Test Anal.* 4, 556–576
55. Li, J.X. *et al.* (2008) Discriminative stimulus effects of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane in rhesus monkeys. *J. Pharmacol. Exp. Ther.* 324, 827–833
56. Appel, J.B. *et al.* (2004) LSD, 5-HT (serotonin), and the evolution of a behavioral assay. *Neurosci. Biobehav. Rev.* 27, 693–701
57. Halberstadt, A.L. and Geyer, M.A. (2010) LSD but not lisuride disrupts prepulse inhibition in rats by activating the 5-HT(2A) receptor. *Psychopharmacology* 208, 179–189
58. Quednow, B.B. *et al.* (2012) Psilocybin-induced deficits in automatic and controlled inhibition are attenuated by ketanserin in healthy human volunteers. *Neuropsychopharmacology* 37, 630–640
59. Schmid, Y. *et al.* (2015) Acute effects of lysergic acid diethylamide in healthy subjects. *Biol. Psychiatry* 78, 544–553
60. Ali, F. *et al.* (2020) Ketamine disinhibits dendrites and enhances calcium signals in prefrontal dendritic spines. *Nat. Commun.* 11, 72
61. Glatfelter, G.C. *et al.* (2022) Structure-activity relationships for psilocybin, baeocystin, aeruginascin, and related analogues to produce pharmacological effects in mice. *ACS Pharmacol. Transl. Sci.* 5, 1181–1196
62. Jefferson, S.J. *et al.* (2023) 5-MeO-DMT modifies innate behaviors and promotes structural neural plasticity in mice. *Neuropsychopharmacology* 48, 1257–1266
63. Pereira, T.D. *et al.* (2020) Quantifying behavior to understand the brain. *Nat. Neurosci.* 23, 1537–1549
64. Goodwin, N.L. *et al.* (2024) Simple behavioral analysis (SimBA) as a platform for explainable machine learning in behavioral neuroscience. *Nat. Neurosci.* 27, 1411–1424
65. Tillmann, J.F. *et al.* (2024) A-SOiD, an active-learning platform for expert-guided, data-efficient discovery of behavior. *Nat. Methods* 21, 703–711
66. Wiltschko, A.B. *et al.* (2020) Revealing the structure of pharmacobehavioral space through motion sequencing. *Nat. Neurosci.* 23, 1433–1443
67. Shao, L. *et al.* (2016) *In vivo* phenotypic drug discovery: applying a behavioral assay to the discovery and optimization of novel antipsychotic agents. *MedChemComm* 7, 1093–1101
68. Torrado Pacheco, A. *et al.* (2023) Acute psilocybin enhances cognitive flexibility in rats. *Neuropsychopharmacology* 48, 1011–1020
69. Conn, K. *et al.* (2024) Psilocybin restrains activity-based anorexia in female rats by enhancing cognitive flexibility: contributions from 5-HT1A and 5-HT2A receptor mechanisms. *Mol. Psychiatry* 29, 3291–3304
70. Shao, L.X. *et al.* (2025) Psilocybin's lasting action requires pyramidal cell types and 5-HT2A receptors. *Nature* 642, 411–420
71. Muir, J. *et al.* (2024) Isolation of psychedelic-responsive neurons underlying anxiolytic behavioral states. *Science* 386, 802–810
72. Shore, R. *et al.* (2024) Behavioural investigations of psilocybin in animals 1962-2021: a scoping review. *bioRxiv* Published online January 05, 2024. https://doi.org/10.1101/2024.01.04.574146
73. Gyles, T.M. *et al.* (2023) Advancing preclinical chronic stress models to promote therapeutic discovery for human stress disorders. *Neuropsychopharmacology* 49, 215–226
74. Lu, O.D. *et al.* (2025) A multi-institutional investigation of psilocybin's effects on mouse behavior. *bioRxiv* Published online April 09, 2025. https://doi.org/10.1101/2025.04.08.647810
75. Woodburn, S.C. *et al.* (2024) Psilocybin facilitates fear extinction: importance of dose, context, and serotonin receptors. *ACS Chem. Neurosci.* 15, 3034–3043
76. Farinha-Ferreira, M. *et al.* (2025) Concurrent stress modulates the acute and post-acute effects of psilocybin in a sex-dependent manner. *Neuropharmacology* 266, 110280
77. Jefsen, O.H. *et al.* (2021) Transcriptional regulation in the rat prefrontal cortex and hippocampus after a single administration of psilocybin. *J. Psychopharmacol.* 35, 483–493
78. Chourbaji, S. *et al.* (2005) Learned helplessness: validity and reliability of depressive-like states in mice. *Brain Res. Brain Res. Protoc.* 16, 70–78
79. Aleksandrova, L.R. *et al.* (2019) Evaluation of the Wistar-Kyoto rat model of depression and the role of synaptic plasticity in depression and antidepressant response. *Neurosci. Biobehav. Rev.* 105, 1–23
80. Liao, C. *et al.* (2025) Structural neural plasticity evoked by rapid-acting antidepressant interventions. *Nat. Rev. Neurosci.* 26, 101–114
81. Holmes, S.E. *et al.* (2019) Lower synaptic density is associated with depression severity and network alterations. *Nat. Commun.* 10, 1529
82. Ly, C. *et al.* (2018) Psychedelics promote structural and functional neural plasticity. *Cell Rep.* 23, 3170–3182
83. Schmidt, M. *et al.* (2025) Psilocin fosters neuroplasticity in iPSC-derived human cortical neurons. *eLife* 14 RP104006
84. Calabrese, E.J. (2008) Enhancing and regulating neurite outgrowth. *Crit. Rev. Toxicol.* 38, 391–418
85. Mothersill, C. *et al.* (2010) Serum serotonin levels determine the magnitude and type of bystander effects in medium transfer experiments. *Radiat. Res.* 174, 119–123
86. Shao, L.X. *et al.* (2021) Psilocybin induces rapid and persistent growth of dendritic spines in frontal corte *in vivo*. *Neuron* 109, 2535–2544.e2534
87. de la Fuente Revenga, M. *et al.* (2021) Prolonged epigenomic and synaptic plasticity alterations following single exposure to a psychedelic in mice. *Cell Rep.* 37, 109836
88. Lu, J. *et al.* (2021) An analog of psychedelics restores functional neural circuits disrupted by unpredictable stress. *Mol. Psychiatry* 26, 6237–6252
89. Hesselgrave, N. *et al.* (2021) Harnessing psilocybin: antidepressant-like behavioral and synaptic actions of psilocybin are independent of 5-HT2R activation in mice. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2022489118
90. Nardou, R. *et al.* (2023) Psychedelics reopen the social reward learning critical period. *Nature* 618, 790–798
91. Davoudian, P.A. *et al.* (2023) Shared and distinct brain regions targeted for immediate early gene expression by ketamine and psilocybin. *ACS Chem. Neurosci.* 14, 468–480
92. Rijsketic, D.R. *et al.* (2023) UNRAVELing the synergistic effects of psilocybin and environment on brain-wide immediate early gene expression in mice. *Neuropsychopharmacology* 48, 1798–1807
93. McReynolds, J.R. *et al.* (2018) What does the Fos say? Using Fos-based approaches to understand the contribution of stress to substance use disorders. *Neurobiol. Stress* 9, 271–285
94. Gonzalez-Maeso, J. *et al.* (2007) Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron* 53, 439–452
95. Zhang, R. *et al.* (2024) Rapid, biochemical tagging of cellular activity history *in vivo*. *Nat. Methods* 21, 1725–1735
96. Liao, C. *et al.* (2025) Single-nucleus transcriptomics reveals time-dependent and cell-type-specific effects of psilocybin on gene expression. *bioRxiv* Published online January 04, 2025. https://doi.org/10.1101/2025.01.04.631335
97. Cameron, L.P. *et al.* (2023) 5-HT2ARs mediate therapeutic behavioral effects of psychedelic tryptamines. *ACS Chem. Neurosci.* 14, 351–358
98. Kiilerich, K.F. *et al.* (2023) Repeated low doses of psilocybin increase resilience to stress, lower compulsive actions, and strengthen cortical connections to the paraventricular thalamic nucleus in rats. *Mol. Psychiatry* 28, 3829–3841
99. Tiwari, P. *et al.* (2024) Ventral hippocampal parvalbumin interneurons gate the acute anxiolytic action of the serotonergic psychedelic DOI. *Neuron* 112, 3697–3714.e3696
100. Wood, J. *et al.* (2012) Disruption of prefrontal cortex large scale neuronal activity by different classes of psychotomimetic drugs. *J. Neurosci.* 32, 3022–3031
101. Vesuna, S. *et al.* (2020) Deep posteromedial cortical rhythm in dissociation. *Nature* 586, 87–94

102. Michaiel, A.M. *et al.* (2019) A hallucinogenic serotonin-2A receptor agonist reduces visual response gain and alters temporal dynamics in mouse V1. *Cell Rep.* 26, 3475–3483.e3474
103. Schmack, K. *et al.* (2021) Striatal dopamine mediates hallucination-like perception in mice. *Science* 372, eabf4740
104. Vargas, M.V. *et al.* (2023) Psychedelics promote neuroplasticity through the activation of intracellular 5-HT2A receptors. *Science* 379, 700–706
105. Strachan, R.T. *et al.* (2014) Divergent transducer-specific molecular efficacies generate biased agonism at a G protein-coupled receptor (GPCR). *J. Biol. Chem.* 289, 14211–14224
106. Guo, S. *et al.* (2022) Recent progress in assays for GPCR drug discovery. *Am. J. Physiol. Cell Physiol.* 323, C583–C594
107. Charlton, S.J. and Vauquelin, G. (2010) Elusive equilibrium: the challenge of interpreting receptor pharmacology using calcium assays. *Br. J. Pharmacol.* 161, 1250–1265
108. Bdioui, S. *et al.* (2018) Equilibrium assays are required to accurately characterize the activity profiles of drugs modulating Gq-protein-coupled receptors. *Mol. Pharmacol.* 94, 992–1006
109. UniProt, C. (2025) UniProt: the Universal Protein Knowledgebase in 2025. *Nucleic Acids Res.* 53, D609–D617
110. Johnson, M.P. *et al.* (1994) Species variations in transmembrane region V of the 5-hydroxytryptamine type 2A receptor alter the structure-activity relationship of certain ergolines and tryptamines. *Mol. Pharmacol.* 45, 277–286
111. Syvanen, S. *et al.* (2009) Species differences in blood-brain barrier transport of three positron emission tomography radioligands with emphasis on P-glycoprotein transport. *Drug Metab. Dispos.* 37, 635–643
112. Tosh, D.K. *et al.* (2024) Potent and selective human 5-HT(2B) serotonin receptor antagonists: 4'-cyano-(N)-methanocarbaadenosines by synthetic serendipity. *J. Med. Chem.* 67, 21264–21291
113. Oksenberg, D. *et al.* (1992) A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT1B receptors. *Nature* 360, 161–163
114. Zajdel, P. *et al.* (2024) Simultaneous 5-HT1BR agonist/5-HT6R antagonist action as a potential treatment of Parkinson's disease and its comorbidities. *J. Pharmacol. Exp. Ther.* 392, 100055
115. Zilberg, G. *et al.* (2024) Structural insights into the unexpected agonism of tetracyclic antidepressants at serotonin receptors 5-HT1eR and 5-HT1FR. *Sci. Adv.* 10, eadk4855
116. Nair, A.B. and Jacob, S. (2016) A simple practice guide for dose conversion between animals and human. *J. Basic Clin. Pharm.* 7, 27–31