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The role of modern neuroscience in the renaissance of psychedelic research

El rol de la neurociencia moderna en el renacimiento de la investigación psicodélica

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Abstract

The primary objective of this review is to highlight the increasing relevance of experimental neuroscience in advancing the mechanistic understanding and translational potential of classic psychedelics and other psychoactive substances of interest. First, we review clinical evidence for the therapeutic potential of psychedelics in the context of depression, anxiety, and addiction. Second, we review recent basic neuroscientific findings regarding the molecular and neural circuit bases of psychedelic action, with a focus on contributions from systems neuroscience. We survey recent advances in experimental neuroscience techniques that are well-poised to advance our mechanistic understanding of psychedelics by identifying the specific brain circuits that contribute to distinct aspects of the psychedelic experience. We end by noting that many promising psychoactive substances are woefully understudied in comparison to the more popular psychedelics. Studying their effects on neural circuits and behavior could be a fruitful and financially feasible direction for burgeoning neuroscience research institutions.

Keywords: psychedelic, psilocybin, serotonin, systems neuroscience, translational neuroscience, basic neuroscience

Resumen

El objetivo de esta revisión es resaltar el rol de la neurociencia experimental en el avance del entendimiento mecanístico y traslacional de los psicodélicos clásicos y otras sustancias psicoactivas de interés. Resumimos los estudios clínicos que han establecido el potencial de los psicodélicos para el tratamiento de varios trastornos neuropsiquiátricos incluyendo el abuso de sustancias, trastorno de estrés postraumático, entre otros. Nos enfocamos en descubrimientos neurocientíficos básicos que empiezan a delinear los circuitos neuronales que promueven el efecto psicodélico y discutimos los avances metodológicos en el subcampo de neurociencia de sistemas que han permitido tales avances. Concluimos por mencionar que muchas clases de sustancias psicoactivas prometedoras han sido

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lamentablemente poco estudiadas en comparación con los psicodélicos más populares y sugerimos que estudiar sus efectos sobre los circuitos neuronales y el comportamiento podría ser un nicho fructífero y económicamente viable para las florecientes instituciones de investigación en neurociencia y ciencias biomédicas en general de países menos establecidos.

Palabras clave: **psicodelia, psilocibina, neurociencia de sistemas, neurociencia traslacional, neurociencia básica.**

Brief background on psychedelics

Psychedelics are a class of psychoactive substances known for their profound effects on consciousness. Classic psychedelics all act as agonists of the serotonin 2A receptor (5-HT2AR), and include lysergic acid diethylamide (LSD), psilocybin (from the mushroom genus *Psilocybe*), dimethyltryptamine (DMT), ayahuasca (a combination of plants containing DMT and monoamine oxidase inhibitors that prevent gastrointestinal metabolism), 5-Methoxy-N,N-dimethyltryptamine (5-MeO-DMT, from the Sonora desert toad), and mescaline (from the peyote cactus). Non-classic psychedelics have diverse pharmacological mechanisms, while sharing some of the experiential effects of the classic psychedelics. Non-classic psychedelics include drugs such as ketamine, 3,4-methylenedioxymethamphetamine (MDMA), and natural compounds such as *Salvia divinorum* and ibogaine (from Tabernanthe iboga).

Psychedelics have been used for centuries in various cultures and religions for spiritual, therapeutic, medicinal, and recreational purposes. Their profound ability to produce intense subjective experiences and produce long-lasting alterations of sensory processing and cognition after a single dose has led to a long-standing interest in using them as tools to probe the human mind (Andersen et al., 2021; Goodwin et al., 2022; Knudsen, n.d.; Millière et al., 2018; Nichols, 2016). Acute effects can range from increased emotion, relaxation, and bodily awareness to total sensorial hallucination, out-of-body experiences, or ego dissolution (Carhart-Harris et al., 2016; Carhart-Harris & Goodwin, 2017; Millière et al., 2018; Nutt et al., 2020; Swanson, 2018).

Psychedelics are well-known for their profound effects on cognition and perception, which span a range of subjective experiences depending on the drug, dose, setting, and individual factors. For the classic psychedelics, these experiences can be broadly categorized into four generalized states that are reliably induced by progressively higher doses. Briefly, these states are 1) The empathogenic experience: characterized by heightened emotional openness, relaxation, and vivid visual effects; 2) The out-of-body experience: encompassing bodily changes such as vivid hallucinations (usually visual), intense emotional states, and the feeling of separating from one's body; 3) The near-death experience: marked by ego dissolution, detachment from reality, and life review-like reflections; 4) The ego-dissolving transcendental experience: features complete ego dissolution and a sense of unity with the universe. These experiences are often accompanied by the certainty of their truth, and feelings of great realization.

While psychedelic research flourished in the 1950s, new laws to control the production and accessibility of psychoactive substances effectively led to a hiatus of this work from the 1970s onwards (Nichols, 2016). Most notably, the 1971 ratification of the United Nations Convention of Psychotropic Substances led to widespread restrictions on the import and export of many drugs, as well the relegation

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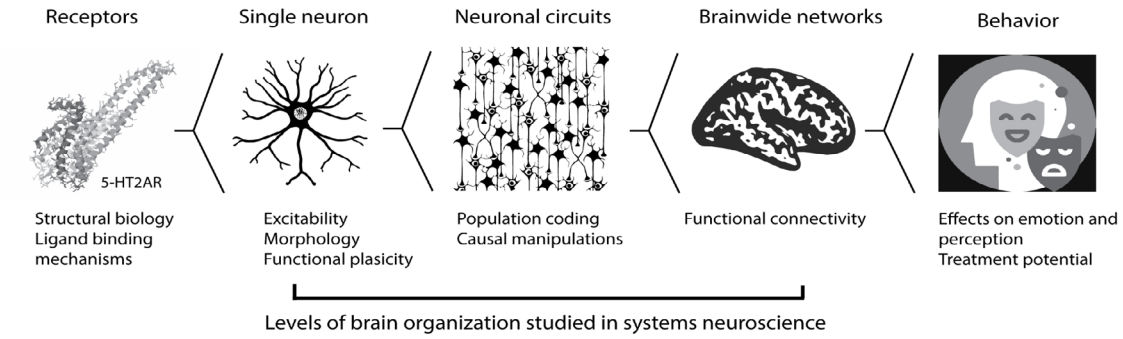
of many psychedelics to a Schedule I classification which designated them as serious risk to public health with no acknowledged therapeutic value (Khan, 1979).

The modern resurgence in psychedelic research

Recently, there has been a remarkable renewal of interest in psychedelics within both the scientific community and the public sphere. This has been in part fueled by growing evidence of the therapeutic potential of psychedelics in treating mental health disorders such as depression, addiction, anxiety, and post-traumatic stress disorder, amongst other diseases (Breeksema et al., 2020; McClure-Begley & Roth, 2022; Vollenweider & Preller, 2020; Zafar et al., 2023). Decriminalization efforts towards psychedelics have also contributed to the resurgence of psychedelic research and exploration. In particular, the U.S. Food and Drug Administration designation of psilocybin, LSD, ketamine, and MDMA as a "breakthrough therapies" for different clinical indications has led to a global funding boost for psychedelic research (Center for Drug Evaluation & Research, 2024). In the first half of this article, we review the data from clinical trials that have contributed to this recent resurgence of interest for psychedelic research. In the second half, we discuss a new generation of experimental neuroscience techniques that has begun to provide crucial insight into the effects that psychedelics have on the brain at the molecular, cellular, circuit, and network levels (see Figure 1).

Figure 1

Illustration depicting levels of organization for neuroscience studies.



The translational promise of psychedelics

Throughout human history, many cultures have prized psychedelic compounds and associated rituals as parts of healing and religious practices (Nichols, 2016). Mushrooms from the genus *Psilocybe,* which contain the psychedelic compounds psilocybin and psilocin, have been used by Mesoamerican cultures for thousands of years (Carod-Artal, 2015). Peyote (*Lophophora williansii*) a cactus containing many psychoactive chemicals such as mescaline, has been used in indigenous North American rituals as early as 3700 BCE (El-Seedi et al., 2005). Though most psychedelic compounds are legally classified by

the U.N. as having "no currently accepted medical use," many recent Phase 2 clinical trials have begun to rigorously examine the therapeutic value of psychedelic treatment in many neuropsychiatric disorders (summarized in Table 1). Below, we summarize recent clinical data regarding the translational potential of psychedelics in the management of anxiety and depression, and substance use disorders (see also (Rucker et al., 2016; Carhart-Harris & Goodwin, 2017; Nichols et al., 2017; Thomas et al., 2017; Dos Santos et al., 2018; Reiff et al., 2020; Andersen et al., 2021; Yao et al., 2024). The list of clinical trials presented in Table 1 is as comprehensive as possible based on the search criteria employed at the time of writing. However, it may not account for all ongoing or recently completed trials due to the dynamic and rapidly evolving nature of clinical research in this field.

Table 1.
Clinical trials investigating classic psychedelics in neuropsychiatric disorders (with results as of April 2024), completed studies only. Sources: International Standard Randomized Controlled Trial Number and clinicaltrials.gov. Search criteria restricted to phase 2, 3, or 4.

Publication, clinical trial registration	Clinical indication, sample size	Psychedelic treatment, study design	Study outcomes
Anxiety and depression			
(Grob et al., 2011) NCT00302744	Anxiety and depression in cancer patients, n = 12	Psilocybin, single dose, DC-RCT, crossover, niacin placebo	Significant reduction in trait anxiety at 1 and 3 months, significant reduction in depression scores at 6 months
(Gasser et al., 2014) NCT00920387	Anxiety associated with life-threatening diseases, n = 12	LSD, two doses, DB-RCT, crossover, VLD LSD control, with psychotherapy	Significant reduction in trait and state anxiety at 2 months (vs VLD control), sustained at 12 months.
(Ross et al., 2016) NCT00957359	Anxiety and depression in cancer patients, n = 29	Psilocybin, single dose, DB-RCT, crossover, niacin placebo	Significant decrease in anxiety and depression (vs niacin group, before crossover), sustained at 6.5 months
(Carhart-Harris et al., 2016) ISRCTN14426797	TRD, n = 12 (study extension to n = 20)	Psilocybin, two doses, open-label	Significant decrease in depression (from baseline) at 1 week, sustained at 6 months.
(Davis et al., 2021) NCT03181529	MDD, n = 27	Psilocybin, two doses, randomized waiting list-controlled, with psychotherapy	Significant decrease in depression metrics in immediate treatment group (vs delayed group).

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Publication, clinical trial registration	Clinical indication, sample size	Psychedelic treatment, study design	Study outcomes
Anxiety and depression			
(Carhart-Harris Robin et al., 2021) NCT03429075	MDD, n = 59	Psilocybin, two doses, DB-RCT, vs daily escitalopram, with psychological support	No significant difference between depression score of psilocybin group (vs escitalopram group) at 6 weeks.
(Goodwin et al., 2022) NCT03775200	Treatment-resistant MDD, n = 233	Synthetic psilocybin, single dose (25 vs 10mg), DB-RCT, VLD control, with psychological support	Higher dose psilocybin group reduced depression scores, but higher rates of adverse events.
(Goodwin et al., 2023) NCT04739865	TRD, n = 19	Single dose synthetic psilocybin adjunct to ongoing SSRI treatment, open label, with psychological support	Significant reduction in depression scores at 3 weeks
(Raison et al., 2023) NCT03866174	MDD, n = 104	Psilocybin, single dose, DB-RCT, niacin placebo, with psychological support	Significant reduction in depression and functional disability (vs niacin group)
Substance use disorders			
(Johnson et al., 2014) EFORMAT (Johnson et al., 2017) NCT01943994	Long term tobacco smoking, n = 15	Psilocybin, up to three doses, open-label, after four CBT sessions	7-day point prevalence abstinence: 80% at 6 months, 67% at 12 months
(Bogenschutz et al., 2015) NCT02061293	Alcohol use disorder, n = 10	Psilocybin, open label, up to two doses, with supportive motivational therapy	Significant decrease in drinking behaviors, sustained at 9 months
(Bogenschutz et al., 2022) NCT02061293	Alcohol use disorder, n = 95	Psilocybin, two doses, DB-RCT, diphenhydramine placebo, with psychotherapy	Significant decrease in percentage of heavy drinking days (vs diphenhydramine group)

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Publication, clinical trial registration	Clinical indication, sample size	Psychedelic treatment, study design	Study outcomes
Other indications			
(Moreno et al., 2006)	Obsessive compulsive disorder, n = 9	Psilocybin, up to four doses, single arm	Improvement of symptoms in all patients, most within 24 hours of treatment
(Schneier et al., 2023) NCT04656301	Body dysmorphic disorder, n = 12	Psilocybin, single dose, open label, with psychotherapy	Significant improvement of body dysmorphic disorder, sustained at 3 months.

Note. CBT: cognitive behavioral therapy; DB-RCT: double-blind randomized controlled trial; LSD: lysergic acid diethylamide; MDD: major depressive disorder; SSRI: selective serotonin reuptake inhibitor; TRD: treatment resistant depression; VLD: very low dose.

Psychedelics as treatments for anxiety and depression

Serotonergic psychedelics have demonstrated rapid and long-lasting antidepressant and anxiolytic effects, including in treatment-resistant populations. A review of over 190,000 respondents to the U.S. National Survey on Drug Use and Health (2008-2012) found that classical psychedelic use was associated with reduced odds of psychological distress and reduced odds of past-year suicidal thinking (Hendricks et al., 2015). Randomized controlled trials in patients with life-threatening illnesses have found that one or two doses of psychedelics can result in sustained reductions in patient-reported and clinician-scored metrics of anxiety and depression (Grob et al., 2011; Gasser et al., 2014; Ross et al., 2016; Griffiths et al., 2016). In a broader patient population, three recent randomized controlled trials have found that psilocybin combined with supportive psychotherapy can be efficacious in the management of major depressive disorder (MDD) (Davis et al., 2021; Goodwin et al., 2022; Raison et al., 2023). One additional study found that psilocybin was at least as effective as escitalopram, a commonly prescribed selective serotonin reuptake inhibitor (Carhart-Harris Robin et al., 2021).

However, one major study found that psilocybin treatment was associated with higher rates of suicidal ideation, self-injurious behaviors, and other adverse events (Goodwin et al., 2022). Further work will be required to determine the patient populations in whom the potential therapeutic benefit of psychedelic treatment outweighs the potential risk for adverse complications. Additional drug development efforts may help minimize the off-target effects of psychedelics while maintaining their clinical utility. For example, preclinical work has led to the development of candidate non-hallucinogenic 5-HT2AR agonists with antidepressant efficacy in mouse models, though this approach may be at odds with clinical trials that have found that the antidepressant efficacy of psilocybin was predicted by the quality of the acute psychedelic experience (Cameron et al., 2018; Cameron et al., 2021; Kaplan et al., 2022).

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Psychedelic treatments for substance use disorders

Despite being categorized as Schedule I drugs with a high potential for abuse, psychedelics have many important distinctions from prototypical drugs of abuse such as alcohol, nicotine, or opioids. Classic psychedelics have limited reinforcing effects and individuals generally report only occasional, intermittent use (Johnson et al., 2018). Early clinical trials (before the prohibitions on psychedelic research) suggested that psychedelics may even be useful in the treatment of drug addiction. A meta-analysis of six randomized controlled trials investigating the efficacy of LSD in the treatment of alcoholism (published from 1966-1970, n = 536 participants), found that a single dose of LSD significantly reduced alcohol misuse (Krebs & Johansen, 2012). A study that enrolled prisoners in Maryland who used heroin (n = 74) found that subjects who were assigned to a single-dose LSD session followed by a six-week residential therapy program were more likely to maintain abstinence from heroin use compared to subjects assigned to standard outpatient clinical therapy, at timepoints up to 12 months later (Savage & McCabe, 1973).

More recently, studies have found that psychedelic treatment can disrupt both drug-taking and patient-reported drug cravings in tobacco, opioid, and alcohol use (reviewed in (Zafar et al., 2023). An open-label study of long-term tobacco smokers (n = 15), found that up to three doses of psilocybin incorporated into a 15-week smoking-cessation protocol resulted in 7-day point prevalence abstinence of 80% at 6 months follow-up, and 67% at 12 months follow-up (Johnson et al., 2014, 2017). Though the study design precludes definitive conclusions about the contribution of psilocybin, the rates of long-term smoking abstinence observed in this pilot study are higher than the ~30% abstinence achieved with typical smoking-cessation pharmacotherapies (Cahill et al., 2013). In the management of alcohol use disorder, a randomized controlled trial (n = 95) based on previous pilot studies has found that two doses of psilocybin combined with supportive psychotherapy led to a significant decrease in heavy drinking days, when compared to a control group that received diphenhydramine and supportive psychotherapy as an active placebo (Bogenschutz et al., 2015, 2022; Nielson et al., 2018; O'Donnell et al., 2022).

The renaissance of psychedelic research is well-motivated by rapidly developing clinical and preclinical work that has begun to demonstrate the translational potential and clinical utility of psychedelic treatments across many neuropsychiatric disorders. By comparison, our basic understanding of the effects of psychedelics on the brain has lagged. In the next sections, we discuss modern experimental neuroscience techniques that have improved greatly in the last decade and that will be critical in developing a better understanding the effects of psychedelics across multiple levels of brain organization (see Figure 1). We will focus on systems neuroscience techniques that are used to study neural circuits in animal models at greater spatial and /or temporal resolution than is generally feasible in humans.

Advances in electrophysiology

Electrophysiological methods to record neural activity have become more accessible, robust, and high-throughput in the last decade (Stevenson & Kording, 2011)'£¨ . Electrophysiological recordings are usually performed by implanting microelectrodes, insulated except for one or more small recording sites. The signals from the electrodes are amplified and digitized in the range of 20–30KHz, a rate required to resolve extracellular action potential waveforms ("spikes") of duration the order of 1-2 msec

(Steinmetz et al., 2018). These methods have allowed for the recording of an ever-increasing number of neurons simultaneously, at lower costs. Moreover, these techniques do not require the expression of transgene constructs via the introduction of viral vectors, nor breeding transgenic mouse lines of a specific genotype. Applications of electrophysiology to the study of psychedelics have revealed electrical oscillations that accompany states of dissociation (Vesuna et al., 2020), fast-timescale changes in inter-areal brain synchrony (Brys et al., 2023), as well as layer-specific effects of psychedelics on cortical neuron processing (Lu et al., 2021). *In vivo* electrophysiological recordings will be key for resolving the complex and heterogenous changes that can be induced by psychedelics within the same brain region, and may help resolve long-standing paradoxical results that have been obtained using brain imaging methods with lower spatiotemporal resolution (Smausz et al., 2022).

Optical sensors of neuronal activity

An alternative to electrophysiological recordings is to use optical imaging methods that allow for high-resolution imaging of neuronal activity. One common approach is to use calcium sensors that fluoresce in response to increases in intracellular calcium concentration, which are known to increase during neuronal action potentials. These sensors can be delivered to cells via dyes, viral vectors, or integrated into the genome of animals (Grienberger & Konnerth, 2012). In contrast to more commonly used non-invasive methods such as functional magnetic resonance imaging (fMRI), or electroencephalography (EEG), optical sensors allow increased temporal and spatial resolution depending on the imaging preparation and microscopy method. Bulk signal from calcium indicators can be recorded using simpler methods such as fiber photometry (Simpson et al., 2024), but many sensors are now bright enough to be detected in single neurons, most commonly via epifluorescence or two-photon microscopy methods that allow researchers to assign signals to putative individual neurons, or even individual axons and dendritic spines (Chen et al., 2022; Chen et al., 2013; Grienberger et al., 2022; Stosiek et al., 2003; Yasuda et al., 2004). One important advantage of these methods is that the sensor can be delivered to genetically defined neuronal populations of interest through cell-type specific promoters in viral constructs, or in combination with transgenic cre-recombinase mouse lines. By using these approaches, researchers have shown that psychedelic compounds strongly influence pyramidal neurons in sensory cortices (which express high levels of 5-HT2Ars), as opposed to neighboring interneurons (Lu et al., 2021). This is only the beginning, as these techniques will enable researchers to focus on how individual neurons in a genetically defined subpopulation within a brain region are affected by psychedelic drugs, instead of relying on correlates of neural activity that are much coarser in spatial and temporal resolution.

Optical sensors to detect neuromodulators and neuropeptides

Agonism of the 5-HT2AR serotonergic receptor subtype is thought to be of particular importance for classic psychedelics because all hallucinogenic compounds exhibit high affinity for 5-HT2Ars, and blocking 5-HT2Ars abolishes many of the behavioral effects of hallucinogenic compounds in mice, rats, and humans (Fiorella et al., 1995; Ray, 2010; Roth et al., 1998; Vollenweider et al., 1998). Other catecholamines such as dopamine and noradrenaline have also been identified as important downstream targets of psychedelic drugs. For example, psychedelic compounds also display high affinity for dopamine receptors which may underlie some of their effects (Marona-Lewicka et al., 2005). Thus, it

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is critical to understand how different psychedelics affect a broad array of neuromodulators across the brain. The ability of neuroscientist to describe these effects has expanded dramatically with the recent inventions of optical sensors for dopamine, acetylcholine, norepinephrine, and serotonin (Sabatini & Tian, 2020; Wang et al., 2023). The most recent versions of these optical sensors are bright enough with sufficient affinity to detect changes in the levels of these neuromodulators in real time, *in vivo*, using low-cost recording techniques including fiber photometry. Sensors such as PsychLight (based on the 5-HT2AR receptor) (Dong et al., 2021), the dopamine sensor dLight (Patriarchi et al., 2018), and the serotonin sensor iSeroSnFR (Unger et al., 2020) have already begun to contribute to our understanding of psychedelic-related phenomena. For example, the interrogation of 5-HT2AR receptor activation using PsychLight was found to predict the hallucinogenic potential of new drug candidates (Dong et al., 2021), and dLight revealed clues as to why non-canonical psychedelics such as ketamine can induce reward, but have lower addiction liability (Brys et al., 2023).

Chemogenetics

Direct manipulations of neuronal activity allow researchers to move beyond observational research and towards causal discoveries. One of the most widely adopted strategies for manipulating neurons is chemogenetics (Campbell & Marchant, 2018; Kang et al., 2023). A commonly used chemogenetic strategy are designer receptors exclusively activated by designer drugs (DREADDS) (Roth, 2016), which are genetically engineered G protein-coupled receptors designed to be activated by a chemical actuator that is otherwise pharmacologically inert. Different types of DREADDs can be used to excite or inhibit neurons using the same experimental paradigm. First, DREADDs are expressed in a neuron population of interest (via viral vector or transgenic animal). Then, the DREADD agonist (e.g., deschloroclozapine, DCZ) (Nagai et al., 2020) is systemically injected into the animal, where it will only bind to DREADDs, and consequently only influence the activity of neurons in which DREADDs are expressed. Chemogenetic approaches have important advantages over other forms of neural activity manipulations: they do not require chronic invasive implants, they can be targeted to genetically defined subsets of neurons, the effects can be tittered based on the concentration or delivery schedule of the selected ligand, and there is no additional required hardware. Thus, chemogenetics are a relatively low-cost and high-throughput method to begin to probe the contribution of specific subsets of neurons to different aspects of the psychedelic experience.

Optogenetics

Another way to manipulate the activity of specific neuronal populations is with the use of optogenetics. Most optogenetic techniques employ light-sensitive ion channels that open or close in response to specific wavelengths of light (Deisseroth, 2015). These genetically engineered ion channels are then expressed in specific populations of neurons using viral vectors or transgenic animals, allowing researchers to control the activity of genetically defined neuronal populations through light delivered via fiber optic implants, cranial windows, or implanted lenses. Optogenetic actuators with a broad range of different light-wavelength sensitivities and ionic selectivity's have been developed, and these tools can be used to rapidly and reversibly excite (Boyden et al., 2005; Klapoetke et al., 2014) or inhibit (Mahn et al., 2018) the activity of neurons in the timescale of milliseconds. A major benefit of optogenetic techniques

is their temporal precision, which allows researchers to evaluate the contribution of specific brain regions or neuronal populations to precisely defined parts of a behavior or task. These techniques have already contributed to psychedelic-related research: optogenetic release of dopamine in the striatum produced hallucination-like behaviors in mice (Cassidy et al., 2018), and optogenetic inhibition of prefrontal cortex neurons partially blocked the prosocial effect of LSD and MDMA (Christoffel et al., 2021; Gregorio et al., 2021). Optogenetic manipulations will be of great value for determining the necessity and sufficiency of activity in different neuronal subpopulations in different aspects of the psychedelic experience.

Automated analyses of animal behavior

Though it is not possible to gauge an animal's subjective psychedelic experience, there are many informative behavioral responses to psychedelics, especially when compared to active pharmaceutical controls or after pretreatment with antagonists of interest. For example, a simple behavioral metric such as the frequency of head twitching in mice is correlated to the hallucinogenic potency of many drugs in humans (Corne & Pickering, 1967; Corne et al., 1963; Halberstadt et al., 2020; Keller & Umbreit, 1956; Osmond, 1957); Psychedelics have also been shown to induce other rodent behaviors such as backwards-walking, exploratory behavior, and pre-pulse inhibition of startle, however these have not yet been correlated with drug effects in humans (Halberstadt & Geyer, 2018).

Nonetheless, behavioral analyses of animals in the lab tend to be based on predetermined and human-observable features which may fail to capture more complex behaviors such as sequences of discrete behavioral elements or three-dimensional behaviors that require multiple camera angles. In the last decade, developments in machine learning have enabled various forms of automated video analysis and behavioral segmentation. These include software suites (many of which are open-source) for marker-less limb tracking in 2D or 3D, including during social interactions and collective behaviors (Mathis et al., 2018; Pereira et al., 2022; Wiltschko et al., 2015). Recent methods for fully automated recording and measurement of psychedelic-induced mouse behaviors have already begun to show promising results (Jaster & González-Maeso, 2023).

As a complement to these behavioral tracking tools, analysis tools for complex behaviors have also been developed, allowing researchers to identify action sequences or kinematics in an unbiased manner, independent from the need of manual behavioral scoring (Hsu & Yttri, 2021; Weinreb et al., 2023). When compared to traditional behavioral scoring, these programs offer increased throughput, efficiency, standardization, scalability, and potentially a more impartial characterization of an animal's behavioral repertoire, all at a lower cost than labor-intensive manual labeling strategies or licensed software that focuses on fewer metrics. A key strength of these new behavioral analysis tools is that they can be low-cost and tailored to the specific requirements and questions of a given study. Most of these analysis packages only require behavioral recordings acquired using commercially available cameras, and computational resources are most important when training a new model (though many pre-trained models are available as starting points (Ye et al., 2022). These behavioral pipelines can therefore be adapted by new labs with minimal financial and labor investment and are well poised to yield important insights as to the behavioral repertoire changes that characterize different types of psychedelics. Indeed, previous studies have used similar approaches to identify the behavioral signatures of neuropsychiatric

disease models and pharmacological manipulations (Abdus-Saboor et al., 2019; Wiltschko et al., 2020; Zhang et al., 2022).

Modern neuroscience and the future of psychedelic research

It remains unclear which brain regions are responsible for the subjective effects of psychedelics. Most likely, the psychedelic experience is driven by a constellation of brain regions that undergo unique and heterogeneous changes. Indeed, the same psychedelic compound can drive opposing changes in different brain regions depending on the postsynaptic expression of 5-HT receptor subtypes, the downstream targets of said brain regions, and the local connectivity of the affected area (Kwan et al., 2022). While systems neuroscience techniques such as those described above have been applied in a limited number of studies in the field of psychedelic drugs, these applications remain rare, and detailed implications are not yet fully explored. Wherever possible, we have cited existing studies to highlight these early examples. However, for many of the described methods, particularly in behavioral and network-level investigations, the manuscript aims to propose their future application to the study of psychedelics. Below, we highlight some of the findings that have emerged from the application of systems neuroscience to psychedelics.

For example, psychedelics can increase the magnitude and variability of stimulus responses in visual cortices (Michaiel et al., 2019; Rose & Horn, 1977), while driving profound inhibition in subcortical nuclei such as the dorsal raphe (McCall, 1982; Sprouse & Aghajanian, 1987; VanderMaelen et al., 1986). They can also drive mixed excitation and inhibition in the hippocampus (Domenico et al., 2021), locus coeruleus (Sprouse & Aghajanian, 1987), and thalamus (Hesselgrave et al., 2021). While paradoxical, many of these observations were made before the advent of tools such as optogenetics or optical sensors which could allow for more precise monitoring and manipulation of candidate neuronal subtypes that underlie the acute and prolonged effects of psychedelics in animal models.

Recent applications of genetically encoded calcium indicators (GECIs) and molecular neurobiology tools have already brought forward more concrete theories regarding the effect of psychedelics on perception. GECIs were used to determine that activity in the apical dendrites of a subset of layer 5 (L5) of sensory cortices in mice is related to the perception of various sensory stimuli. Intriguingly, 5-HT2ARs are highly concentrated in the apical dendrites of L5 neurons (Jakab & Goldman-Rakic, 1998; Masson et al., 2012), suggesting that receptor activation via psychedelics could increase the excitability of neurons involved in sensory perception (Ekins et al., 2023; Shao et al., 2021; Takahashi et al., 2020, 2016). Manipulating the activity of apical dendrites shifted the perceptual threshold, demonstrating that an active dendritic mechanism is causally linked to perceptual detection, though less is known about the subsequent plasticity that occurs afterwards (Takahashi et al., 2020, 2016). Thus, applying modern systems neuroscience methods to the study of psychedelics holds promise for uncovering further mechanistic insights into how the cellular and circuit actions of psychedelics are linked to cognitive variables.

Recent studies have begun to investigate such induced cortical plasticity. Psychedelics have been found to induce major changes in cortical neurons *in vitro* and *in vivo* (in rodents), inducing increases in neurite and spine count as well as augmenting function (Ly et al., 2018). This type of neuroplasticity has

been found to proceed through 5-HT2AR-mediated intracellular signaling pathways that are specific to psychedelics and no other serotonergic agonists (Vargas et al., 2023). Changes in cortical spine density are a hallmark of several neuropsychiatric diseases (Forrest et al., 2018), and the psychedelics' ability to promote cortical neuron growth highlight the potential therapeutic implications of psychedelic-induced neural plasticity in treating such diseases (Grieco et al., 2022; Kwan et al., 2022).

Conclusion

As interest in psychedelics continues to rise, other promising psychoactive compounds continue to emerge which have yet to be studied with modern neurotechnologies. These include: designer psychoplastogens – synthetic derivatives of naturally occurring psychedelics specifically designed to promote or reduce effects (Dong et al., 2021; Kargbo, 2023; Vargas et al., 2021); understudied natural psychedelics such as ibogaine and bufotenine (Gonçalves et al., 2021; Sadgrove, 2022; Uthaug et al., 2019); and modified versions of existing psychoactive compounds (Cameron et al., 2021; Cao et al., 2022; Wallach et al., 2023). Due to the recent methodological advances discussed above, systems neuroscience is now poised to provide much needed insight into the neural circuit mechanisms that are responsible for the psychedelic experience and its therapeutic potential. The increased accessibility and reduced costs of these methods further opens a path for burgeoning neuroscience institutions to join these efforts.

Authors contribution

Kathryn Evans wrote the sections related to the clinical impact of psychedelic use. Crystian Massengill wrote the sections on the neural mechanisms of action of psychedelics. Jonnathan Singh Alvarado conceived the article and wrote the sections on systems neuroscience approaches to the study of psychedelics.

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