2022

Investigating the Effects of Psilocybin on Models of Anxiety, Recognition Memory, and Depression-Like Behavior and the Role of the 5-HT2A Receptor in Mediating Psilocybin's Impact on Behavioral Despair

Nikita Thakur
Virginia Commonwealth University

Investigating the Effects of Psilocybin on Models of Anxiety, Recognition Memory, and Depression-Like Behavior and the Role of the 5-HT_{2A} Receptor in Mediating Psilocybin's Impact on Behavioral Despair

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Physiology and Biophysics at Virginia Commonwealth University

By:
Nikita Thakur
B.A. Biology, University of Virginia, 2018

Director: Javier González-Maeso, Ph.D.
Professor
Department of Physiology and Biophysics

Virginia Commonwealth University
Richmond, Virginia
June 14, 2022

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my advisor, Dr. Javier González-Maeso, who has always made himself available to ensure I am on the right track in fulfilling my goals. I am grateful for his continued mentorship and advice, and for challenging me to think more critically. Designing and successfully carrying out experiments in addition to preparing a thesis is no easy feat, and I would not have been able to achieve these goals without his guidance. Second, I would like to thank Dr. Mario de la Fuente Revenga and Alaina Jaster for their patience in teaching me and for their assistance throughout my project. I also thank Dr. Jason Younkin and Dr. Somdatta Saha for always encouraging me to ask questions and for being patient whenever I did ask too many.

To all of my other lab colleagues, it has been a great honor for me to work alongside you. I am in constant awe of your wealth of knowledge, accomplishments, and your persistent dedication to the process of scientific inquiry. Observing firsthand the intricacies of research, from the inception of an idea to its execution, has given me a greater appreciation for this field. I also thank you dearly for your helpful advice and pointers leading up to my defense. Above all, being in your company has made my stay in Richmond all the more enjoyable. I am also indebted to my committee members, Dr. Peter Hamilton and Dr. Gretchen Neigh. Their support, valuable insight, and feedback have been crucial throughout this process. I am thankful to the Wolstenholme Lab as well for sharing their space and for allowing me to use their equipment to run my experiments.

Last but certainly not least, I would like to extend my heartfelt gratitude to my parents, Ganesh and Veena, and brother, Nitish. They have unconditionally supported me in all of my

endeavors and have served as a continuous source of love and encouragement. I feel truly blessed to have you in my life.

Thank you all so much, none of this would have been possible without your support.

Adopted Image Permission:

Table of Contents

List of Abbreviations

5-HT	5-Hydroxytryptamine or Serotonin
$5\text{-HT}_{2A}\text{R}$	Serotonin 2A Receptor
DMT	N,N-dimethyltryptamine
DOI	2,5-dimethoxy-4-iodoamphetamine
DOM	2,5-dimethoxy-4-methylamphetamine
FST	Forced Swim Test
GAD	Generalized Anxiety Disorder
GPCR	G Protein-Coupled Receptor
HTR	Head-Twitch Response
KO	Knockout
LSD	Lysergic Acid Diethylamide
MDD	Major Depressive Disorder
M100,907	Volinanserin
NOR	Novel Object Recognition
OFT	Open Field Test
WT	Wild Type

List of Figures

Abstract

INVESTIGATING THE EFFECTS OF PSILOCYBIN ON MODELS OF ANXIETY, RECOGNITION MEMORY, AND DEPRESSION-LIKE BEHAVIOR AND THE ROLE OF THE 5-HT_{2A} RECEPTOR IN MEDIATING PSILOCYBIN'S IMPACT ON BEHAVIORAL DESPAIR

By Nikita Thakur

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Physiology and Biophysics at Virginia Commonwealth University

Virginia Commonwealth University, 2022

Director: Javier González-Maeso, Ph.D.
Professor
Department of Physiology and Biophysics

Psychedelics are a class of hallucinogenic substances that exert their effects through serotonin (5-HT) receptor agonism, particularly at the 5-HT_2 receptor, which is further characterized into the 2A, 2B, and 2C subtypes. While some research studies indicate that psychedelics mediate their effects via 5-HT_{2A} receptor agonism, others show that it is not necessary to induce these effects. Recently, there has been a growing interest in psychedelic-assisted therapy as a promising alternative for treatment of anxiety and depression, two of the most common psychiatric disorders worldwide associated with significant morbidity. Although antidepressants and pharmacological interventions for anxiety have revolutionized management, they often have mixed efficacy and fail to provide satisfactory emotional relief. Clinical studies show that psychedelics like psilocybin and LSD produce antidepressant and anxiolytic effects, but there is still an active debate on whether or not these effects are mediated via action at the 5-HT_{2A} receptor.

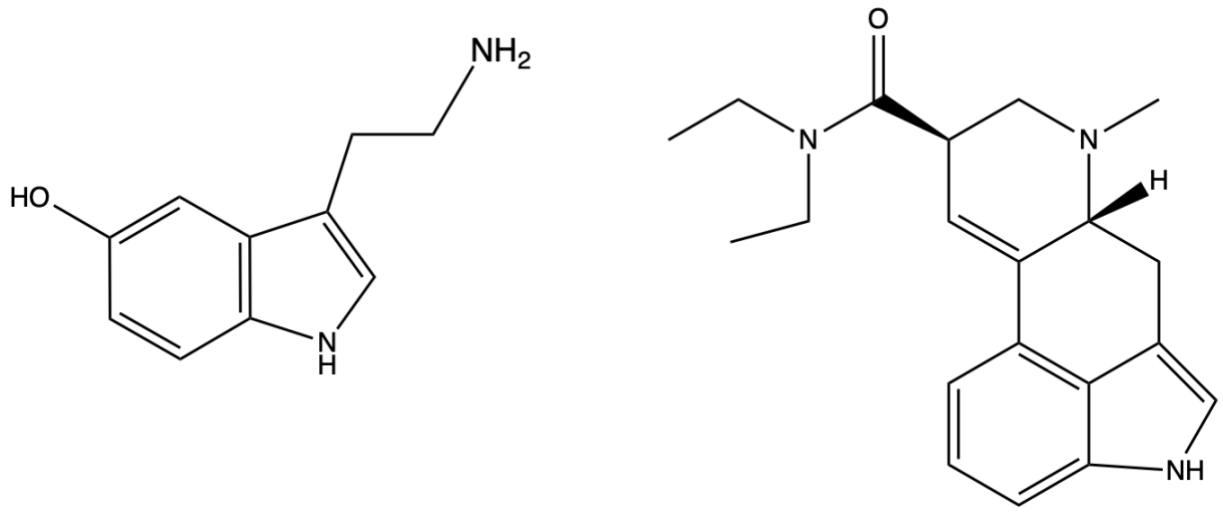
In this study, my first aim was to examine psilocybin's effects on the following behavioral assays in male WT C57BL/6 mice: head-twitch response (HTR), a 5-HT_{2A}-specific rodent model of hallucinogenic action; locomotor activity, a model of anxiety; novel object recognition (NOR), to assess recognition memory; light-dark box preference, an additional model of anxiety; and forced swim test (FST), a model of depression-like behavior. HTR assessed the acute effects of psilocybin, while the remaining assays measured psilocybin's post-acute effects (i.e. 24 hrs following administration). Second, I aimed to determine whether or not psilocybin's post-acute antidepressant-like effects are mediated by the 5-HT_{2A} receptor by conducting FST assays using the 5-HT_{2A}-specific antagonist volinanserin (M100,907). Acutely, psilocybin evoked a greater HTR relative to the vehicle group, but post-acutely it did not produce a significant difference in locomotion, NOR, or light-dark exploratory behavior. However, mice given psilocybin in the first FST had both lower immobility and higher climbing times relative to controls. In the second FST, mice administered M100,907 shortly before injection with psilocybin exhibited no significant difference in immobility and climbing times compared to controls. Interestingly, in the third FST, mice given M100,907 alone had a significantly lower climbing time, while immobility and swimming times between the vehicle and experimental groups were nonsignificant. The results of this study suggest that the 5-HT_{2A} receptor may indeed play a central role in mediating psychedelics' post-acute effects, and that antagonizing it impairs these effects. These findings highlight the need for additional studies that examine the post-acute effects of a variety of psychedelics and further investigate the level of involvement of the 5-HT_{2A} receptor using genetically modified animal models.

Chapter 1: Introduction

Psychedelics

Psychedelics are psychoactive substances that affect mood, perception, thought, and numerous other cognitive processes. They are also known as classic serotonergic hallucinogens, as they exert their effects through brain serotonin 2A (5-HT_{2A}) receptors (Nichols, 2016). Although classification systems may vary, in general there are three categories of psychedelics based on biochemical and behavioral characteristics: ergolines, phenethylamines, and tryptamines (Winter, 2009). While lysergic acid diethylamide (LSD) is classified as an ergoline (Nichols, 2004), it is important to note that its structure contains both a phenethylamine and the indole nucleus common to tryptamines. The phenethylamine hallucinogens include mescaline, 2,5-dimethoxy-4-methylamphetamine (DOM), and 2,5-dimethoxy-4-iodoamphetamine (DOI). Those belonging to the tryptamine class include N,N-dimethyltryptamine (DMT), 5-methoxy-N,N-dimethyltryptamine (MDMT), psilocybin (found in "magic" mushrooms), and its active derivative psilocin (Winter, 2009).

Although the realm of hallucinogenic research is relatively young, historical records indicate that psychedelics have been used for thousands of years and were frequently employed by early cultures in sociocultural and ritualistic practices. However, the introduction of psychedelics into modern research began in 1943, when Swiss chemist Albert Hoffman accidentally discovered LSD and its powerful psychological effects. Not long after, serotonin was discovered in the mammalian brain, and the tryptamine moiety in LSD was found to be the scaffold for serotonin's chemical structure (Nichols, 2016). Hoffman's discovery was followed by significant investigations of the therapeutic applications of LSD, psilocybin, mescaline, and other psychedelic substances for various psychiatric illnesses (Belouin & Henningfield, 2018).



A. *5-hydroxytryptamine* ***B.*** *Lysergic acid diethylamide*

Figure 1: *Comparison of the chemical structures of serotonin and LSD. Both* ***(A)*** *5-hydroxytryptamine (serotonin) and* ***(B)*** *lysergic acid diethylamide (LSD) contain the tryptamine moiety, suggesting that LSD functions in the brain via interaction with a serotonin receptor. ChemDraw Professional 21.0.*

Serotonin Hypothesis of Psychedelic Action

Following the discovery of LSD's similar chemical and pharmacological profiles to serotonin (*Fig. 1*), there was an increasing interest in understanding the role of serotonin (5-HT) in behavior. Initially, Gaddum et al. (1953) found that LSD antagonized the effects of 5-HT in peripheral tissues. Specifically, whereas 5-HT induced vasoconstriction and bronchoconstriction in perfused cat lungs, LSD abolished these effects. LSD was also shown to antagonize the effects of 5-HT on rat uterus (Gaddum et al., 1953). This evidence of LSD's antimetabolite action on smooth muscle, in addition to its structural similarities to 5-HT, led Woolley and Shaw (1954) to propose that LSD induces altered mental states because of its antagonism of 5-HT action in the

brain. Furthermore, they inferred that if LSD can produce alternate states of consciousness by interfering with serotonin, then serotonin supposedly plays an important role in maintaining normal mental processes (Woolley & Shaw, 1954).

As cited in Nichols and Nichols (2008), Shaw and Woolley later modified their original inference, since subsequent assays performed two years later showed that LSD could mimic the effects of 5-HT. For instance, LSD was found to stimulate clam heart and cause an increase in the amplitude of the beat, similar to 5-HT. Additionally, LSD acted like 5-HT in raising the blood pressures of anesthetized dogs, suggesting that it may be a 5-HT receptor agonist (Shaw & Woolley, 1956). Eventually, it was determined that LSD primarily functions as an agonist at 5-HT receptors, and that its hallucinogenic effects are mediated through this action. The serotonin hypothesis of psychedelic action was expanded to include psychedelic substances of other classes, such as mescaline and psilocin (Aghajanian & Marek, 1999).

5-HT_{2A} Receptor as a Primary Target of Psychedelics

In 1957, Gaddum and Picarelli reported the existence of two different 5-HT receptor subtypes from their studies in the guinea pig ileum. They characterized these subtypes as D and M, since they could be blocked with dibenzyline and morphine, respectively (Gaddum & Picarelli, 1957). However, it was not until the mid-1970s when additional 5-HT receptors were identified in mammalian brain samples using newly developed radioligand binding techniques (Peroutka & Snyder, 1979). Following the extensive development of molecular biological techniques, additional 5-HT receptor subtypes were discovered during the 1980s up through the beginning of the 1990s. Currently, there are 14 structurally and pharmacologically distinct mammalian 5-HT receptors arranged into seven families. With the exception of the 5-HT_3

receptor (a ligand-gated ion channel), all 5-HT receptors are G protein-coupled receptors (GPCRs) (Barnes & Sharp, 1999).

The GPCRs are a superfamily of receptors with a common motif of seven transmembrane alpha helices (comprised of hydrophobic amino acids) that couple with heterotrimeric GTP-binding proteins, or G proteins. Although initially divided into three classes, GPCRs are now categorized into six receptor families based on sequence and function: A (rhodopsin), B (secretin), C (metabotropic glutamate), D (fungal mating pheromone), E (cAMP), and F (frizzled and smoothened) (Basith et al., 2018). Intracellular signaling through GPCRs occurs upon binding of the appropriate ligand, which activates the G protein. G proteins are composed of α, β, and γ subunits, where the α and $\beta\gamma$ subunits are responsible for signaling. There are different signaling cascades depending on the specific type of G protein associated with a receptor. For instance, G_s is stimulatory, $G_{i/o}$ is inhibitory, and $G_{q/11}$ is stimulatory but also results in a downstream increase in intracellular calcium concentration (Alexander et al., 2011).

Of the 5-HT receptors, the 5-HT_2 receptor family in particular has been shown to mediate the hallucinogenic effects of psychedelics. The 5-HT_2R family comprises three different receptor subtypes: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} (Alexander et al., 2011). In several drug discrimination assays, pretreatment of rats with the 5-HT_2R antagonists ketanserin and pirenperone blocked the discriminative stimulus effects of the psychedelic DOM. These antagonists were also effective in preventing DOM-stimulus generalization to mescaline and LSD (Glennon et al., 1983). Moreover, subsequent studies found a strong correlation between 5-HT_2R binding affinity in rat cortical tissues and human hallucinogenic potency for various psychedelic substances (Glennon

et al., 1984). Eventually, the focus of research shifted to the 5-HT_{2A} receptor (5-HT_{2A}R) as being the necessary component in generating hallucinogenesis and mediating related behavioral responses in animal models (López-Giménez & González-Maeso, 2018). The 5-HT_{2A}R, like all members of the 5-HT_2 receptor family, is a class A GPCR that is $G_{q/11}$-coupled. This pathway activates phospholipase C and produces inositol triphosphate (IP_3) and diacylglycerol (DAG), ultimately increasing intracellular calcium levels (McCorvy & Roth, 2015).

Several important studies highlighted the involvement of 5-HT_{2A}R in inducing psychedelics' effects. In the mid-1990s, Fiorella et al. indicated that antagonists blocking the effects of psychedelics were more selective for the 5-HT_{2A} receptor as opposed to the 5-HT_{2C} receptor. Additionally, the 5-HT_{2A} selective antagonist volinanserin (M100,907), but not the 5-HT_{2C} selective antagonist SB 200,646A, was shown to block the effects of the psychedelic DOI in rats (Fiorella et al., 1995). 5-HT_{2A}R was also found to play a role in head-twitch response (described in a later section), a rodent behavior often expressed in response to 5-HT receptor agonists. In this study, DOI was injected bilaterally into the medial prefrontal cortex of rats to induce head-twitches. Pretreatment with the 5-HT_{2A} antagonists ketanserin and M100,907 inhibited DOI-induced head-twitches, but not in rats pretreated with a selective 5-$HT_{2C/2B}$ antagonist (Willins & Meltzer, 1997). Another study by González-Maeso et al. (2003) used genetically modified animal models to confirm that DOI- and LSD-induced head-twitches present in wild-type mice are abolished in 5-HT_{2A}R null-mutant mice. Intriguingly, a 1998 study

in humans showed that psilocybin-induced psychotomimetic effects were blocked by ketanserin and the atypical antipsychotic risperidone (Vollenweider et al., 1998). Not only was this data consistent with previous animal studies, but it also served as the first evidence in humans of 5-HT_{2A}R's central role in generating hallucinogenic effects.

Clinical Applications of Psychedelics

Depression and anxiety are two of the most common psychiatric disorders worldwide and are both associated with significant morbidity. In the United States alone, approximately 8.4% of adults reported having at least one major depressive episode in 2020 (National Institute of Mental Health, 2022). Anxiety disorders are the most common mental illness in the U.S., affecting about 18.1% of the population every year (Anxiety and Depression Association of America, 2021).

The DSM-5 diagnostic criteria for major depressive disorder (MDD) include: depressed mood; loss of interest or pleasure (anhedonia); significant weight loss (when not dieting) or weight gain; insomnia or hypersomnia; fatigue; feelings of worthlessness, or excessive or inappropriate guilt; diminished ability to think or concentrate; and recurrent thoughts of death or suicidal ideation, or a suicide attempt. For an MDD diagnosis, five or more of these symptoms must be present nearly every day during the same two-week period, with at least one being depressed mood or anhedonia (American Psychiatric Association, 2013). While there are multiple types of anxiety disorders, generalized anxiety disorder (GAD) is more common in the U.S. GAD is characterized by excessive anxiety or worry often associated with: restlessness or fatigue, irritability, muscle tension, or difficulty concentrating. According to the DSM-5, these

symptoms must persist for at least six months to receive a diagnosis (American Psychiatric Association, 2013).

While the discovery of antidepressant therapy transformed the management of depression, up to 60% of patients remain inadequately treated. This can be attributed to several factors, such as non-compliance following undesirable side effects, the drugs' delayed therapeutic effect, or an inherent non-responsiveness to the drugs. Likewise, pharmacological and psychosocial interventions used for anxiety have mixed efficacy and often fail to provide satisfactory emotional relief to patients (Muttoni et al., 2019). Recently, there has been a growing interest in psychedelic-assisted therapy as a promising alternative for treatment of these disorders.

Research indicates that amygdala hyperactivity is associated with depressive symptoms, and antidepressant therapy normalizes its function (Sladky et al., 2015). As 5-HT receptor agonists, psychedelics like psilocybin have been found to enhance amygdala inhibition, which correlates with an increase in positive mood (Kraehenmann et al., 2015). Psychedelics may also act by modulating glutamatergic neurotransmission via 5-HT_{2A}R agonism (Moreno et al., 2011). Higher levels of cortical glutamate concentration indirectly increase brain-derived neurotrophic factor (BDNF) expression, which is associated with increased neurogenesis and neuroplasticity (Vollenweider & Kometer, 2010). Brain imaging has shown that patients with depression exhibit deficient neurogenesis and downregulation of neurotrophic activity. Therefore, restoration of normal BDNF levels with the help of psychedelics could have a therapeutic effect (Duman, 2004). In addition to their neurobiological mechanisms, psychedelics elicit personally meaningful and spiritually significant experiences conducive to their therapeutic potential (Griffiths et al., 2006). In fact, clinical trials with psilocybin have shown that up to 87% of

participants attribute an increased life satisfaction or wellbeing to this psychospiritual experience. Moreover, at the 6.5-month follow-up, psilocybin was associated with enduring anxiolytic and antidepressant effects (Ross et al., 2016).

Overall, psychedelics have been shown to induce post-acute therapeutic effects by modulating neural circuits implicated in mood and affective disorders via 5-HT receptor agonism (*Fig. 2*) and by evoking profound psychospiritual experiences (Muttoni et al., 2019). Incorporating psychedelics to create a new approach for treating depression and anxiety could potentially help patients who suffer debilitating side effects or are unresponsive to conventional methods.

On the contrary, there have been a few studies suggesting that these effects of psychedelics may not be 5-HT_{2A}R-dependent. For instance, using chronically stressed male mice, Hesselgrave et al. (2021) found that a single dose of psilocybin reversed anhedonic responses assessed with the sucrose and female urine preference tests while also strengthening excitatory synapses in the hippocampus. However, pretreatment with the 5-HT_{2A}R antagonist ketanserin did not prevent these behavioral or electrophysiological responses to psilocybin (Hesselgrave et al., 2021). Another study found that while ketanserin pretreatment 10 min prior to administration of psilocybin completely abolished head-twitch responses in mice, it did not block psilocybin-induced structural plasticity (Shao et al., 2021). These findings indicate that psychedelics' mechanism of action may be independent of 5-HT_{2A}R activation. Nonetheless, the extent of 5-HT_{2A} receptor involvement in mediating the effects of psychedelics is an ongoing discussion that warrants further research.

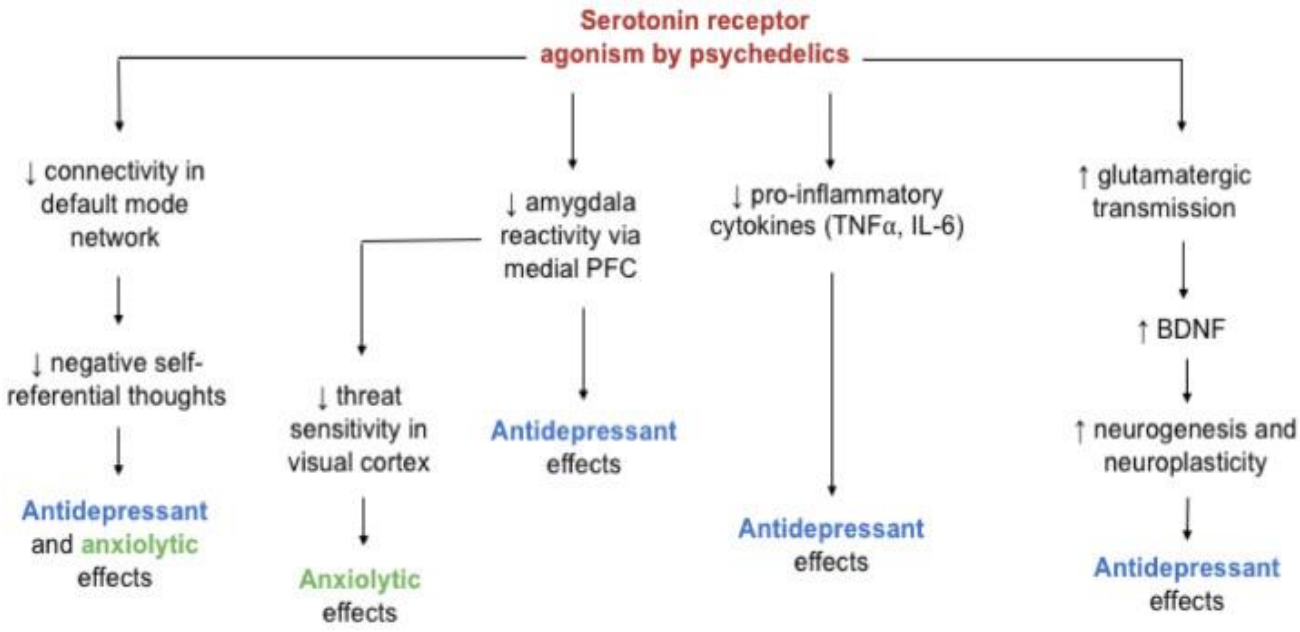


Figure 2: *Copyright © 2019 Silvia Muttoni et al. From "Classical psychedelics for the treatment of depression and anxiety: A systematic review," by S. Muttoni, M. Ardissino, and C. John, 2019, Journal of Affective Disorders, Volume 258. Diagram of the multiple neurobiological mechanisms mediating psychedelics' antidepressant and anxiolytic effects, as a result of serotonin (5-HT) receptor agonism.*

Assessing Psychedelic Action with Behavioral Models

The use of animal models can provide valuable insight into better understanding various physiological and behavioral processes in vivo. However, when studying neuropsychological effects, there may be certain limitations to animal models, such as varied drug dose regimens, differing sensory systems, and an inability to measure subjective experiences. Nevertheless, models exhibiting effects analogous to human behavioral responses induced by psychedelics may be beneficial in elucidating anatomical and molecular pathways underlying these behaviors (Hanks & González-Maeso, 2013). Five behavioral models of hallucinogenic drug action were

the focus of the present study: head-twitch response, locomotor activity, novel object recognition, light-dark box preference, and forced swim test.

Head-Twitch Response

The head-twitch response (HTR) is a rapid side-to-side rotational head movement that occurs in rodents as an acute response to serotonergic hallucinogens and other 5-HT_{2A}R agonists (Canal & Morgan, 2012). Typically, mice will initiate a head twitch while balanced on their hind paws or in a quadruped stance, with the body slightly hunched back and head extended forward. Over the course of an HTR, the trunk and neck progressively elongate, with the head increasingly extending away from the body. Movement during head twitches is often confined to the neck and does not involve the torso. Although multiple receptors and neurotransmitter systems can induce or regulate HTR, many studies have confirmed that this behavior is specifically linked to activation of 5-HT_{2A}R (Halberstadt & Geyer, 2013a). In fact, research has shown that head-twitch frequency abruptly increases when rodents are given 5-HT_{2A}R agonists like the psychedelic DOI (Canal & Morgan, 2012). Furthermore, HTR is blocked by selective 5-HT_{2A}R antagonists and is absent in 5-HT_{2A}R KO mice (González-Maeso et al., 2007). Due to this specificity, HTR is often used as an animal behavioral assay for 5-HT_{2A}R activation and hallucinogenic effects.

There are several methods used to assess HTR in the laboratory setting. The more common method involves a trained rater directly observing the animals and counting the number of head twitches. Alternatively, the animals are recorded with a video camera and their behavior is assessed offline. Both methods may be subject to inter-rater variability, and neither is feasible

for behavioral studies requiring very long durations. However, de la Fuente Revenga et al. (2019) recently developed a magnetometer-based HTR detection system that provides a high-throughput assay and offers high sensitivity and specificity. In this procedure, mice are anesthetized and a small neodymium magnet is surgically implanted on the cranial surface. After a brief recovery period, the mice are placed in glass beakers wrapped with magnet wire. When coil voltage amplification and Fourier analysis are complete, individual HTRs are identified by manually searching for sinusoidal wavelets that meet certain criteria indicating rapid rotational head movement (de la Fuente Revenga et al., 2019).

Locomotor Activity

The open field test (OFT) was initially developed by American psychologist Calvin Hall in 1934 as a test to measure emotionality in rodents (Seibenhener & Wooten, 2015), and is now used as a common measure of general activity and exploratory behavior. It allows for the measurement of various qualitative and quantitative aspects of movement, including: total distance, movement along the walls (thigmotaxis) relative to center, distance moved over different time periods, hole-poking, freezing, and rearing. In the present study, horizontal activity and time spent in the center and periphery relative to total time were used as parameters of locomotor activity. OFT is also often used to assess the stimulant, sedative, or toxic effects of pharmacological substances. Typically, the open field apparatus is circular, square, or rectangular in shape with surrounding walls to prevent the animal's escape. In early studies, the OFT traditionally would last a short duration (between 2 and 10 minutes), mainly due to the method of having to manually acquire data. However, more recent approaches permit a much higher throughput and longer periods of monitoring. Examples include video recording and the

use of software that tracks movement by the number of infrared beam breaks (Gould et al., 2009, pp. 1-3).

Interestingly, hallucinogenic substances used in OFT assays have had varying effects on locomotor and exploratory behavior in rodents. When assessed acutely in rats, Krebs-Thomson et al. (1998) found psychedelics like DOI and LSD to decrease the amount of locomotor activity and increase avoidance of the central portion of the open field. In mice, acute effects of psychedelics involve a reduction in the amount of investigatory behavior (i.e. rearing and hole-poking). However, low to moderate doses produce a delayed increase in distance traveled while high doses reduce activity at the beginning of testing (Halberstadt et al., 2013b). When the effects of hallucinogens are assessed post-acutely, total exploratory distance remains unchanged relative to controls (de la Fuente Revenga et al., 2021).

Novel Object Recognition

Novel object recognition (NOR) is commonly used to investigate learning and memory in rodents. It was originally described by Ennaceur and Delacour (1988) and tested on rats, but has since been successfully adapted for mice. NOR is relatively fast and efficient, and relies on as few as three sessions: habituation to the arena, training, and testing. Training entails visual exploration of two identical objects, and testing involves replacing one of the previously explored objects with a novel one (Lueptow, 2017). Rodents' preference for a novel object indicates that a representation of the familiar object exists in memory (Ennaceur, 2010). One of the main advantages of NOR is its reliance on rodents' natural proclivity for exploring novelty. Moreover, there is no need for positive or negative reinforcement nor numerous training sessions to motivate behavior, and it requires significantly less time than other memory tests (i.e. Morris

water maze or Barnes maze). Additionally, it can be modified to assess different phases of learning and memory, such as acquisition, consolidation, or recall. The versatility of NOR allows for the study of different pharmacological agents that disrupt or enhance memory (Lueptow, 2017).

Multiple studies have documented the acute and post-acute effects of psychedelics on NOR. In one study performed six days after injection, a single dose of LSD was found to produce an increase in exploratory time for the novel object when compared to saline controls. This preference was significant for young (2 months) and adult (9 months), but not old (12-18 months), rats (Cini et al., 2019). In another study testing NOR 20 min after injection, rats given either saline or psychedelic exhibited a significant increase in exploration time for the novel object relative to the familiar one. However, there was no significant difference in novel object exploratory preference between these groups (Wojtas et al., 2021). In mice, both DOI and saline produced significant increases in novel object exploration relative to the familiar object 24 hrs post-injection. However, there was no difference in exploratory preference between both cohorts (de la Fuente Revenga et al., 2021).

Light-Dark Box Preference

The light-dark test is based on both rodents' innate aversion to brightly illuminated areas and on their spontaneous exploratory behavior in response to mild stressors, such as a novel environment and light. When exposed to a novel environment, a natural conflict arises between the tendency to explore and an initial tendency to avoid the unfamiliar. In the light-dark box, a square apparatus is equally divided into dark and light compartments with a small opening connecting both sides. This assay can be used to examine the anxiogenic or anxiolytic properties

of different pharmacological substances. For instance, a drug-induced increase in behaviors in the white (light) relative to the dark compartment reflects anxiolytic activity. In the first light-dark studies, photocells across the partition were used to detect transitions between the two areas. Later studies relied on human observers to document transitions and the amount of time spent on each side from video recordings. A more modern technique involves computer-controlled detection systems equipped with infrared beam sensors. Here, infrared beam interruptions are automatically recorded and used to measure different parameters, such as locomotion, rearing, time spent in light and dark zones, and shuttle crossings between zones (Bourin & Hascoët, 2003).

Psychedelics have been found to exert differing effects on the light-dark behavior of rodents. In one study by Wojtas et al. (2021) measuring acute effects, both rats given control or the psychedelic 25B-NBOMe spent a longer time in the dark as opposed to the light compartment. However, the time spent in the dark zone was longer and statistically significant in rats treated with the psychedelic. Likewise, the time spent by all animals in the light zone was shorter and specifically decreased in the rats given 25B-NBOMe relative to controls. These effects were dose-dependent, with higher doses of psychedelic having a more significant impact on light-dark exploratory behavior compared to the control group (Wojtas et al., 2021). Another study in mice showed that the psychedelic DOI had no statistically significant post-acute effect on time spent in the light zone when compared to mice given vehicle (de la Fuente Revenga, 2021).

Forced Swim Test

The forced swim test (FST), sometimes called the Porsolt swim test, was initially developed for rats and later modified for mice (Porsolt et al., 1978). It is one of the most commonly used assays to measure behavioral despair and passivity as a model of depression-like behavior, and it involves placing the rodent in an inescapable container filled with water. The FST operates on the assumption that when placed in a container with water, an animal will first attempt to escape but will eventually exhibit immobility. This immobility may be considered to reflect a measure of behavioral despair. Several other parameters can also be measured, such as the time spent struggling/climbing or swimming. Typically, the test duration is 6 minutes long and behavioral coding occurs during the last 4 minutes (mice) or 5 minutes (rats). This test has been extensively used because it exposes the animals to stress, which has been shown to have a role in the tendency for major depression. The main advantage of FST is that it is relatively simple to perform and the results are easily and quickly analyzed. Furthermore, it has a high predictive validity due to its sensitivity to a broad range of antidepressant drugs (Yankelevitch-Yahav et al., 2015).

The effects of psychedelic substances on FST have been well-documented. One study showed that N,N-dimethyltryptamine (DMT), the principle hallucinogenic component of ayahuasca, significantly reduced immobility and increased swimming times in rats relative to the control group (Cameron et al., 2018). Another study found that both psilocybin and LSD produced significant reductions in immobility time and increased swimming time, with LSD also increasing the climbing time relative to rats given saline (Hibicke et al., 2020). In mice, DOI significantly reduced the immobility time compared to controls, indicating antidepressant-like effects (de la Fuente Revenga et al., 2021).

Rationale for This Study

While psychedelics have been shown to produce long-lasting antidepressant and anxiolytic effects, it is still an open question on whether or not these effects are 5-HT_{2A} receptor-dependent. First, I aimed to take a closer look at the psychedelic psilocybin's acute effects on head-twitch response (HTR) as well as its post-acute effects on the following behavioral models: locomotor activity, novel object recognition (NOR), light-dark box preference, and forced swim test (FST). I hypothesized that psilocybin will acutely increase the number of head twitches and post-acutely decrease immobility time in the FST compared to controls, while post-acute behaviors in the locomotion, NOR, and light-dark box assays will remain unaffected. Since psilocybin was found to have a significant effect on immobility time in the FST, my next goal was to determine if the 5-HT_{2A}R played a necessary role in mediating these post-acute effects using the 5-HT_{2A}R-specific antagonist volinanserin (M100,907). I hypothesized that mice given M100,907 shortly prior to injection with psilocybin will exhibit greater immobility (or behavioral despair) compared to mice who were administered psilocybin alone. With this study, my hope was to better understand not only the post-acute antidepressant-like and anxiolytic effects of psilocybin, but also to examine whether or not these effects are in fact 5-HT_{2A}R-dependent. The results from this study can be used a model for future studies to explore the magnitude of the 5-HT_{2A} receptor's role in mediating different psychedelics' post-acute effects.

Chapter 2: Materials and Methods

Animals

Adult (10-20 weeks old) male WT C57BL/6 mice were used for all head-twitch response, locomotive function, novel object recognition, light-dark box preference, and forced-swim tests. Mice were ordered from Jackson Laboratory. All mice were housed in a vivarium in cages with up to four mice total on a 12-hour light/dark cycle at 23°C with food and water ad libitum, except during behavioral testing (always during the light cycle). Routine monitoring and veterinary care were provided with the assistance of the Virginia Commonwealth University Division of Animal Research staff. All experiments were conducted in accordance with NIH guidelines and were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and the number of animals used in these experiments.

Drugs

The IUPAC name and source of the drugs used for these procedures and experiments is as follows: 3-[2-(dimethylamino)ethyl]-1*H*-indol-4-yl]dihydrogen phosphate (psilocybin) from Usona Institute; and (*R*)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (volinanserin or M100,907) from Sigma-Aldrich. Drugs were dissolved in 0.9% saline and injection volumes and concentrations were determined based on body weight (0.005 mL/gram). All drugs were administered intraperitoneally (i.p.). Any indication for use of a vehicle refers to an equivalent volume of 0.9% NaCl administered to the animal.

Head-Twitch Response

Surgical implantation of the head-mounted magnet was performed as previously described (de la Fuente Revenga et al., 2019). First, mice were anesthetized using 2% isoflurane. Magnetic ear tag devices were created by attaching small, circular neodymium magnets to the end of standard colored ear tags with superglue. The south pole was glued to the tag with the north pole facing upwards. Ear tags were attached to the lower half of both ears, close to the head and not obstructing the ear canal. Mice underwent a week-long recovery period prior to testing.

As outlined by de la Fuente Revenga et al. (2019), data collection for automated head-twitch detection was performed in non-overlapping ~500-turn enameled wire (30 AWG) coiled around plastic cylinders (11 cm diameter x 14 cm tall). The terminals of each coil were connected to a phono preamplifier (Pyle PP444). A total of six mice could be run during one trial, with one mouse per chamber. Mice were placed into these chambers and recorded for 30 min to measure basal activity and allow for acclimation to the chamber. Mice were then removed, injected with either psilocybin (1 mg/kg, i.p.) or vehicle, immediately placed again into chambers, and monitored for 90 min. The chambers were cleaned with 1% Roccal-D in between sessions. While in the chambers, head and body movements were amplified and recorded as changes in voltage occurring with varying frequencies. The amplified signal output was recorded with MATLAB software version R2018b (MathWorks). These wavelet signals were transformed into unipolar peaks that were then identified as true HTR events based on certain criteria regarding frequency, duration, and intensity of the peak. After completion of the experiment, mice were returned to their home cages.

Locomotor Activity

Locomotor activity was monitored as previously described (de la Fuente Revenga et al., 2021). Animals were injected with either psilocybin (1 mg/kg, i.p.) or vehicle and immediately returned to their home cage. Evaluation of locomotor activity was conducted 24 hrs after drug administration. Mice were allowed to acclimate to the behavioral room for 1 hr prior to monitoring their individual locomotor activity. Mice were placed in individual locomotion chambers (25 x 25 x 20 cm open-field Plexiglas cages) with an automated detection system composed of 16 infrared light beam rays in the X and Y axes. A total of four mice could be run during one trial, with one mouse per chamber. Each trial lasted 90 min. These chambers were connected to a computerized three-dimensional activity monitoring system, Fusion (v5.3; Omnitech Electronics Inc.), that interpolates the animal's activity from interruption of infrared beams traversing the different planes of space. These interruptions are detected and can be quantified into different metrics, such as horizontal/vertical activity, rearing, and total distance. Horizontal activity was measured as the collapsed amount of beam interruptions in the X-Y planes in 5-min increments. Time spent in the central (defined as the center 8 x 8 square matrix) and peripheral (defined as the exterior 4 x 16 and 16 x 4 matrices) zones of the apparatus relative to the total test duration were also quantified. The chambers were cleaned with 1% Roccal-D in between sessions. After completion of the experiment, mice were returned to their home cages.

Novel Object Recognition

Study was conducted as previously described (de la Fuente Revenga et al., 2021). The testing arena consisted of an opaque rectangular plastic container with an open top (32 x 20 x 23 cm). The objects used (tissue culture flasks filled with wood chips and opaque white light bulbs)

were of comparable volume and height (approx. 10 cm). Objects were attached to the bottom of the container (using double-sided tape) 10 cm away from the walls. Both the objects and chambers were thoroughly cleaned with diluted ethanol between tests to remove olfactory cues. Preference for a novel object, compared to a familiar one, was tested 24 hrs after mice received either psilocybin (1 mg/kg, i.p.) or vehicle. Mice were allowed to acclimate to the behavioral room for the duration of the whole session (at least 1 hr prior to testing). A total of four mice could be evaluated during each test, with one mouse per container.

The test consisted of three stages: a 20 min habituation to the field; a 5 min acquisition trial in which two identical objects were present in the field; and a 5 min recognition trial in which one of the identical objects was replaced with a novel one. All stages were separated by 5 min during which the animals were returned to their home cage. Object exploration was defined as the animal licking, sniffing, or touching the object with forepaws while sniffing. Climbing an object was not considered in the exploration time. The exploration time for each side during acquisition (same object on both sides) and the exploration time for the novel object during recognition was assessed via digital video camera recordings. The exploratory preference for the novel object was calculated as the percentage of total exploratory time spent exploring the novel object during the recognition stage. Place preference was controlled by alternating the position of the novel object between left and right during the recognition stage. After completion of the experiment, mice were returned to their home cages.

Light-Dark Box Preference

Animals were injected with either psilocybin (1 mg/kg, i.p.) or vehicle and immediately returned to their home cage. Preference for light or dark environment was studied 24 hrs after

drug administration using a commercial open-field activity box in which space is equally divided into light and dark areas (25 x 12 x 20 cm each) connected through a small (5 x 5 cm) opening (Pais et al., 2019). Mice were allowed to acclimate to the behavioral room for 1 hr prior to testing. Four mice could be run during a trial, with one mouse per chamber. Each trial lasted 90 minutes. The boxes were connected to Fusion, which inferred animal activity and position from interruptions to infrared beams traversing the different spatial planes. The study monitored their preference for each area (light/dark). Time spent during the first three 5-min fractions and total time spent in the light zone were quantified. The chambers were cleaned with 1% Roccal-D in between sessions. After completion of the experiment, mice were returned to their home cages.

Forced Swim Test

Assay occurred as previously described (de la Fuente Revenga et al., 2021). 2 L (12.5 cm diameter x 21 cm height) beakers were two-thirds filled with water (approx. 23ºC). Mice were carefully introduced to the containers while being held by the tail so that the head would remain above water during the immersion. A habituation FST was conducted 24 hrs prior to drug injection. Procedure was conducted 24 hrs after injection with either psilocybin (1 mg/kg, i.p.) or vehicle. This protocol was repeated for two additional studies: FST 24 hrs post-injection with either M100,907 (0.5 mg/kg, i.p.) administered 15 min prior to psilocybin (1 mg/kg, i.p.) or vehicle alone; and FST 24 hrs post-injection with either M100,907 (0.5 mg/kg, i.p.) or vehicle. Mice were acclimated to the behavioral room for the duration of the whole session (at least 1 hr prior to testing). There was at least a one-week period between each FST to ensure drug washout.

Sessions were recorded on a digital video camera and immobility, climbing, and swimming times were scored during the last 4 min of the 6 min session. Immobility was defined

as passive floating with no additional activity except that needed to maintain the animal's head above water level. Climbing was defined as vertical movements of the forelimbs against the walls. Swimming was defined as horizontal movements of the fore- or hindlimbs across the water surface (Bogdanova et al., 2013). Three mice could be evaluated during each trial, with one mouse per beaker. Beakers were cleaned with 1% Roccal-D and refilled with water in between sessions. After each trial, mice were allowed to dry in a paper towel-lined empty cage placed atop a heating pad before being returned to their home cages.

Statistical Analysis

Data from the HTR test was first processed using MATLAB software. Data from the locomotion and light-dark box studies were analyzed with Fusion software. Data from Fusion for each of these studies was compiled into a large data set that measured activity based on several different metrics. Horizontal activity count and time spent in central and peripheral zones (relative to total duration) were selected as measures of locomotor activity. Time duration in the light zone was used as a metric of light-dark preference. Data from NOR and FST were collected from digital video camera recordings. Animals were randomly allocated into the different experimental groups.

Results from all studies were inputted into GraphPad Prism software version 9 for graphing and statistical analysis. Statistical significance of experiments involving time courses and two treatments was assessed by two-way ANOVA. When appropriate, Bonferroni multiple comparisons *post hoc* test was used for additional statistical analysis to examine several points of comparison. Statistical significance of experiments involving two groups was assessed using

student's unpaired t-test. The level of significance chosen was $p < 0.05$. All data are presented as mean ± standard error of the mean (S.E.M.).

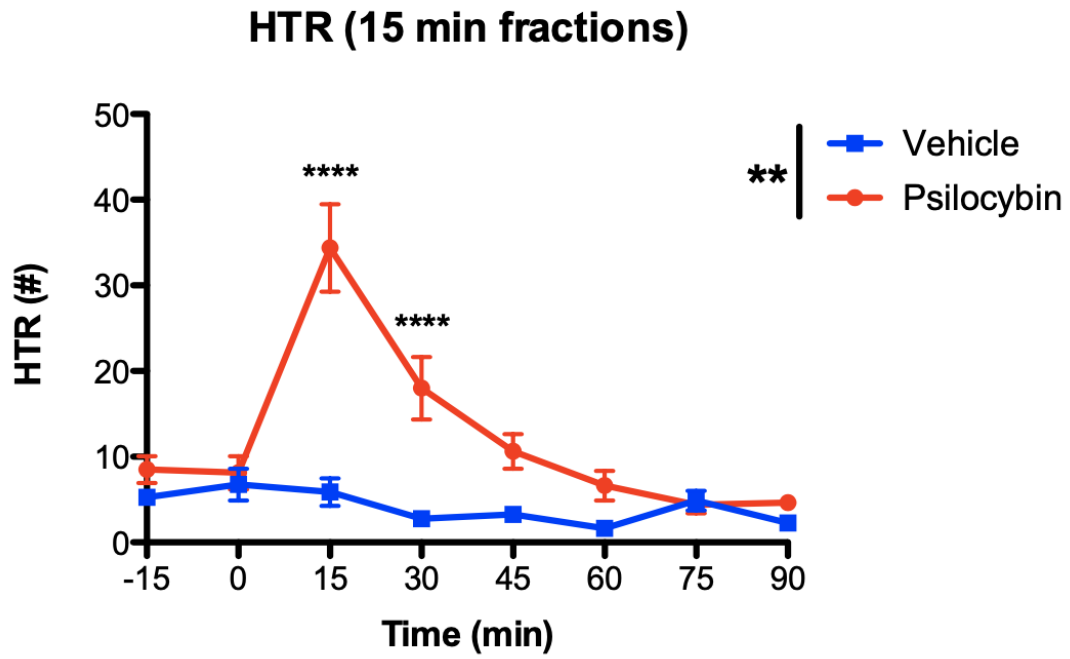
Chapter 3: Results

Effect of Psilocybin on HTR

Male WT C57BL/6 mice were prepared for the head-twitch experiment by attachment of magnet-implanted ear tags. After a week-long recovery process, they were first measured for a 30-min basal HTR. Animals were then injected with vehicle (0.9% saline) or psilocybin (1 mg/kg) and replaced into the chambers for 90 min. Data was collected in 15-min fractions and in a 30-min block following an initial 15-min habituation period.

During the 15-min fractions (*Fig. 3A*), a significant difference in head-twitch counts was observed between the vehicle and psilocybin groups (two-way ANOVA: $F(1, 14) = 13.90$, $p = 0.0022$) with an effect of time (two-way ANOVA: $F(1.878, 26.30) = 28.28$, $p < 0.0001$). There was a significant time x drug interaction (two-way ANOVA: $F(7, 98) = 22.77$, $p < 0.0001$). Bonferroni *post hoc* analysis showed significance particularly at the 15-min ($p = 0.0050$) and 30-min ($p = 0.0300$) time points. Composite head-twitch counts (*Fig. 3B*) between vehicle and psilocybin groups were also significant (student's unpaired t-test: $t = 4.941$, $df = 14$, $p = 0.0002$). These findings verified that the dose of psilocybin (1 mg/kg) was biologically active and justified its use in subsequent assays.

A.



B.

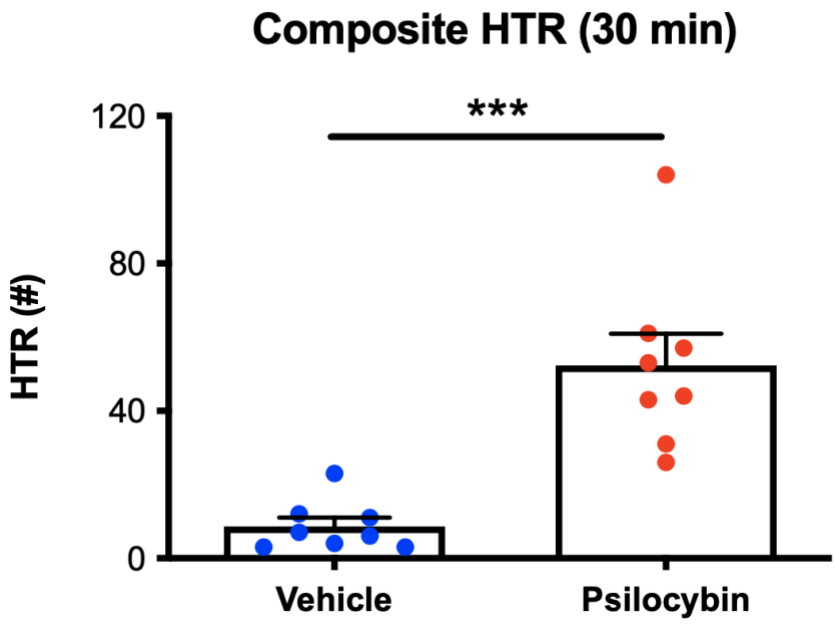


Figure 3: *Effect of psilocybin on head-twitch response in male WT C57BL/6 mice. Mice were administered vehicle or psilocybin (1 mg/kg) 24 hrs prior to testing. Number of HTR events shown in* ***(A)*** *15-min fractions (30 min baseline + 90 min testing), two-way ANOVA with*

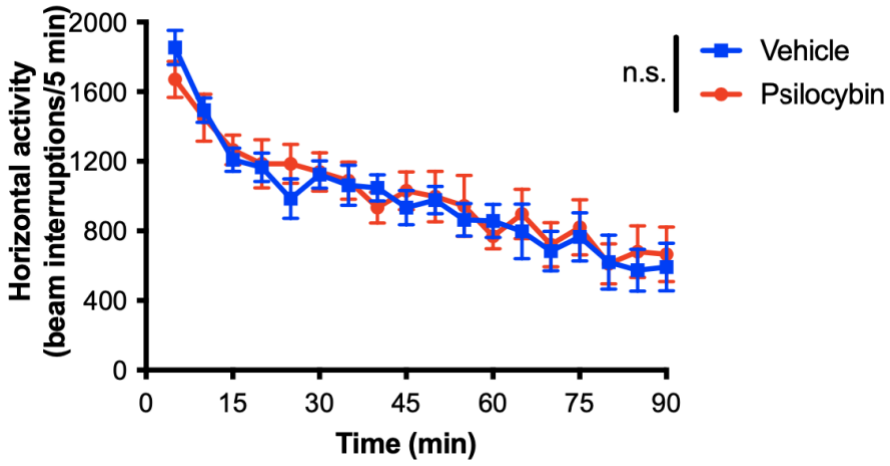
*Bonferroni post hoc test; and **(B)** a 30-min block after 15 min of habituation, student's unpaired t-test. n = 8 for each group. Data are presented as group mean ± S.E.M. (** p < 0.01, *** p < 0.001, **** p < 0.0001)*

Effect of Psilocybin on Locomotor Activity

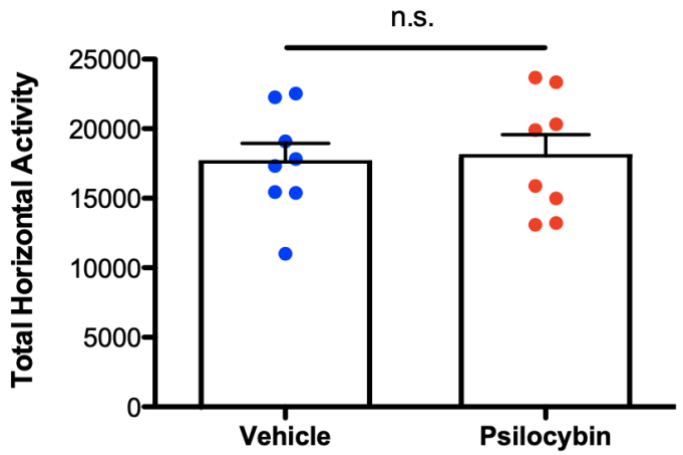
Male WT C57BL/6 mice were administered vehicle (0.9% saline) or psilocybin (1 mg/kg) 24 hrs prior to testing and were acclimated to the behavioral room for at least 1 hr before monitoring individual locomotor activity. Each mouse was placed in an individual Plexiglas locomotion chamber with an automated detection system for a duration of 90 min. Data was collected as horizontal activity (beam interruptions in 5 min fractions), total horizontal activity, and percent of total time spent in the central and peripheral zones.

During the 5 min fractions (*Fig. 4A*), there was no significant difference observed between the vehicle and psilocybin groups (two-way ANOVA: $F(1, 14) = 0.04884$, $p = 0.8283$). While time had a significant effect (two-way ANOVA: $F(7.140, 99.95) = 22.73$, $p < 0.0001$), there was no significant time x drug interaction (two-way ANOVA: $F(17, 238) = 0.5044$, $p = 0.9496$). Similarly, during the total horizontal activity (*Fig. 4B*), there was no significance between both groups (student's unpaired t-test: $t = 0.2210$, $df = 14$, $p = 0.8283$). Additionally, no difference was observed when assessing time spent in the center and in the periphery (student's unpaired t-test: $t = 0.9684$, $df = 14$, $p = 0.3493$) relative to total time (*Figs. 4C-D*, respectively).

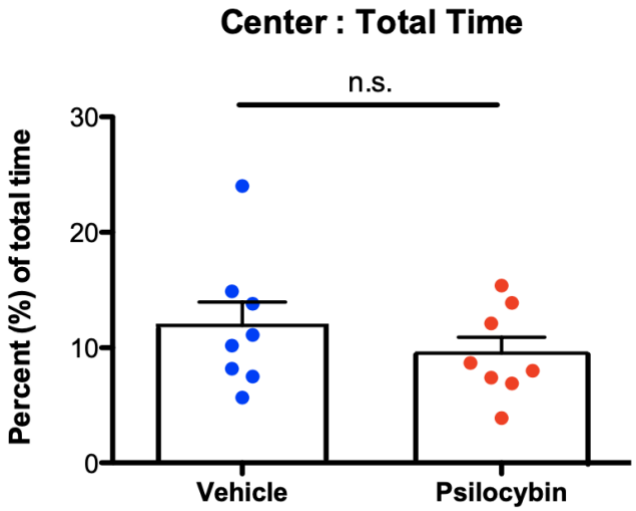
A.


Horizontal activity
(beam interruptions/5 min)
2000
1600
1200
800
400
0
0
15
30
45
60
75
90
Time (min)
n.s.
Vehicle
Psilocybin

B.


n.s.
Total Horizontal Activity
25000
20000
15000
10000
5000
0
Vehicle
Psilocybin

C.


Center : Total Time
n.s.
Percent (%) of total time
30
20
10
0
Vehicle
Psilocybin

D.

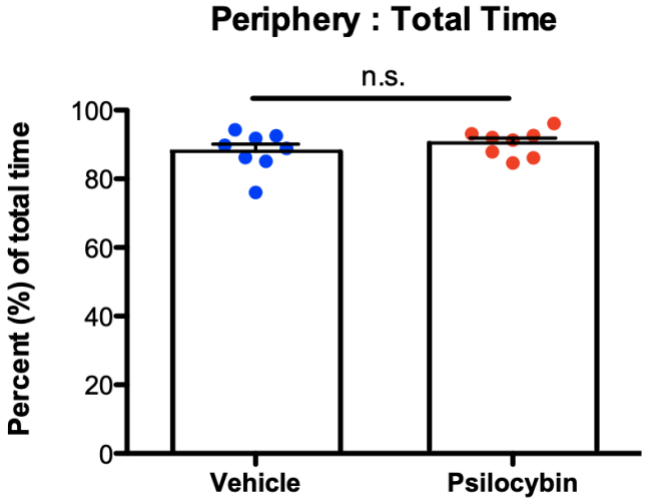

Periphery : Total Time
n.s.
Percent (%) of total time
100
80
60
40
20
0
Vehicle
Psilocybin

Figure 4: *Effect of psilocybin on locomotor activity in male WT C57BL/6 mice. Mice were administered vehicle or psilocybin (1 mg/kg) 24 hrs prior to testing.* ***(A)*** *Horizontal activity measured in 5-min fractions, two-way ANOVA with Bonferroni post hoc test.* ***(B)*** *Total horizontal activity;* ***(C)*** *Center-to-total time; and* ***(D)*** *Periphery-to-total time analyzed with student's unpaired t-test. n = 8 for each group. Data are presented as group mean ± S.E.M. (n.s. = not significant)*

Effect of Psilocybin on NOR

Male WT C57BL/6 mice were administered vehicle (0.9% saline) or psilocybin (1 mg/kg) 24 hrs prior and were acclimated to the behavioral room for at least 1 hr before testing. Each mouse was placed in an opaque rectangular plastic container with an open top, and objects of comparable height and volume were used. Testing consisted of three stages: habituation to the arena, acquisition (using two identical objects), and recognition (one familiar object replaced with a novel one). Exploratory preference was calculated for both the acquisition and recognition stages for both groups of mice.

As shown in *Fig. 5*, there was a significant difference in exploratory preference between the acquisition and recognition stages for both experimental groups (two-way ANOVA: $F(1,10) = 102.7$, $p < 0.0001$), indicating greater preference for the novel object compared to the familiar one. However, there was no significant effect of the drug (two-way ANOVA: $F(1, 10) = 1.312$, $p = 0.2787$) nor time x drug interaction (two-way ANOVA: $F(1, 10) = 0.9749$, $p = 0.3468$).

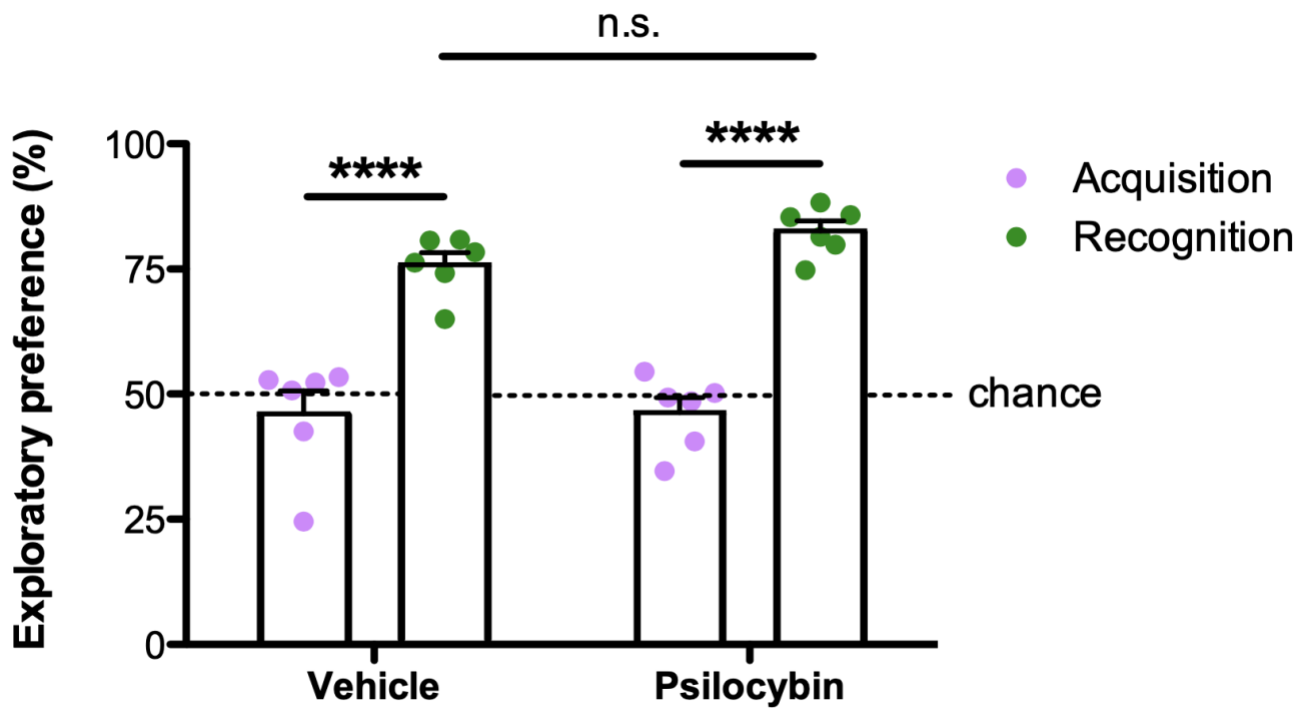


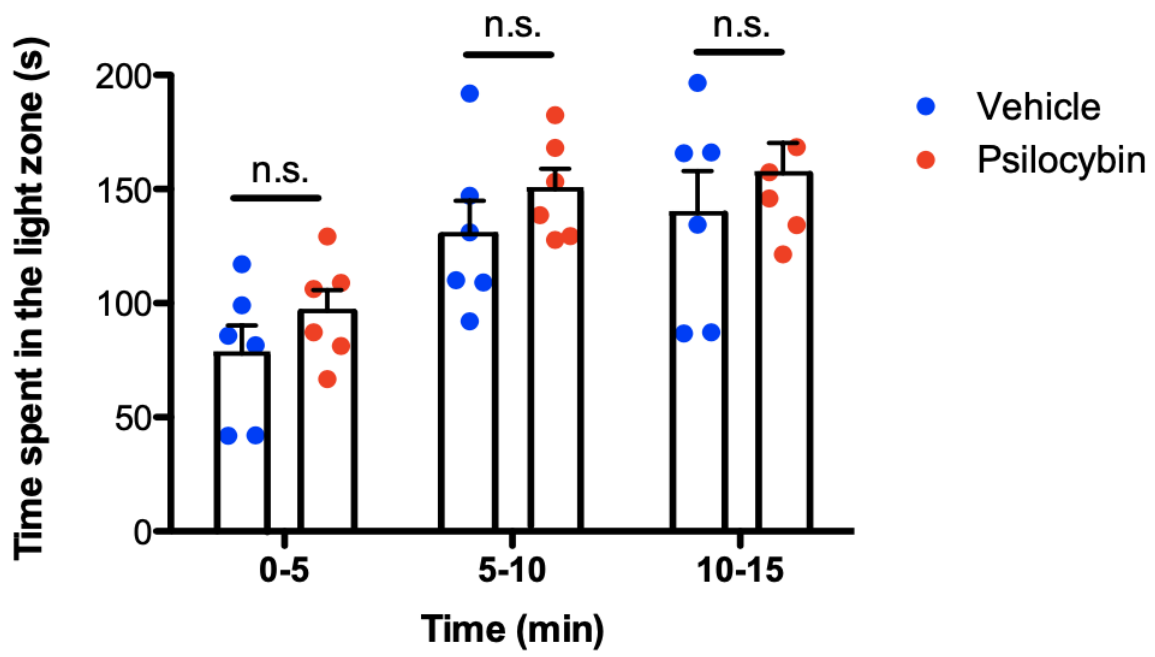
Figure 5: *Effect of psilocybin on novel object recognition in male WT C57BL/6 mice. Mice were administered vehicle or psilocybin (1 mg/kg) 24 hrs prior to testing. Exploratory preference (%) for each side during acquisition and for the novel object during recognition was assessed from digital camera recordings. n = 6 for each group. Data are presented as group mean ± S.E.M. Two-way ANOVA with Bonferroni post hoc test (**** $p < 0.0001$, n.s. = not significant).*

Effect of Psilocybin on Light-Dark Box Preference

Male WT C57BL/6 mice were administered vehicle (0.9% saline) or psilocybin (1 mg/kg) 24 hrs prior and acclimated to the behavioral room for at least 1 hr. Mice were placed in individual chambers and light-dark activity was monitored for 90 min. Each chamber was equally divided into light and dark zones with a small opening connecting both areas. Data was collected for the first three 5 min fractions and for the total 90 min duration.

During the first three 5 min fractions (*Fig. 6A*), psilocybin did not have a significant effect on time spent in the light zone (two-way ANOVA: $F(1, 10) = 1.864$, $p = 0.2021$). Although time had a significant effect (two-way ANOVA: $F(1.444, 14.44) = 17.84$, $p = 0.0003$), there was no significant time x drug interaction (two-way ANOVA: $F(2, 20) = 0.004993$, $p = 0.9950$). Likewise, there was no significant difference in total exploratory time spent in the light zone (*Fig. 6B*) between vehicle and psilocybin groups (student's unpaired t-test: $t = 1.479$, $df = 10$, $p = 0.1698$).

A.



B.

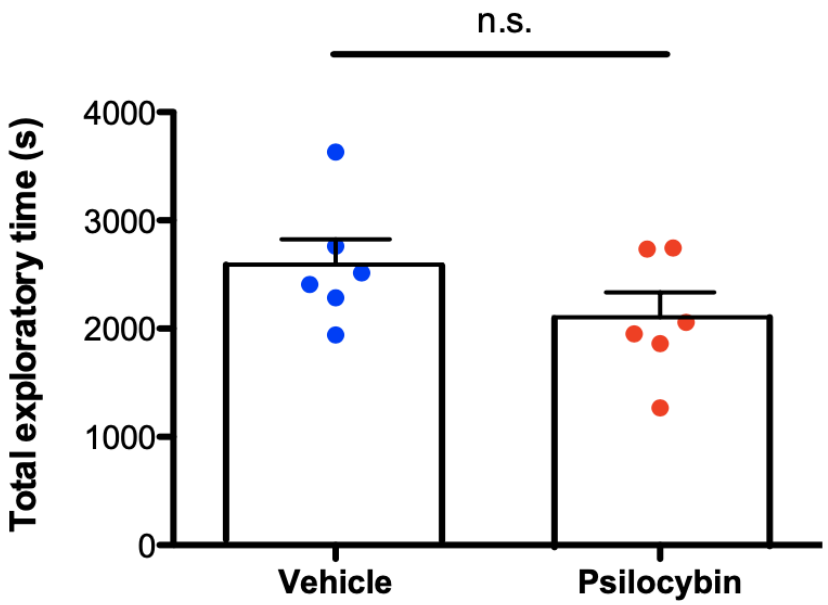


Figure 6: *Effect of psilocybin on light-dark box preference in male WT C57BL/6 mice. Mice were administered vehicle or psilocybin (1 mg/kg) 24 hrs prior to testing. Time (s) spent in light zone shown* ***(A)*** *in the first three 5-min fractions of testing, two-way ANOVA with Bonferroni*

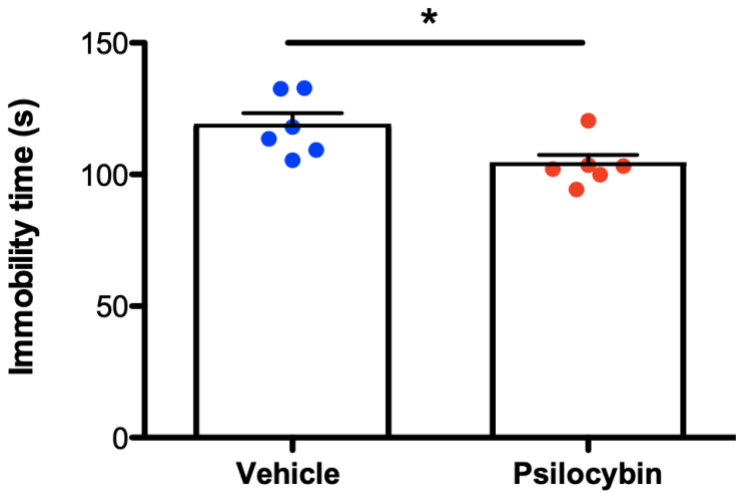
*post hoc test; and **(B)** over the total 90-min of testing, student's unpaired t-test. n = 6 for each group. Data are presented as group mean ± S.E.M. (n.s. = not significant)*

Effect of Psilocybin on FST

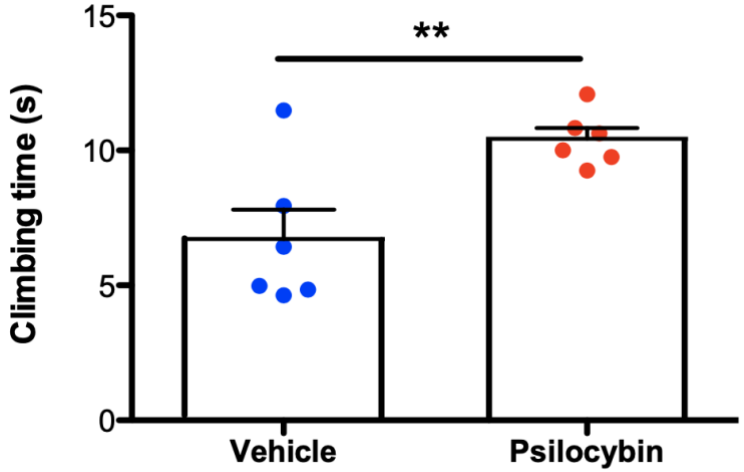
Male WT C57BL/6 mice underwent a habituation FST 24 hrs prior to administration of either vehicle (0.9% saline) or psilocybin (1 mg/kg). Drugs were injected 24 hrs prior and animals were acclimated to the behavioral room for at least 1 hr before conducting the recorded FST. Mice were placed into individual beakers that were ~2/3 filled with water and immobility, climbing, and swimming times were scored during the last 4 min of the 6 min test duration.

Mice in the psilocybin group had a significantly lower immobility time (*Fig. 7A*; student's unpaired t-test: $t = 2.462$, $df = 10$, $p = 0.0335$) and higher climbing time (*Fig. 7B*; student's unpaired t-test: $t = 3.205$, $df = 10$, $p = 0.0094$) relative to the controls. There was no significant difference in swimming time (*Fig. 7C*) between the two groups (student's unpaired t-test: $t = 1.915$, $df = 10$, $p = 0.0845$).

A.


*
150
100
50
0
Immobility time (s)
Vehicle
Psilocybin

B.


**
15
10
5
0
Climbing time (s)
Vehicle
Psilocybin

C.

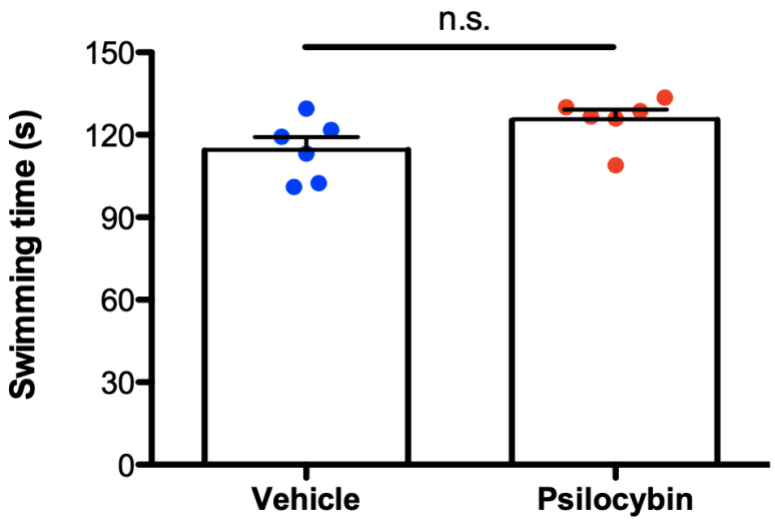

n.s.
150
120
90
60
30
0
Swimming time (s)
Vehicle
Psilocybin

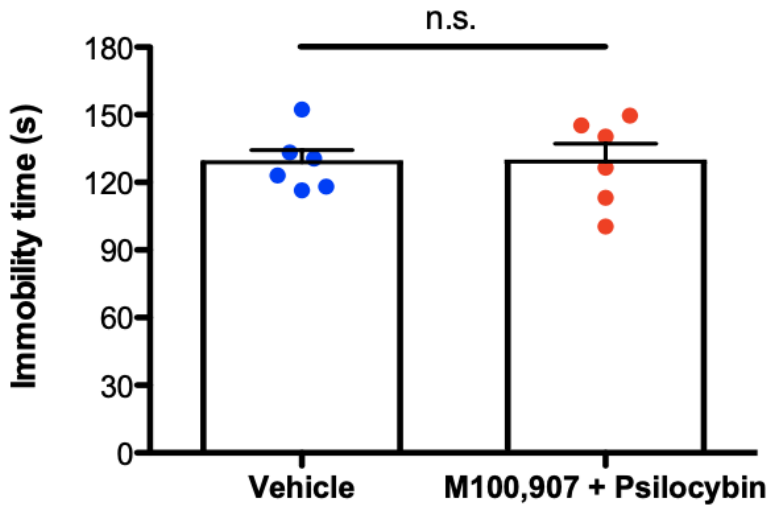
Figure 7: *Effect of psilocybin on forced swim test in male WT C57BL/6 mice. Mice were administered vehicle or psilocybin (1 mg/kg) 24 hrs prior to testing.* ***(A)*** *Immobility;* ***(B)*** *Climbing; and* ***(C)*** *Swimming time (s) analyzed with student's unpaired t-test. n = 6 for each group. Data are presented as group mean ± S.E.M. (* $p < 0.05$, ** $p < 0.01$, n.s. = not significant)*

Effect of M100,907 + Psilocybin on FST

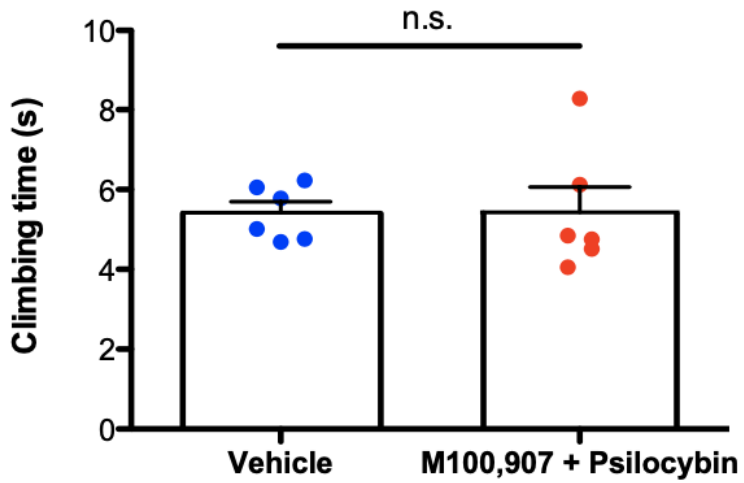
I hypothesized that psilocybin's effects on FST are 5-HT_{2A}R-dependent, and that these effects would be diminished with a selective receptor antagonist. To test this, the 5-HT_{2A}-specific antagonist volinanserin (M100,907) was administered to mice 15 min prior to injection with psilocybin. Male WT C57BL/6 mice underwent a habituation FST 24 hrs prior to administration of either vehicle (0.9% saline) or M100,907 (0.5 mg/kg) + psilocybin (1 mg/kg). Drugs were injected 24 hrs prior and animals were acclimated to the behavioral room for at least 1 hr before conducting the recorded FST. Mice were placed into individual beakers that were ~2/3 filled with water and immobility, climbing, and swimming times were scored during the last 4 min of the 6 min test duration.

There was no significant difference in immobility (*Fig. 8A*; student's unpaired t-test: $t = 0.02917$, $df = 10$, $p = 0.9773$), climbing (*Fig. 8B*; student's unpaired t-test: $t = 0.0096$, $df = 10$, $p = 0.9925$), or swimming time (*Fig. 8C*; student's unpaired t-test: $t = 0.03077$, $df = 10$, $p = 0.9761$) between both groups.

A.


n.s.
Immobility time (s)
180
150
120
90
60
30
0
Vehicle
M100,907 + Psilocybin

B.


n.s.
Climbing time (s)
10
8
6
4
2
0
Vehicle
M100,907 + Psilocybin

C.

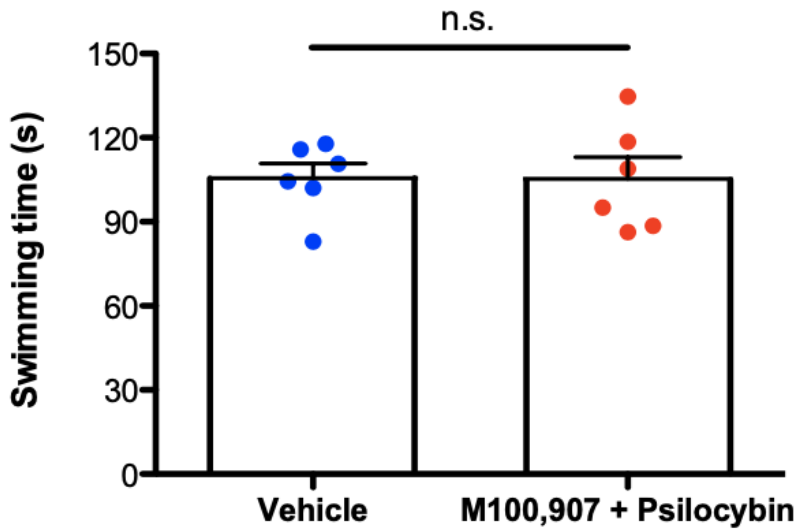

n.s.
Swimming time (s)
150
120
90
60
30
0
Vehicle
M100,907 + Psilocybin

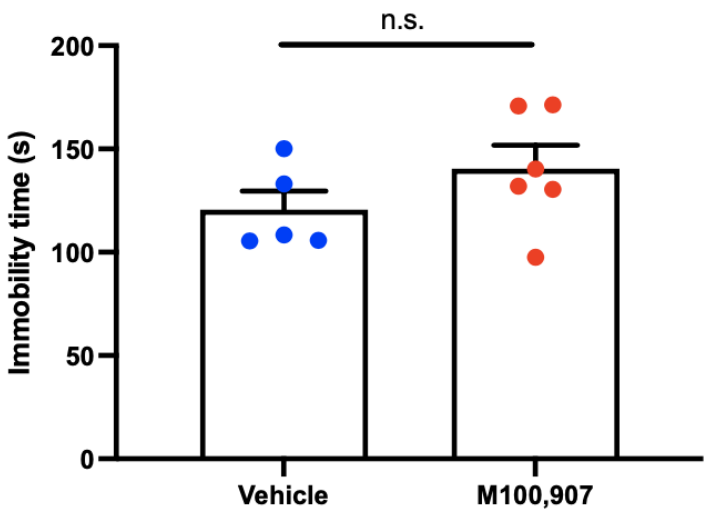
Figure 8: *Effect of M100,907 + psilocybin on forced swim test in male WT C57BL/6 mice. Mice were administered vehicle or M100,907 (0.5 mg/kg) 15 min prior to psilocybin (1 mg/kg), 24 hrs prior to testing.* ***(A)*** *Immobility;* ***(B)*** *Climbing; and* ***(C)*** *Swimming time (s) analyzed with student's unpaired t-test. n = 6 for each group. Data are presented as group mean ± S.E.M. (n.s. = not significant)*

Effect of M100,907 on FST

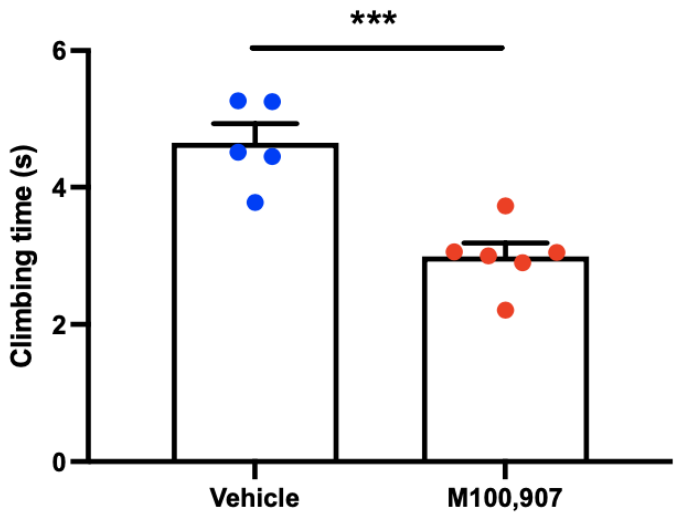
Next, I wanted to see if M100,907 alone would have an effect on immobility, climbing, or swimming time. Male WT C57BL/6 mice underwent a habituation FST 24 hrs prior to administration of either vehicle (0.9% saline) or M100,907 (0.5 mg/kg). Drugs were injected 24 hrs prior and animals were acclimated to the behavioral room for at least 1 hr before conducting the recorded FST. Mice were placed into individual beakers that were ~2/3 filled with water and immobility, climbing, and swimming times were scored during the last 4 min of the 6 min test duration.

There was no significant difference in immobility time (*Fig. 9A*; student's unpaired t-test: $t = 1.325$, $df = 9$, $p = 0.2179$) nor in swimming time (*Fig. 9C*; student's unpaired t-test: $t = 1.204$, $df = 9$, $p = 0.2594$) between the vehicle and M100,907 groups. However, mice given M100,907 had a significantly lower climbing time (*Fig. 9B*) relative to controls (student's unpaired t-test: $t = 4.980$, $df = 9$, $p = 0.0008$).

A.


n.s.
200
150
100
50
0
Immobility time (s)
Vehicle
M100,907

B.


***
6
4
2
0
Climbing time (s)
Vehicle
M100,907

C.

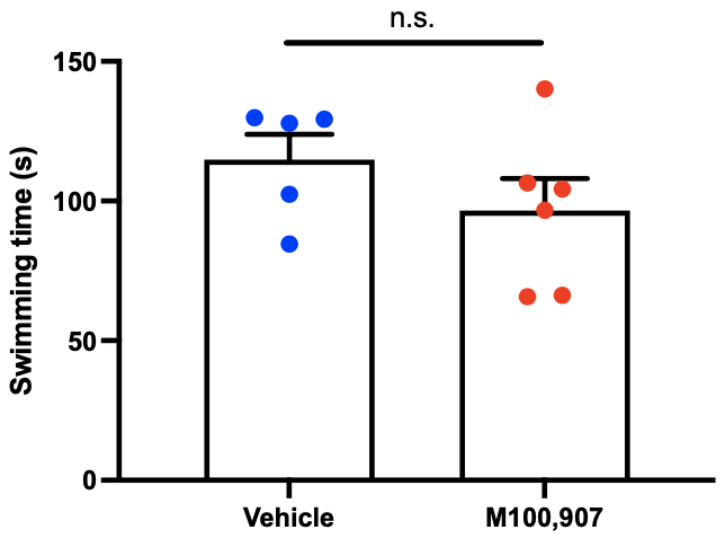

n.s.
150
100
50
0
Swimming time (s)
Vehicle
M100,907

Figure 9: *Effect of M100,907 on forced swim test in male WT C57BL/6 mice. Mice were administered vehicle or M100,907 (0.5 mg/kg) 24 hrs prior to testing.* ***(A)*** *Immobility;* ***(B)*** *Climbing; and* ***(C)*** *Swimming time (s) analyzed with student's unpaired t-test. n = 5 for vehicle and n = 6 for M100,907. Data are presented as group mean ± S.E.M. (*** $p < 0.001$, n.s. = not significant)*

Chapter 4: Discussion

Psychedelics have been shown to induce antidepressant and anxiolytic effects, indicating their potential as promising alternatives for the management of depression and anxiety. Importantly, many studies have found the 5-HT_{2A} receptor to play a critical role in mediating these effects. For instance, de la Fuente Revenga et al. (2021) showed the 5-HT_{2A} receptor to mediate DOI's acceleration of fear extinction using 5-HT_{2A}R WT and KO male mice. Another study using male rats found that DOI- and mescaline-induced, but not LSD- nor psilocybin-induced, intracranial self-stimulation (ICSS) depression was blocked with the 5-HT_{2A}R-specific antagonist volinanserin (M100,907) (Jaster et al., 2022). These varying results on ICSS depression blockade across different psychedelics underscore the need for additional work regarding the specific molecular target responsible for the effects of psychedelics. On the other hand, a few studies suggest that 5-HT_{2A}R involvement is not necessary for psychedelic-induced behavioral and electrophysiological changes (Hesselgrave et al., 2021; Shao et al., 2021). The purpose of this study was to examine the post-acute effects (i.e. 24 hrs after injection) of the psychedelic psilocybin on models of anxiety, recognition memory, and depression-like behavior. Additionally, I wanted to determine whether psilocybin's effects on behavioral despair in the FST depend on 5-HT_{2A} receptor activation by employing the selective antagonist M100,907.

As expected, psilocybin acutely induced a highly significantly number of head-twitch events compared to the vehicle group. This finding confirmed that the dose of psilocybin used (1 mg/kg) was indeed a biologically active dose and justified its use in our subsequent behavioral tests measuring psilocybin's post-acute effects. However, in our assays of locomotion,

psilocybin- and vehicle-treated mice showed no difference in horizontal activity nor in the time spent in the center and periphery of the open-field relative to the total duration. Likewise, compared to their control counterparts, mice administered psilocybin did not exhibit significant differences post-acutely in the time spent exploring the light zone in the light-dark box preference test. Additionally, while psilocybin and control mice showed strong preference for the novel object over the familiar object in the NOR assay, the exploratory preference between the two experimental groups was not significant. Together, these results indicate that psilocybin did not affect behaviors of anxiety or recognition memory when assessed post-acutely. This is consistent with previous studies using the phenethylamine psychedelic DOI, which show that behaviors in models of anxiety and recognition memory remain unchanged at least 24 hrs after administration of a single dose (de la Fuente Revenga et al., 2021).

Interestingly, psilocybin was found to significantly reduce immobility time and increase climbing/struggling time in the FST. This finding implies that a single dose of psychedelic reduces behavioral despair as a model of depression-like behavior at least 24 hrs after its administration. Even more surprisingly, administration of the 5-HT_{2A}-specific antagonist M100,907 (0.5 mg/kg) 15 min prior to psilocybin in subsequent FST assays abolished the effects of psilocybin alone on immobility and climbing times. This result indicates that psilocybin's antidepressant-like effects are in fact dependent on 5-HT_{2A} receptor activation, in contrast to prior studies claiming that psychedelic-induced behavioral changes do not require 5-HT_{2A} involvement (Hesselgrave et al., 2021; Shao et al., 2021). Furthermore, the FST with M100,907 alone revealed a significantly lower climbing time in the group receiving antagonist, while the

immobility and swimming times between the control and experimental groups were not significant.

Nevertheless, the present study had several potential limitations. These included the use of only one strain and sex of mice. Studies have shown that different strains of mice have varying responses to drugs in aspects such as locomotor activity and prepulse inhibition (van den Buuse, 2010). Thus, future studies may incorporate strains such as 129Sv or BALB/c to better characterize the hallucinogenic effects of psychedelics across a wider spectrum of genetic backgrounds. Additionally, this study only utilized male mice. Intriguingly, studies including female mice have found that psychedelics like DOI have different effects on startle amplitude and percent of prepulse inhibition compared to males (Vohra et al., 2021). Another study has shown that DMT induces distinct changes in neuronal structure in female, but not male, rats, such as reduced dendritic spine density (Cameron et al., 2019). Going forward, the inclusion of female mice could unveil sex-specific behavioral responses that were not observed in the present study. Another limitation of this study was that the mice used in the FST assays were not drug-naïve. That is, mice receiving psychedelic in the first FST received vehicle in the second FST, but were again administered drug in the third FST. Since the mice were not drug-naïve, this may explain the varying baseline of the vehicle group in the FST parameters tested. Furthermore, each mouse underwent the FST more than once (since there were three separate assays), which may have resulted in learning. This, along with the lack of drug-naivety, could have also contributed to the altering baseline values amongst the different FST assays. These limitations can be rectified in future studies by ensuring that each mouse only undergoes the FST once, and by using separate mice for each experimental group (so that all mice used are drug-naïve). Doing

so may reveal differences between the vehicle and experimental groups that were not present otherwise in this study.

In regard to future directions, there are several avenues that warrant further exploration. Given the results of the FST, which showed the necessity of 5-HT$_{2A}$ activation in order to induce psilocybin's effects on behavioral despair as a model of depression-like behavior, it would be particularly interesting to carry out these behavioral assays in mice with WT 5-HT$_{2A}$ compared to mice with null or mutant 5-HT$_{2A}$. While HTR is strongly correlated with 5-HT$_{2A}$ receptor activation and has been shown to be significantly reduced or absent in null-mutant mice (González-Maeso et al., 2003), additional studies could better examine the extent of 5-HT$_{2A}$ involvement in mediating psychedelics' effects in models of anxiety, recognition memory, and depression-like behavior. Future studies could also investigate the role of 5-HT$_{2A}$ in mediating psychedelics' effects on dendritic spine density. Structural and functional modification of dendritic spines are central to brain plasticity. Generally, increased synaptogenesis and functional plasticity are highly correlated with the size and shape of a dendritic spine (de la Fuente Revenga et al., 2021). Clinical studies have demonstrated that depression is associated with a reduction in synaptic density in the frontal cortex and other brain regions regulating mood and anxiety (Savalia et al., 2021). Very interestingly, Ly et al. (2018) found that a single dose of DMT significantly increased the dendritic spine density of cortical pyramidal neurons 24 hrs after administration. Further research would allow for a more comprehensive understanding of the degree of 5-HT$_{2A}$R involvement in psychedelic-induced behavioral and electrophysiological changes. Additionally, the present study only looked at the effects of psilocybin on the above-

mentioned behavioral models. Including a wider variety of hallucinogens, such as LSD and DMT, would permit a better evaluation and comparison of the behavioral effects of different psychedelics. Future directions may also measure the behavioral outcomes of acute vs. chronic doses, and could thus determine the dose-dependent effects of psychedelics.

Overall, this study not only reveals a post-acute effect of psilocybin on behavioral despair as a model of depression-like behavior, but also that this antidepressant-like activity is dependent on 5-HT_{2A} receptor activation. While there was no significant post-acute effect on models of anxiety or recognition memory, it highlights the need for future studies that expand on the dose-dependent effects of psychedelics on these behaviors. More importantly, this study sheds light on the central role of the 5-HT_{2A}R in mediating psychedelics' effects and opens the door for further research on the extent of its involvement through the use of genetically modified animal models.

References

Aghajanian, G. K., & Marek, G. J. (1999). Serotonin and hallucinogens. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 21(2 Suppl), 16S–23S. https://doi.org/10.1016/S0893-133X(98)00135-3

Alexander, S. P., Mathie, A., & Peters, J. A. (2011). Guide to Receptors and Channels (GRAC), 5th edition. British journal of pharmacology, 164 Suppl 1(Suppl 1), S1–S324. https://doi.org/10.1111/j.1476-5381.2011.01649_1.x

American Psychiatric Association. (2013). Diagnostic and Statistical Manual of Mental Disorders, 5(5). American Psychiatric Association. https://doi.org/10.1176/appi.books.9780890425596.dsm05

Anxiety and Depression Association of America. (2021). Facts & statistics. ADAA; Anxiety and Depression Association of America. https://adaa.org/understanding-anxiety/facts-statistics

Barnes, N. M., & Sharp, T. (1999). A review of central 5-HT receptors and their function. Neuropharmacology, 38(8), 1083–1152. https://doi.org/10.1016/s0028-3908(99)00010-6

Basith, S., Cui, M., Macalino, S., Park, J., Clavio, N., Kang, S., & Choi, S. (2018). Exploring G Protein-Coupled Receptors (GPCRs) Ligand Space via Cheminformatics Approaches: Impact on Rational Drug Design. Frontiers in pharmacology, 9, 128. https://doi.org/10.3389/fphar.2018.00128

Belouin, S. J., & Henningfield, J. E. (2018). Psychedelics: Where we are now, why we got here, what we must do. Neuropharmacology, 142, 7–19. https://doi.org/10.1016/j.neuropharm.2018.02.018

Bogdanova, O. V., Kanekar, S., D'Anci, K. E., & Renshaw, P. F. (2013). Factors influencing behavior in the forced swim test. Physiology & behavior, 118, 227–239. https://doi.org/10.1016/j.physbeh.2013.05.012

Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. European journal of pharmacology, 463(1-3), 55–65. https://doi.org/10.1016/s0014-2999(03)01274-3

Cameron, L. P., Benson, C. J., DeFelice, B. C., Fiehn, O., & Olson, D. E. (2019). Chronic, Intermittent Microdoses of the Psychedelic N,N-Dimethyltryptamine (DMT) Produce Positive Effects on Mood and Anxiety in Rodents. ACS chemical neuroscience, 10(7), 3261–3270. https://doi.org/10.1021/acschemneuro.8b00692

Cameron, L. P., Benson, C. J., Dunlap, L. E., & Olson, D. E. (2018). Effects of N, N-Dimethyltryptamine on Rat Behaviors Relevant to Anxiety and Depression. ACS chemical neuroscience, 9(7), 1582–1590. https://doi.org/10.1021/acschemneuro.8b00134

Canal, C. E., & 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 testing and analysis, 4(7-8), 556–576. https://doi.org/10.1002/dta.1333

Cini, F. A., Ornelas, I., Marcos, E., Goto-Silva, L., Nascimento, J., Ruschi, S., Salerno, J., Karmirian, K., Costa, M., Sequerra, E., Araújo, D. de, Tófoli, L. F., Rennó-Costa, C., Martins-de-Souza, D., Feilding, A., Rehen, S., & Ribeiro, S. (2019). D-lysergic acid diethylamide has major potential as a cognitive enhancer. BioRxiv. https://doi.org/10.1101/866814

de la Fuente Revenga, M., Shin, J. M., Vohra, H. Z., Hideshima, K. S., Schneck, M., Poklis, J. L., & González-Maeso, J. (2019). Fully automated head-twitch detection system for the

study of 5-HT2A receptor pharmacology in vivo. Scientific reports, 9(1), 14247. https://doi.org/10.1038/s41598-019-49913-4

de la Fuente Revenga, M., Zhu, B., Guevara, C. A., Naler, L. B., Saunders, J. M., Zhou, Z., Toneatti, R., Sierra, S., Wolstenholme, J. T., Beardsley, P. M., Huntley, G. W., Lu, C., & González-Maeso, J. (2021). Prolonged epigenomic and synaptic plasticity alterations following single exposure to a psychedelic in mice. Cell reports, 37(3), 109836. https://doi.org/10.1016/j.celrep.2021.109836

Duman R. S. (2004). Role of neurotrophic factors in the etiology and treatment of mood disorders. Neuromolecular medicine, 5(1), 11–25. https://doi.org/10.1385/NMM:5:1:011

Ennaceur A. (2010). One-trial object recognition in rats and mice: methodological and theoretical issues. Behavioural brain research, 215(2), 244–254. https://doi.org/10.1016/j.bbr.2009.12.036

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behavioural brain research, 31(1), 47–59. https://doi.org/10.1016/0166-4328(88)90157-x

Fiorella, D., Rabin, R. A., & Winter, J. C. (1995). The role of the 5-HT2A and 5-HT2C receptors in the stimulus effects of hallucinogenic drugs. I: Antagonist correlation analysis. Psychopharmacology, 121(3), 347–356. https://doi.org/10.1007/BF02246074

Gaddum, J. H., Hebb, C. O., Silver, A., & Swan, A. A. (1953). 5-Hydroxytryptamine; pharmacological action and destruction in perfused lungs. Quarterly journal of experimental physiology and cognate medical sciences, 38(4), 255–262. https://doi.org/10.1113/expphysiol.1953.sp001037

Gaddum, J. H., & Picarelli, Z. P. (1957). Two kinds of tryptamine receptor. British journal of pharmacology and chemotherapy, 12(3), 323–328. https://doi.org/10.1111/j.1476-5381.1957.tb00142.x

Glennon, R. A., Young, R., & Rosecrans, J. A. (1983). Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT2 antagonists. European journal of pharmacology, 91(2-3), 189–196. https://doi.org/10.1016/0014-2999(83)90464-8

Glennon, R. A., Titeler, M., & McKenney, J. D. (1984). Evidence for 5-HT2 involvement in the mechanism of action of hallucinogenic agents. Life sciences, 35(25), 2505–2511. https://doi.org/10.1016/0024-3205(84)90436-3

González-Maeso, J., Weisstaub, N. V., Zhou, M., Chan, P., Ivic, L., Ang, R., Lira, A., Bradley-Moore, M., Ge, Y., Zhou, Q., Sealfon, S. C., & Gingrich, J. A. (2007). Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. Neuron, 53(3), 439–452. https://doi.org/10.1016/j.neuron.2007.01.008

González-Maeso, J., Yuen, T., Ebersole, B. J., Wurmbach, E., Lira, A., Zhou, M., Weisstaub, N., Hen, R., Gingrich, J. A., & Sealfon, S. C. (2003). Transcriptome fingerprints distinguish hallucinogenic and nonhallucinogenic 5-hydroxytryptamine 2A receptor agonist effects in mouse somatosensory cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience, 23(26), 8836–8843. https://doi.org/10.1523/JNEUROSCI.23-26-08836.2003

Gould T.D., Dao D.T., Kovacsics C.E. (2009) The Open Field Test. In: Gould T. (eds) Mood and Anxiety Related Phenotypes in Mice. Neuromethods, vol 42. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-60761-303-9_1

Griffiths, R. R., Richards, W. A., McCann, U., & Jesse, R. (2006). Psilocybin can occasion mystical-type experiences having substantial and sustained personal meaning and spiritual significance. Psychopharmacology, 187(3), 268–292. https://doi.org/10.1007/s00213-006-0457-5

Halberstadt, A. L., & Geyer, M. A. (2013a). Characterization of the head-twitch response induced by hallucinogens in mice: detection of the behavior based on the dynamics of head movement. *Psychopharmacology*, *227*(4), 727–739. https://doi.org/10.1007/s00213-013-3006-z

Halberstadt, A. L., Powell, S. B., & Geyer, M. A. (2013b). Role of the 5-HT_2A receptor in the locomotor hyperactivity produced by phenylalkylamine hallucinogens in mice. Neuropharmacology, 70, 218–227. https://doi.org/10.1016/j.neuropharm.2013.01.014

Hanks, J. B., & González-Maeso, J. (2013). Animal models of serotonergic psychedelics. *ACS chemical neuroscience*, *4*(1), 33–42. https://doi.org/10.1021/cn300138m

Hesselgrave, N., Troppoli, T. A., Wulff, A. B., Cole, A. B., & Thompson, S. M. (2021). Harnessing psilocybin: antidepressant-like behavioral and synaptic actions of psilocybin are independent of 5-HT2R activation in mice. Proceedings of the National Academy of Sciences of the United States of America, 118(17), e2022489118. https://doi.org/10.1073/pnas.2022489118

Hibicke, M., Landry, A. N., Kramer, H. M., Talman, Z. K., & Nichols, C. D. (2020). Psychedelics, but Not Ketamine, Produce Persistent Antidepressant-like Effects in a Rodent Experimental System for the Study of Depression. ACS chemical neuroscience, 11(6), 864–871. https://doi.org/10.1021/acschemneuro.9b00493

Jaster, A. M., Elder, H., Marsh, S. A., de la Fuente Revenga, M., Negus, S. S., & González-Maeso, J. (2022). Effects of the 5-HT2A receptor antagonist volinanserin on head-twitch response and intracranial self-stimulation depression induced by different structural classes of psychedelics in rodents. Psychopharmacology, 10.1007/s00213-022-06092-x. Advance online publication. https://doi.org/10.1007/s00213-022-06092-x

Kraehenmann, R., Preller, K. H., Scheidegger, M., Pokorny, T., Bosch, O. G., Seifritz, E., & Vollenweider, F. X. (2015). Psilocybin-Induced Decrease in Amygdala Reactivity Correlates with Enhanced Positive Mood in Healthy Volunteers. Biological psychiatry, 78(8), 572–581. https://doi.org/10.1016/j.biopsych.2014.04.010

Krebs-Thomson, K., Paulus, M. P., & Geyer, M. A. (1998). Effects of hallucinogens on locomotor and investigatory activity and patterns: influence of 5-HT2A and 5-HT2C receptors. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 18(5), 339–351. https://doi.org/10.1016/S0893-133X(97)00164-4

López-Giménez, J. F., & González-Maeso, J. (2018). Hallucinogens and Serotonin 5-HT2A Receptor-Mediated Signaling Pathways. Current topics in behavioral neurosciences, 36, 45–73. https://doi.org/10.1007/7854_2017_478

Lueptow L. M. (2017). Novel Object Recognition Test for the Investigation of Learning and Memory in Mice. Journal of visualized experiments : JoVE, (126), 55718. https://doi.org/10.3791/55718

Ly, C., Greb, A. C., Cameron, L. P., Wong, J. M., Barragan, E. V., Wilson, P. C., Burbach, K. F., Soltanzadeh Zarandi, S., Sood, A., Paddy, M. R., Duim, W. C., Dennis, M. Y., McAllister, A. K., Ori-McKenney, K. M., Gray, J. A., & Olson, D. E. (2018).

Psychedelics Promote Structural and Functional Neural Plasticity. Cell reports, 23(11), 3170–3182. https://doi.org/10.1016/j.celrep.2018.05.022

McCorvy, J. D., & Roth, B. L. (2015). Structure and function of serotonin G protein-coupled receptors. Pharmacology & therapeutics, 150, 129–142. https://doi.org/10.1016/j.pharmthera.2015.01.009

Moreno, J. L., Holloway, T., Albizu, L., Sealfon, S. C., & González-Maeso, J. (2011). Metabotropic glutamate mGlu2 receptor is necessary for the pharmacological and behavioral effects induced by hallucinogenic 5-HT2A receptor agonists. Neuroscience letters, 493(3), 76–79. https://doi.org/10.1016/j.neulet.2011.01.046

Muttoni, S., Ardissino, M., & John, C. (2019). Classical psychedelics for the treatment of depression and anxiety: A systematic review. Journal of affective disorders, 258, 11–24. https://doi.org/10.1016/j.jad.2019.07.076

National Institute of Mental Health. (2022). Major Depression. https://www.nimh.nih.gov/health/statistics/major-depression

Nichols D. E. (2004). Hallucinogens. Pharmacology & therapeutics, 101(2), 131–181. https://doi.org/10.1016/j.pharmthera.2003.11.002

Nichols D. E. (2016). Psychedelics. Pharmacological reviews, 68(2), 264–355. https://doi.org/10.1124/pr.115.011478

Nichols, D. E., & Nichols, C. D. (2008). Serotonin receptors. Chemical reviews, 108(5), 1614–1641. https://doi.org/10.1021/cr078224o

Pais, A. B., Pais, A. C., Elmisurati, G., Park, S. H., Miles, M. F., & Wolstenholme, J. T. (2019). A Novel Neighbor Housing Environment Enhances Social Interaction and Rescues

Cognitive Deficits from Social Isolation in Adolescence. Brain Sciences, 9(12), 336. https://doi.org/10.3390/brainsci9120336

Peroutka, S. J., & Snyder, S. H. (1979). Multiple serotonin receptors: differential binding of [3H]5-hydroxytryptamine, [3H]lysergic acid diethylamide and [3H]spiroperidol. Molecular pharmacology, 16(3), 687–699.

Porsolt, R. D., Anton, G., Blavet, N., & Jalfre, M. (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. European journal of pharmacology, 47(4), 379–391. https://doi.org/10.1016/0014-2999(78)90118-8

Ross, S., Bossis, A., Guss, J., Agin-Liebes, G., Malone, T., Cohen, B., Mennenga, S. E., Belser, A., Kalliontzi, K., Babb, J., Su, Z., Corby, P., & Schmidt, B. L. (2016). Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial. Journal of psychopharmacology (Oxford, England), 30(12), 1165–1180. https://doi.org/10.1177/0269881116675512

Savalia, N. K., Shao, L. X., & Kwan, A. C. (2021). A Dendrite-Focused Framework for Understanding the Actions of Ketamine and Psychedelics. Trends in neurosciences, 44(4), 260–275. https://doi.org/10.1016/j.tins.2020.11.008

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. Journal of visualized experiments : JoVE, (96), e52434. https://doi.org/10.3791/52434

Shao, L. X., Liao, C., Gregg, I., Davoudian, P. A., Savalia, N. K., Delagarza, K., & Kwan, A. C. (2021). Psilocybin induces rapid and persistent growth of dendritic spines in frontal

cortex in vivo. Neuron, 109(16), 2535–2544.e4. https://doi.org/10.1016/j.neuron.2021.06.008

Shaw, E., & Woolley, D. W. (1956). Some serotoninlike activities of lysergic acid diethylamide. Science (New York, N.Y.), 124(3212), 121–122. https://doi.org/10.1126/science.124.3212.121

Sladky, R., Spies, M., Hoffmann, A., Kranz, G., Hummer, A., Gryglewski, G., Lanzenberger, R., Windischberger, C., & Kasper, S. (2015). (S)-citalopram influences amygdala modulation in healthy subjects: a randomized placebo-controlled double-blind fMRI study using dynamic causal modeling. NeuroImage, 108, 243–250. https://doi.org/10.1016/j.neuroimage.2014.12.044

van den Buuse M. (2010). Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. Schizophrenia bulletin, 36(2), 246–270. https://doi.org/10.1093/schbul/sbp132

Vohra, H. Z., Saunders, J. M., Jaster, A. M., de la Fuente Revenga, M., Jimenez, J., Fernández-Teruel, A., Wolstenholme, J. T., Beardsley, P. M., & González-Maeso, J. (2021). Sex-specific effects of psychedelics on prepulse inhibition of startle in 129S6/SvEv mice. Psychopharmacology, 10.1007/s00213-021-05913-9. Advance online publication. https://doi.org/10.1007/s00213-021-05913-9

Vollenweider, F. X., & Kometer, M. (2010). The neurobiology of psychedelic drugs: implications for the treatment of mood disorders. Nature reviews. Neuroscience, 11(9), 642–651. https://doi.org/10.1038/nrn2884

Vollenweider, F. X., Vollenweider-Scherpenhuyzen, M. F., Bäbler, A., Vogel, H., & Hell, D. (1998). Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2

agonist action. Neuroreport, 9(17), 3897–3902. https://doi.org/10.1097/00001756-199812010-00024

Willins, D. L., & Meltzer, H. Y. (1997). Direct injection of 5-HT2A receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. The Journal of pharmacology and experimental therapeutics, 282(2), 699–706.

Winter, J.C. Hallucinogens as discriminative stimuli in animals: LSD, phenethylamines, and tryptamines. Psychopharmacology 203, 251–263 (2009). https://doi.org/10.1007/s00213-008-1356-8

Wojtas, A., Herian, M., Skawski, M., Sobocińska, M., González-Marín, A., Noworyta-Sokołowska, K., & Gołembiowska, K. (2021). Neurochemical and Behavioral Effects of a New Hallucinogenic Compound 25B-NBOMe in Rats. Neurotoxicity research, 39(2), 305–326. https://doi.org/10.1007/s12640-020-00297-8

Woolley, D. W., & Shaw, E. (1954). A BIOCHEMICAL AND PHARMACOLOGICAL SUGGESTION ABOUT CERTAIN MENTAL DISORDERS. Proceedings of the National Academy of Sciences of the United States of America, 40(4), 228–231. https://doi.org/10.1073/pnas.40.4.228

Yankelevitch-Yahav, R., Franko, M., Huly, A., & Doron, R. (2015). The forced swim test as a model of depressive-like behavior. Journal of visualized experiments : JoVE, (97), 52587. https://doi.org/10.3791/52587