Approaches to the discovery of psychedelic-like drugs without the hallucinogenic side effects in the preclinical setting

Fabiano V. Costa[1*], Konstantin N. Zabegalov[1], Denis B. Rosemberg[2], Konstantin A. Demin[3,4], Murilo S. de Abreu[5*], Elena V. Petersen[5] and Allan V. Kalueff [1, 3-9*]

[1]Neurobiology Program, Sirius University of Science and Technology, Sochi, Russia.

[2]Laboratory of Experimental Neuropsychobiology, Department of Biochemistry and Molecular Biology, Natural and Exact Sciences Center, Graduate Program in Biological Sciences: Toxicological Biochemistry, Federal University of Santa Maria, Santa Maria, RS, Brazil.

[3]Institute of Translational Biomedicine, Saint Petersburg State University, Saint Petersburg, Russia

[4]Almazov National Medical Research Centre, Ministry of Healthcare of Russian Federation, Saint Petersburg, Russia

[5]Moscow Institute of Physics and Technology, Moscow, Russia

[6]Ural Federal University, Ekaterinburg, Russia

[7]Scientific Research Institute of Neurosciences and Medicine, Novosibirsk, Russia

[8]Laboratory of Preclinical Bioscreening, Granov, Russian; Research Center of Radiology and Surgical Technologies, Ministry of Healthcare of Russian Federation, Pesochny, Russia

[9]COBRAIN Center, M. Heratsi Yerevan State Medical University, Yerevan, Armenia.

*Correspondence to:

Allan V Kalueff, Institute of Translational Biomedicine, St. Petersburg State University, St. Petersburg, Russia. **Email:** avkalueff@gmail.com

Fabiano V Costa, Neurobiology Program, Sirius University of Science and Technology, Sochi, Russia. **Email:** fvcosta88@gmail.com

ORCID iD

Allan V Kalueff https://orcid.org/0000-0002-7525-1950

Fabiano V Costa https://orcid.org/0000-0001-6218-9660

Denis B Rosemberg https://orcid.org/0000-0003-2538-8761

Konstantin A Demin https://orcid.org/0000-0003-0258-2801

Abstract

Introduction: Mounting clinical and preclinical evidence suggests serotonergic psychedelic drugs as a novel effective treatment of several key brain disorders, including anxiety, depression and addiction. Various animal experimental models are commonly used to understand the mechanisms of action of psychedelic drugs. Recognizing the importance of translational psychedelic research, we discuss the existing challenges in this field, and outline novel promising approaches to using animal models to probe neurotropic effects of psychedelic drugs with and without hallucinogenic properties.

Areas covered: This review summarizes shared, evolutionarily conserved psychotropic effects evoked by psychedelic drugs across various model organisms and taxa. We also discuss recent advances in using animal models to develop psychedelic drugs devoid of hallucinogenic side effects.

Expert opinion: Currently, much remains to be done to facilitate the search for safer and more effective psychedelic drugs for the treatment of psychiatric disorders. With the creation of new experimental models, using a wide range of model species and novel tools from chemical biology, as well as applying both conventional and alternative animal models, this goal may become reachable.

Keywords: Psychedelic drugs; hallucinogens; non-hallucinogenic drugs; animal models

Introduction

Psychedelic drugs uniquely and potently affect human behavior, perception and cognition [1,2]. After decades of stagnation, the renaissance of clinical and preclinical research of serotonergic psychedelics - tryptamines (*e.g*., psilocin and N,N-dimethyltryptamine, DMT), lysergamines (*e.g*., lysergic acid diethylamide, LSD) and phenethylamines (*e.g*., mescaline) **[3,4]** - paves the way towards establishing this class of drugs as effective therapies for neuropsychiatric disorders **[5,6].** For instance, psilocybin (0.3 mg/kg) reduces anxiety, depression, hopelessness, demoralization and death anxiety in patients with life-threatening cancer **[7],** LSD (200 µg) blunts anxiety associated in patients with life-threatening diseases **[8]**, and DMT (0.36 mg/kg) lowers depression in treatment-resistant depression patients **[9]**.

Beyond improving clinical symptoms per se, psychedelic drugs can also promote the growth of pyramidal cortical neurons, suggesting the possibility to treat neuronal atrophy (e.g., common in the prefrontal cortex PFC) in stress-related brain disorders **[10,11]**. However, these neuroprotective effects are associated with strong subjective effects, such as hallucinations, delirium and psychosis **[12],** some of which can be harmful and/or dangerous. Thus, the main problem with using psychedelic drugs clinically is their likely psychotogenic/hallucinogenic side effects, and the fact that it is unclear whether the latter are truly necessary for psychedelics to produce their lasting therapeutic responses **[13,14]**.

Furthermore, therapeutic effects of psychedelics seemingly correlate with their subjective effects [15,16] whose intensity depends on the serotonin 5-HT2A receptor occupancy in the brain [17]. On the one hand, it may be logical to directly link the 5-HT2A receptor activity to potential therapeutic effects of psychedelic drugs [18]. However, given the likelihood of a mere correlational link, and because psychedelics have complex pharmacology, the existence of much more intricate mechanisms of psychedelic therapeutic effects beyond their hallucinogenic properties, may be possible. Indeed, recent findings (discussed further) begin to question the beneficial effects of 5-HT2A activation and the necessity of hallucinogenic states, to promote

plastic neuroprotective changes in the brain. Collectively, this suggests a paradigm shift towards the search for putative *psychoplastogens* – a proposed novel group of psychedelic drugs that do not induce unwanted hallucinogenic, psychotic and/or dissociative effects [10].

Experimental animal models are a critical tool in translational neuroscience research **[19],** and conventional psychedelic drugs often involve clear-cut and easy-to-detect characteristic behavioral responses. For example, intraperitoneal injection of traditional psychedelics (*e.g.*, 2,5-dimethoxy-4-iodoamphetamine DOI, LSD, mescaline and psilocybin) in rodents induces a specific behavior, head-twitch response (HTR) **[20,21]**. This phenotype is particularly useful as a rodent behavioral proxy of human psychedelic action, due to its high predictive validity for a wide range of serotonergic psychedelics **[22]**.

Moreover, like in humans, well-described anxiolytic effects following psychedelic drug administration also commonly occur in rodents **[23,24]**. Besides characteristic behavioral responses, psychedelic drugs-induced physiological responses in rodents also resemble those in mammals. For example, intraperitoneal psilocybin administration (0.1 mg/kg) increases neurogenesis in mouse dentate gyrus, but decreases it at 1 mg/kg **[25],** whereas chronic administration of DMT (0.26 mg/kg) increases brain-derived neurotrophic factor (BDNF) levels in the hippocampus of female rats **[26]** – the changes in neuro/synaptogenesis already linked to clinical mood and anxiety disorders **[27,28]**.

Although rodents are widely used for preclinical drug screening, including psychedelic drug research, other non-mammalian vertebrates (e.g., zebrafish, *Danio rerio*) **[29,30]** and invertebrates (e.g., *Drosophila* **[10]),** show much promise. **Table 1** summarizes clinical and pre-clinical CNS effects of classical psychedelics (LSD, mescaline, psilocybin, and DMT) and selected non-hallucinogenic psychedelics (TBG and AAZ-A-154). Recognizing the importance of translational psychedelic research, here we discuss the existing challenges in the field, and outline novel promising approaches using animal models to probe a fuller spectrum of neurotropic effects of psychedelic drugs.

Understanding the effects of psychedelic drugs in rodents and zebrafish

As already noted, classical psychedelic drugs exert their psychoactive activity primarily through the serotonin 5-HT2A receptor agonism, extensively probed in various rodent models. The 5-HT2A receptor is a G-protein-coupled receptor (GPCR) and the most widely expressed type of serotonin receptors in mammals [31]. Found in multiple tissues (e.g., the immune, muscle, endothelial and endocrine), this receptor can potentially affect a wide range of systems and functions beyond neuronal [31]. Furthermore, while psychedelic drugs can activate multiple GPCRs (e.g., dopaminergic, noradrenergic and trace amine/TAARs receptors) and some other signaling systems, 5-HT2A receptors are recognized as the main contributor to the common behavioral and subjective hallucinogenic effects of psychedelics [20,32-36]. Nevertheless, LSD and similar psychedelic drugs also bind to other serotonin receptors (e.g., 5-HT1A and 5-HT2C), as well as show dopaminergic and adrenergic effects at high doses [31].

In rodents, psychedelic drugs commonly induce anxiolytic-like states in low-to-moderate doses [23,37,38] (also see reduced anxiety-related ultrasonic vocalizations in rat pups by DOI [39]). However, there is also some experimental evidence of increased anxiety-like behavior by DOI [40], which corroborates clinical observations that hallucinogens may often induce fear and anxiety [41]. Genetic studies further implicate 5-HT2A receptor-mediated signaling in anxiety-related behaviors, since 5-HT2A knockout mice display elevated anxiety, whereas normalizing 5-HT2A expression rescues this phenotype [42]. Psychedelic drugs also enhance fear conditioning and fear memory extinction, depending on the time of exposure [43], and often increase impulsivity-related behavior in different tests [24,44,45] (note, however, that impulsivity-elevating effects of LSD is associated with dopamine D2, but not 5-HT2A, receptors [46]). Long-term LSD exposure also evokes lasting behavioral abnormalities (including, hyperactivity and hyperirritability, increased locomotor activity, anhedonia and social deficits) that persists for months and are unrelated to LSD discontinuation [47].

Drug-induced HTR paradigm is one of the most widely studied psychedelic assays in rodents that distinguishes, with relatively high predictive validity, 5-HT2A psychedelics from other compounds [22]. HTR is also an easily distinguishable from other related phenotypes following the exposure to dissociative drugs (head-weaving) [48] or opioid withdrawal (wet-dog shakes) [49]. In general, hallucinogenic psychedelic drugs typically facilitate HTR, whereas non-psychedelic 5-HT2A receptor agonists lack this effect [20,50-55]. In line with this, 5-HT2A receptor pharmacological [52,53,55-57] and genetic inactivation [20,58,59] can both predictably block hallucinogen-induced HTR.

Finally, much attention is currently paid to the ability of classical psychedelics to induce pronounced antidepressant responses in rodents, paralleling human studies (e.g., [1-3]). For example, psilocybin, LSD [60] and chronic low-dose DMT exert persistent antidepressant-like effects in rodent forced swim test (FST). Interestingly, psilocybin also elicits similar antidepressant-like action in this test (albeit independently from the 5-HT2A receptor activation), suggesting that other putative mechanisms may co-contribute to antidepressant effects of serotonergic psychedelics in animal models [61].

Complementing rodent studies, non-traditional vertebrate models, such as zebrafish, are emerging as a sensitive powerful in-vivo systems to probe the effects of psychedelic drugs **[62-64]**. Although zebrafish express two copies of 5-HT2A receptor (5-HT2Aa and 5-HT2Ab) **[65]**, other elements of their serotonergic system, such as the serotonin transporter and enzymes of synthesis and catabolism, are remarkably evolutionarily conserved **[66-70].** The sensitivity to a wide range of serotonergic agents, including major psychedelic drugs, makes zebrafish an invaluable tool to study mechanisms underlying their CNS effects and role in various psychiatric diseases **[71-73]**. For example, in the novel tank diving test paradigm, mescaline (5–20 mg/L) is likely anxiolytic as it increases preference for the upper half of the tank and reduces immobility **[29]**. While LSD exerts a biphasic profile in rodents and humans, acting as a 5-HT2A receptor agonist during an early anxiogenic phase and as a dopaminergic D4 receptor agonist at a later,

pro-arousal phase **[74]**, this effect is not observed in zebrafish, where LSD is overtly anxiolytic within minutes **[30]**. Clearly, such differences in profiles call for improved understanding of the 5-HT2A receptor role in psychedelic effects across species, also helping distinguish between various psychedelic drug-evoked phenotypes in vivo.

Conclusion

Recognizing risks of hallucinogenic effects of serotonergic drugs, the search for safer and effective psychedelic drugs for the treatment of psychiatric disorders continues. With the increasing spectrum of established animal models, the availability of tools for characterizing behavioral- and molecular-level effects of psychedelic drugs (especially related to 5-HT2A receptor signaling) is also growing. Mounting evidence, only briefly discussed here, continues to generate important insights into molecular, physiological, and neurobehavioral mechanisms of psychedelic drugs. Recent breakthroughs in studying serotonin receptors also markedly increase our understanding of their molecular interactions with others neuronal pathways (e.g., the 5-HT2A receptor-mediated potentiation of GABAergic inhibition **[75]**). Finally, if such attempts are successful, altered neuroplasticity in the absence of psychedelic-induced hallucinogenic effects may be sufficient for the treatment of neuropsychiatric disorders, and a larger number of patients may potentially benefit from using non-hallucinogenic medicines inspired by psychedelic science.

Expert Opinion

Perspectives on developing novel putative non-hallucinogenic psychedelic drugs

Conventional psychedelic serotonergic drugs (LSD, DMT, psilocybin and mescaline) commonly generate a wide range of effects on perception, emotion and cognition **[12,76]**. However, the mechanisms of action of psychedelics at molecular and brain circuitry levels remain poorly understood **[77]**. For example, a non-hallucinogenic psychedelic analogue of ibogaine, tabernanthalog (TBG), promotes neuroplasticity, reduces alcohol- and heroin-seeking behavior, and evokes antidepressant-like effects without HTR in rodents **[78]**. Similarly, sub-

hallucinogenic doses of DMT prevent mood- and anxiety-like deficits in rodents **[79]**. Importantly, while TBG and DMT are 5-HT2A receptor agonists **[78,80]** and some TBG effects are blocked by a 5-HT2A antagonist ketanserin [78], TBG exhibits lesser 5-HT2A activity than classical psychedelic compounds, but more potently binds to the serotonin transporter (SERT) [18,81].

To understand the main cause of subjective hallucinogenic effects of psychedelic drugs, it is critical to recognize critical structures that generate such responses. For instance, the simplification of the drug can enhance synthetic tractability, improve physicochemical properties and safety of psychedelic drugs **[78]**. Indeed, smaller molecules like 5-F-DMT and 5-Cl-DMT are hallucinogenic, while a similar (but bigger) molecule, 5-Br-DMT, is not **[77]**. Besides the complexity of the drug per se, one must consider the participation of a wider circle of molecules in these hallucinogenic responses. For example, various scaffolding proteins are described as important regulators of 5-HT2A receptors **[82-86],** primarily by influencing their subcellular localization **[87]**. Therefore, both complexity of the molecule and the interactions of 5-HT2A receptors with scaffolding proteins can determine subjective CNS effects of psychedelic drugs. As such, novel ligands can be developed based on modulating CNS receptor function by targeting their scaffolding proteins and other molecular interactors (Fig. 1).

The 5-HT2A receptors belong to the A-1 class of GPCR family that participate in the most versatile group of biological processes **[88]**. First, since different G-proteins may couple to the same serotonin receptor (Fig. 1), its activation can thereby trigger distinct molecular pathways **[89]**. Second, there are also GPCR dimers **[90],** including, for example, heterodimeric serotonin/glutamate 5-HT2A/mGlu2 receptors that are co-immunoprecipitated in rodent brain areas related to the effects of antipsychotics **[91,92]**. Such heterodimers may acquire unique biochemical and functional proprieties, including novel pharmacology, ortho- or allosteric binding sites, signaling responses to agonists or antagonists, and unexpected allosteric-like cross-talk between the protomers **[93,94]**. Moreover, since small changes in the structure of ligands

can also cause distinct cellular signaling profiles, different ligands may promote differing signaling-to-effector pathways within cells **[95]**. All these factors may clearly contribute to CNS effects of various psychedelic drugs, and may help distinguish between hallucinogenic vs. non-hallucinogenic psychedelics.

Although such variables (*e.g.*, scaffolding proteins, varying G-proteins, different ligands and potential heterodimerization; **Fig. 1**) can hamper the resolution and efficacy of pharmacological screening of non-hallucinogenic psychedelics, there are innovative methodologies capable of improving such a task. For instance, the microbial periplasmic binding proteins-based 5-HT sensor (iSeroSnFR) enables imaging serotonin dynamics in brain slices and freely moving rodents **[96]**. This biosensor images real-time serotonin release triggered by fear, social interaction, and behavioral modulation in multiple rodent brain regions, and detects changes in serotonin efflux mediated by human SERT in the presence of 3,4-methylenedioxymethamphetamine (MDMA) in vitro, in the HEK293T cell culture **[96]**.

On the one hand, iSeroSnFR does not assess serotonin receptor activity, making it impossible to perform high-throughput pharmacological screening. On the other hand, another sensor, the psychLight, can convert ligand-induced conformational changes of the 5-HT2A receptor into fluorescence readouts in freely moving rats **[77]**. Pharmacological analysis using psychLight reveals high affinity for 5-HT2 receptors for AAZ-A-154, which also increases dendritic outgrowth in cultured rat embryonic cortical neurons, induces FST antidepressant-like responses and ameliorates anhedonia (sucrose preference) in rats, similar to psychedelic drugs [77]. Although its pharmacological profile has not been fully established yet, previous reports show that AAZ-A-154 does not exert hallucinogenic-like effects **[77]**.

While both iSeroSnFR and psychLight biosensors have been applied to rodents, they can also be used in zebrafish. For example, iSeroSnFR may help analyze molecular dynamics of serotonin related to the two copies of zebrafish 5-HT2A (a and b) receptors. In turn, psychLight may help observe the contrast between molecular mechanisms of these two receptor isoforms in

zebrafish, e.g., probing the difference in ligand-induced conformational changes between these isoforms. However, using zebrafish for this may also meets some limitations, compared to rodents. For example, while rodent behavioral analyses are performed through optical cables inserted by craniotomy holes, zebrafish move in 3D space that complicates analyses, presently seemingly limited to *in vitro* zebrafish assays.

Further untangling signaling cascades

A key strategic area of psychedelic research is studying a wide range of 5-HT2A molecular, transcriptomic and genetic effects in the brain. 5-HT2A receptor activity is primary associated with Gq protein alpha subunit pathway that activates multiple downstream signaling cascades, including phospholipase C (PLS), diacylglycerol (DAG) and inositol triphosphate signaling (IP3), which in turn stimulate the protein kinase C (PKC) activity, Ca2+ release and β-arrestin signal transduction [20,97,98]. Arrestins are a small family of homologous regulatory proteins that act as modifiers of GPCR signaling, supporting receptors internalization and simultaneously activating independent (e.g., tyrosine kinase Src- and MAPK-related) pathways [99-101]. Different GPCR ligands show biased efficiency of activation of G protein- and arrestins-related pathways, including psychedelic drugs via 5-HT2A receptors [102]. Interestingly, serotonin and DOI both act on 5-HT2A receptor through different (β-arrestin-2-dependent and independent, respectively) pathways, to induce mouse HTR [56]. Similarly, LSD acts upon β2-arrestin signaling more than non-hallucinogenic lysergamides, suggesting the importance of β2-arrestin signaling to produce hallucinatory states [97]. Overall, this suggests that searching for ligands that are biased toward different GPCR-related signaling cascades may foster the development of novel psychedelics without pronounced hallucinogenic effects.

Based on this notion, several arrestin-biased ligands of 5-HT2A receptor have recently been developed using structure-based discovery utilizing crystal structures of the 5-HT2A receptor complexed with psychedelics and other serotonergic compounds, to estimate different binding modes, allowing to predict ligand bias [103]. Two novel highly biased (IHCH-7112 and

IHCH7120, bias factors = 6.70 and 12.76 respectively) and one moderately biased (IHCH-7113, bias factor = 1.52) for β2-arrestin signaling drugs have been suggested [103]. Interestingly, IHCH-7079 and IHCH-7086 (but not IHCH-7113) fail to produce HTR at doses as high as 10 mg/kg in mice, and also abolish LSD-induced HTR, similarly to 5-HT2A receptor selective antagonist MDL100907 [103]. Finally, both IHCH-7079 and IHCH-7086 induce pronounced antidepressant responses in various mouse depression models, paralleling data suggesting that hallucinogenic activity is not neccessary for LSD to induce antidepressant effects in mice [103]. Collectively, this strongly supports the use of novel efficient structure-based approaches to improve success of psychedelic drug discovery.

The use of alternative model organisms in psychedelics research

As already mentioned, zebrafish are an emergent tool to investigate the neuropsychiatric disorders, and also have multiple practical advantages, including space-efficiency, easy experimental manipulations and a low-cost maintenance **[120].** Their high reproduction rate, external fertilization, transparency of embryos and rapid development **[121]** foster studying genetic and epigenetic mechanisms of neuropsychiatric disorders, especially given multiple transgenic lines created by gene-editing (*e.g.,* CRISPR-Cas9 or transcription activator-like effector nucleases/TALENS) methods **[122,123]**. However, zebrafish possess some limitations in the translatability of their data due to certain differences from mammals, such as metabolic physiology (cold-blooded fish vs. warm-blooded mammals) and brain anatomy (e.g., zebrafish lack cortex) **[124]**. Because of its small size, the long-term monitoring of endocrine levels from blood is also problematic as it is difficult to obtain a sufficient amount of blood without euthanizing the animal **[125]**. In addition, genome duplication event in teleost fishes **[126]** may complicate genetic analyses of neuropsychiatric phenotypes since some genes may exist in two copies in zebrafish (however, if the gene in question is vital, having two copies of it in zebrafish may help generate viable knockouts if only on copy of the gene is ablated).

Unlike in rodents, there are still multiple questions related to psychedelic-induced behavioral and molecular studies in animals (Table 2). For example, it is unclear to what extent various molecular factors (*e.g.*, different ligands, scaffolding proteins, difference in the G-protein, heterodimerization) can influence hallucinogenic responses? Moreover, it remains to be studied which behavioral tests are most appropriate assays to characterize hallucinogenic-like phenotypes in both popular and novel model organisms, such as rodents and zebrafish? Likewise, novel methodological approaches (*e.g.*, psychLight and iSeroSnFR) can elucidate why LSD does not show the biphasic effect on anxiety (seen in rodents and humans) when tested in zebrafish. The psychLight may also help clarify the differences in molecular mechanisms between the two copies of zebrafish 5-HT2A (5-HT2Aa and 5-HT2Ab) receptor, whereas iSeroSnFR can clarify the differences in the pharmacokinetics of serotonin between these two receptor isoforms.

Understanding the effects of psychedelics on impulsivity

Interestingly, non-hallucinogenic psychedelics, including lisuride and some 5-HT2A receptor β-arrestin–biased agonists [104], somewhat differ from hallucinogenic drugs due to the ability to increase impulsivity, often experienced by humans taking hallucinogenic psychedelics [105-107]. Rodents treated with serotonergic hallucinogens also display increased impulsivity in traditional locomotor and impulsive choice tests [108], especially in a five-choice serial reaction time task (5-CSRTT) [24,109,110]. Although data is scarce on zebrafish performance in 5-CSRTT following hallucinogenic psychedelic drugs, this test was successfully applied to other neurobehavioral and neuropharmacological studies in this model organism [111-113]. In contrast to serotonergic hallucinogens (e.g., DOI), their non hallucinogenic analogues (*e.g.*, lisuride) decrease impulsive decision-making in rats tested in delay discounting paradigm [114], conceptually similar to 5-CSRTT and comparing preference for small instant vs. larger delayed reward [115]. Furthermore, lisuride displays remarkable antiseizure activity in zebrafish Dravet syndrome (DS) model, which again may be relevant to rescuing hyper-impulsivity phenotype in this CNS model [116]. While impulsive behavior following non-hallucinogenic serotonergic

psychedelic drugs remains poorly studied, these drugs may be analyzed in rodent and zebrafish models using both 5-CSRTT and delay discounting task. For example, an emphasis should be made on further screening of impulsivity-related effects of 5-HT2A β-arrestin-biased agonists [117,118] and non-classical psychedelics like TBG [119], which have already shown positive antidepressant and anti-addictive potential in rodents [78].

Finally, rigorous and thorough animal modeling of psychedelic drug effects may benefit from the implementation of artificial intelligence and machine deep learning algorithms, to generate new insights for rapid and large-scale phenotyping using zebrafish. Thus, future methods of behavioral fingerprinting and automated video-based animal tracking will be developed for large-scale drug screening applications, including psychedelic drugs. For example, impulsivity and hyperactivity phenotypes related to hallucinogenic action may be detected by increased locomotor parameters (and especially acceleration and turning endpoints) by automated video-tracking, empowered by artificial intelligence-based pattern recognition algorithms. Together, this may help create efficient databases for high-throughput drug screenings, which can, in turn, ultimately translate into improved success rates of clinical trials. Using this strategy, ineffective non-hallucinogenic psychedelic drug candidates can be reliably identified and/or discarded.

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Author contributions

F.V.C. and A.V.K. were involved in the conception and design, interpretation of the data; draflting (F.V.C.) and editing (A.V.K.) the manuscript; and the final approval of the version to be published. K.N.Z., D.B.R., K.A.D., M.S.A and E.V.P. were involved in analyses and interpretation of the data; the drafting of the paper; and the final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

Figure 1. Different signaling molecular profiles of the serotonergic 5-HT2A receptor activation, including different G-proteins (a), scaffolding proteins (b) or structure of the ligands (c) that may initiate distinct significant cascades. EGR1 - Early growth response protein 1; EGR2 - Early growth response protein 2; Ip3 - Inositol trisphosphate; PLC - Phospholipase C; PLD - Phospholipase D; AC - Adenylyl cyclase; PKC - Protein Kinase C; ERK - Extracellular signal-regulated kinase; mOTR - Mammalian target of rapamycin; PKA - Protein kinase A (based on [86,127,128])

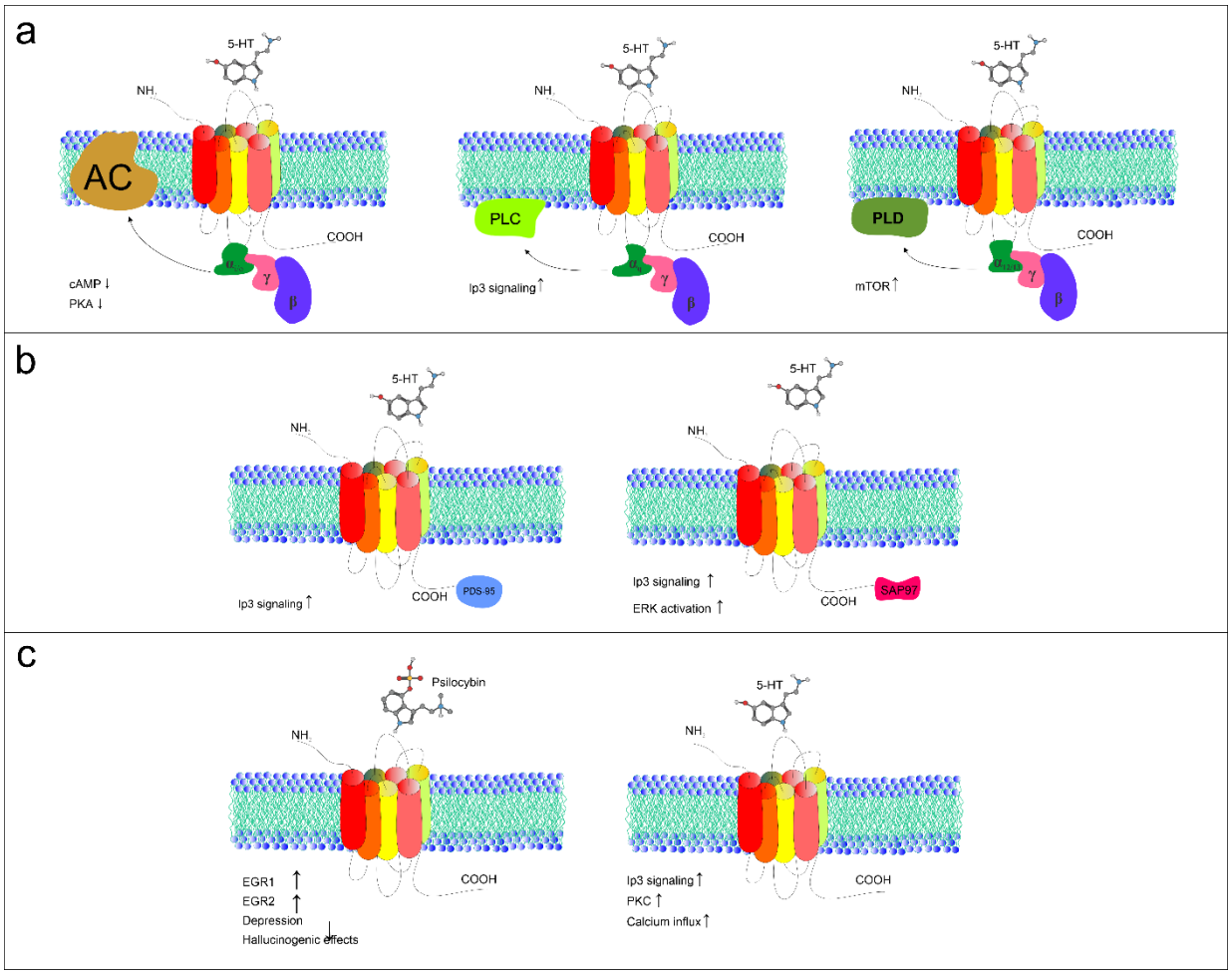

Table 1. Clinical and pre-clinical CNS effects of classical psychedelic and non-hallucinogenic psychedelic drugs injected using intraperitoneal (i.p.), intraventricular (i.v.), subcutaneous (s.c.), oral (p.o.) and intramuscular (i.m.) methods.

Drugs	**CNS effects in different species**		
	Zebrafish	**Rodents**	**Humans**
Classical psychedelic drugs			
LSD	↑time in top, top transitions, ↓latency to top, ↓freezing episodes and duration, reversed dark preference, ↑thigmotaxis, ↑cortisol, ↓shoaling (0.25 mg/L) [30]	Head-twitch response (HTR) and shaking behavior (0.025 mg/kg 7 times/4 days i.p.) [12], ↓ depression (0.15-0.13 mg/kg i.p.) [60,129]; ↓ locomotion (0.06 mg/kg i.p.) [130]	Visual hallucination, audio-visual synesthesia, ↑wellbeing, trust, happiness, blood pressure, heart rate, body temperature, cortisol, oxytocin and epinephrine, ↓ anxiety (0.2 mg/kg p.o.) [131]
Mescaline	↑time in top, top transitions, shoaling, ↓latency to top, immobility, anxiety (20 mg/L) [29]	↑locomotion (100 mg/kg i.p. 60 min after injection) [132]; ↑ aggression (10–50 mg/kg i.p.) [133], HTR (10-100 mg/kg i.p.) [50,134], ataxia, ↓learning (25-75 mg/kg 30-40 min after injection i.p.) [135]	Visual and auditory hallucination, synesthesia, distortions in time perception, depersonalization, ↑anxiety, euphoria, catatonia, altered cognitive performance, psychosis (0.5 mg/kg p.o.) [136-138]
Psilocybin	↑ shoaling, ↑ cortisol [139]	HTR (1.1 mg/kg s.c.) [140], ↓depression-like behavior [60], ↑fear extinction (1 mg/kg i.p.) [25]	Visual, auditory, proprioceptive hallucinations, distorted time perception, mood and memory, spiritual/mystical experience (2 mg i.v, 30 mg/kg p.o.) [141,142], ↓anxiety, depression (22 mg p.o.) [143], ↑mood, perception (0.045–0.315 mg/kg p.o.) [144], ↓alcohol and nicotine addiction (20-30 mg p.o.) [145,146]
DMT	↓memory (0.1-0.5 mL/L chronic)**,** locomotion (0.5 mg/L acute) [147]*, velocity and distance, ↑freezing, bottom dwelling (1-3 mg/L)**,** ↓bottom dwelling, anxiety-like behavior (0.1 mg/L) [148]*	↑anxiety (10 mg/kg i.p.) [149], ↓fear, anxiety, depression (1 mg/kg i.p.) [79]	↑mental wellbeing (25 mg smoked) [150], ↓ depression (2.2 mL/kg p.o.) [151]*, ↑feelings of relaxation (0.7 mg/kg i.m.) [152], ↓anxiety [153]*
Non-hallucinogenic drugs			
TBG	Behaviorally inert in larvae (1–200 μM) [78]	↓depression (50 mg/kg i.p.), ↓heroin seeking (40 mg/kg i.p.) [78]	No data regarding CNS effects
AAZ-A-154	No data regarding CNS effects	↓depression (20 mg/kg i.p.), ↓anhedonia (15 mg/kg i.p.) [77]	No data regarding CNS effects
IHCH-7113	No data regarding CNS effects	HTR (0.125 mg/kg i.p.) [103]	No data regarding CNS effects
IHCH-7079	No data regarding CNS effects	Lack of HTR (<=10 mg/kg i.p.), ↓depression (2-10 mg/kg i.p.) [103]	No data regarding CNS effects
IHCH-7086	No data regarding CNS effects	Lack of HTR (<=10 mg/kg i.p.), ↓depression (2-10 mg/kg i.p.) [103]	No data regarding CNS effects

*These studies used ayahuasca instead of pure DMT (ayahuasca is a complex mixture of DMT and alkaloids, and is used to treat depression clinically).

Table 2. Selected open questions related to psychedelic drug neurobiology and drug screening

Questions
• How can different molecular factors (e.g., different ligands, scaffolding proteins, G-proteins and heterodimerization, as in Fig. 1) modulate hallucinogenic vs. general psychedelic effects? • What are reliable phenotypes of rodent or zebrafish hallucinogenic-like behavior? Are there appropriate behavioral tests to assess hallucinogenic responses in such animal models? • Are there reliable molecular determinants of hallucinogenic responses? Similarly, are there molecular (neurochemical, biochemical, genomic) biomarkers associated with hallucinogenic vs. non-hallucinogenic action? • How can novel methodological approaches (e.g., psychLight and iSeroSnFR) probe hallucinogenic vs. non-hallucinogenic effects? • Are there differences in genomic/transcriptomic profiles between hallucinogenic vs. non-hallucinogenic psychedelic drugs? • What are epigenetic mechanisms underlying hallucinogenic vs. non-hallucinogenic psychedelic drug action? • Are there neuroinflammation-related and/or neuro-apoptotic mechanisms that contribute to hallucinogenic vs. non-hallucinogenic drug action? How do psychedelic drugs interact with neuronal and glial factors (e.g., BDNF, GDNF, NGF)? • Do psychedelic drugs affect neuroimmune response in mammals and zebrafish? Do microglial cells and astrocytes play similar (or distinct) roles in psychedelic hallucinogenic vs. non-hallucinogenic drugs? Can serotonergic psychedelic drugs induce adult neurogenesis? Can these drugs affect neural progenitor cells? • What is the addictive potential of hallucinogenic vs. non-hallucinogenic psychedelic drugs? If so, can psychedelic drugs induce overt withdrawal syndrome in animal models? Are there differences between hallucinogenic vs. non-hallucinogenic psychedelic drugs? • How can zebrafish models be used to mimic core mechanisms underlying psychoses induced by psychedelic drugs? • Are there drug interactions between hallucinogenic and non-hallucinogenic psychedelics?

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