Understanding Psychedelics as Novel Therapeutics in the Treatment of Stress-Induced Psychopathology: A Mechanistic and Behavioral Exploration

By

Zarmeen Zahid

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

(Neuroscience)

At the

UNIVERSITY OF WISCONSIN – MADISON

2024

Date of Final Oral Examination: 07/19/2024

The dissertation is approved by the following members of the Final Oral Committee:
Matthew I. Banks, Professor, Anesthesiology
Cody J. Wenthur, Associate Professor, Pharmacy
Mary Halloran, Professor, Genetics
Vaishali P. Bakshi, Professor, Psychology
Christopher R. Nicholas, Associate Professor, Family Medicine and Community Health
Charles L. Raison, Professor, Psychiatry

For Mom, Dad, Umair, and Lilah

Mom and Dad, you made an immense sacrifice by moving across the world, away from your family, home, and everything familiar, all in the mere hope of providing your children with better circumstances than you had. I will spend the rest of my life being worthy of your sacrifice. Thank you for the never-ending love and support. Umair, you're my brother and my best friend, without you I would never have gotten here, thank you for the fights, the friendship, and the love. Lilah, I look at you and I see sunshine in your eyes. My sweet summer girl, this is all for you, the world is yours.

میرے والدین، عمیر، اور لیلہ کے لیے

امی اور ابو، آپ اپنے والدین سے دور جا کے سمندر پار رہے، اپنے خاندان کی کفالت کی اور اتنی زیادہ محنت کی۔ یہ سب کچھ اپنے بچوں کی زندگی کی بہتری کے لیے کیا۔ میں اپنی باقی عمر ان قربانیوں کے لائق ہونے میں گزروں گی۔ آپ کی بے انتہا محبت اور حمایت کا شکریہ۔

،عمیر، تم میرے بھائی اور سب سے اچھے دوست ہو، تمہارے بغیر میں کبھی بھی یہاں نہیں پہنچ سکتی، تمہاری لڑائیوں دوستی، اور محبت کا شکریہ۔

لیلہ، تمہاری آنکھوں میں سورج دکھتا ہے۔ میری پیاری بہن، یہ سب تمہارے لیے ہے، دنیا تمہاری ہے۔

Table of Contents

Acknowledgements

I have sat here, wrote, and deleted this starting sentence so many times that I could've written a brand-new thesis by now. I wanted something with impact, with grace, and with poise. So, let's just imagine this and get to the next little bit shall we? In this journey of life, in this journey of graduate school, I have met so many versions of myself. Some that I love and am immensely proud of and some that I think I'll leave behind here. In all of them, I find that I am a compilation of the parts that I loved in those that touched my life. The pieces of them I could not and will not let go of. A living testament to the impact that those around me have had and will continue to have long after they are gone. To sit here and bare every piece of myself to you and tell you who it comes from would likely take another thesis sized work, so I'll do my best to keep it succinct. Besides, if you know me and you're on this page, I have at least once "I appreciate you" and "I couldn't have done this without you'd" to death. I believe in expressing my appreciation often and without limits. Our time on this earth comes with a countdown clock we cannot see, and I'll be damned if I let the buzzer get one over on me.

With this, I'd like to start by acknowledging where the biggest piece of my scientific self comes from. My PI's, Dr. Matthew Banks and Dr. Cody Wenthur. Its everyone's nightmare to end up with not one, but two insanely intelligent and highly regarded bosses, and don't get me wrong, I was terrified in the beginning. Now, at the end of this all, I'm happy to report that I truly got lucky in this life. I could not have even conceived of the things I have been able to accomplish in my wildest dreams, and it is all because of what they have taught me. I very literally could not have been given the tools, the knowledge, and the confidence to conduct the explorations that I did without their guidance. Matt and Cody have never wavered in their high expectations for how good science is conducted and their high expectations for my own work. They have been present and very active mentors in my scientific journey; they let not one small data point or comma in a paper go unnoticed. In this, they have instilled in me the enormity of the work we do, the dignity and importance with which we must treat every facet of the science we conduct. They have taught me to have the utmost respect for our craft and have imparted upon me the critical thinking necessary to go out into the world without them and find success in this same way. Every meeting I would have with my lab members we would discuss some figures or some written piece and be all jazzed about how good it looks until the meeting would come to a screeching halt when one of us would inevitably ask "what would Matt/Cody say?". There would then be an audible sigh and a promise to reconvene after the flurry of changes we identified with that one question were completed. I can truly say, I am leaving here with a developed voice of reason and critical thinking in my head, but also with the voice of Matt and Cody in my head (which is arguably more impactful). It has been a pleasure and an honor to learn from you both and so from the deepest reaches of my heart, thank you for this journey.

I would be remiss if I didn't acknowledge the guiding hand of my committee members in the completion of my work. Dr. Vaishali Bakshi, our coffee chats shaped my early experiences here, thank you for your time, your wisdom, and your mentorship. Dr. Nicholas and Dr. Raison, the example of success that you have set for me forms the foundation of my aspirations. You both showed me that you can truly do it all and do it well. Thank you for your guidance, mentorship,

and support. Dr. Mary Halloran, from the very beginning your grounded point of view and insightful feedback has kept me true to the science. Thank you for your expertise and guidance in my scientific endeavors.

To my labs, thank you for all the work you have put in to make our science great. Sean Grady, your role in my work cannot be overstated. Every aspect of my early training had your guiding hand. For the time, energy, and dad jokes you have imparted over the years, thank you. Dr. Bryan Krause, our resident stats wiz, thank you for teaching me that there is always a better way. To always go back and look over the stats because you can't say that two groups are different without the tests to support this claim. To the undergrads, Jack Zheng, Aidan Zeimet, Mallory Kuppe, Leonardo Suarez, Michael Sutherland, Daleep Sandhu and Olivia Moroney, your contributions have led to this success. I can't wait to see your impact on this world. To Dr. Jill Kyzer, your patience, your feedback, and your friendship has been monumental these past years, thank you for all you do for all of us. Dr. Nathan Jones, we started this journey together and now we end together, congratulations to you and it's been an honor to do this damn thing. John Razidlo, your perseverance is inspiring, thank you for your dedication to our field and your support.

Outside of graduate school, I have been lucky enough to receive mentorship that has shaped my past and present. Dr. Anne Baldwin, the impact you have had on me is immeasurable. From the very first day I talked to you about my interests, you saw in me a future that I had yet to see for myself. If it were not for your insistence that I am capable, I would never have gotten here. For your endless love and support, thank you. Dr. Michael Lynch, you were the first person to believe in me as a scientist. Your confidence in my ideas and in our science was the catalyst for the rest of my life. You taught me that there are endless possibilities for one that is curious enough about the world and for that, I thank you. Dr. Vincent Markowski and Dr. Terence Bazzett, you gave me opportunities that shaped my neuroscientific interests. Without you, this version of myself would not exist and for that, I thank you.

Now, if we were to conduct an actual principal components analysis on my being, you would find that the above people would make up a majority of the pieces of my scientific self. However, I am a creature with many facets and now I must acknowledge my other selves. I must acknowledge those who helped me create the other versions of myself that I love and have gotten me to this point today.

To the chosen family that I met on my journey here: Ziyad Sultan, you have been here for the good times and the bad. In you, I have realized the importance of friendship, we truly cannot do any of this alone. Dr. Caitlin Murphy, you have given me confidence, power, and perspective when I so desperately needed it. Your support is a pillar of my journey and you're the confidence weapon I keep in my back pocket. Dr. Will Mayner, our bonfire hangs will be played in the highlight reel of my life. Thank you for the camaraderie we found in navigating our struggles. Aidan, you were one of the first people to work with me in the lab and the first person to realize my doubts, fears, and challenges in the work we do. You are my overthinker friend, and the one I will go to when I need kindness, understanding, and a strength I often lack. Michael, you were next to join, and it became quickly obvious to me that you would become a permanent fixture in my life. You have a fierce

intelligence and a heart made for adventure. Your ability to truly live in the moment and *see* the world has shown me that it's not *all* that bad. Mallory, my go-with the-flow California girl. Your curiosity and your calm are inspiring. You have taught me that life is long and beautiful. Leo, I truly don't know where to begin with you. Our midnight summer moped rides were my therapy. The tears I spilled into our McFlurries were some of the best times of my life. Your joy and wonder at life are all consuming. You have taught me how to laugh at everything.

To the chosen family that has been with me for many past versions of myself: Denise, talking to you is like slipping into my favorite worn cozy sweater. You know every piece of me and have shaped the ones that have made my adult self. You are the sister of my soul, the missing piece of my puzzle, and someone I cannot and will not step through life without. Your love and unwavering confidence in me have shaped me from the timid little girl I was when we met at 9, to the woman and scientist I am today 17 years later. We were girls together, and in my mind, we will always be carefree brimming with the possibilities of life playing with Polly Pockets in your basement. Kevin, my twin and my other half. You have constantly pushed the boundaries of life and redefined what is possible and what is not. Your ability to conquer what you set your mind to inspires me and has lit a fire in me to be better. Your fierce loyalty to the ones you love is not seen every day and I aspire to have has as much conviction in life as you do. Ladynel, I truly believe that every person in this world is born with multiple soulmates. Some are lucky enough to meet them in this lifetime, and I am one such person. You understand me better than I do at times. Your ability to see into my soul leaves me feeling so heard and seen it's a blessing from the universe that I was given your light in this lifetime. Your sunshine has touched every facet of my being and has left it better than when you found me. Once I get a job, you will be getting therapist fees backdated to the day I met you.

To these friends, I owe a debt I will never be able to repay. From the deepest reaches of my soul thank you for being here. I have accomplished this today because of you and would never exist in this iteration were it not for you. To you I look when my steps falter and my voice wavers.

To the family that I was blessed with: Mom, you have given me the very thing that I strive to understand most, consciousness. You sacrificed your life and your happiness for the betterment of your children. You lived a life in which you were taught that as a woman, you have the ability to occupy only a few roles: as a homemaker and as a mother. To that you said, **** no. You moved across the world with a baby (me), learned a new language by making mistake after mistake, learned independence, and gained freedom from the chains that bound you. There is no person in the world who has overcome more to achieve so much. I hope to be a fraction of the woman you are today. Thank you for instilling in me my need for freedom and independence, not just for myself, but for those who cannot attain it for themselves. For you, I will always fight. Dad, you are a mirror held up to my soul. My entire personality is perhaps copy and pasted from your own. You left behind your family, your own hopes and dreams, to do the practical thing by coming to a country across the world to work. The countless nights you went without so we could have now. Your strength to continue on despite, or perhaps in spite, of what the world throws at you has given me the sharp knife-like ability to cut through the bullshit in life that I so desperately needed. You were doubted at every turn and not once did you stop and remeasure your steps. I am who I am in whole because of you. Umair, my empathetic deeply feeling brother. You and I have experienced

it all together, and what a ride it has been so far. The closeness we share, the bond that we have goes beyond words. You have our dad's ruthlessness and our mom's compassion. In you, I find my strength to keep moving every day. You might be my younger brother, but I will always look up to you. Finally, my baby sister, the greatest love of my life, Lilah. One day you will read this and on that day, I hope you know these things about yourself that I so clearly see already. You will be the best of us. You have a heart that astounds me with how much it can feel and hold. You have a sharp wit and cleverness that shows me you are going to truly be able to accomplish whatever you set your mind to. Hold on tight to your inquisitiveness, for it does not come around often. Mom, Dad, Umair, and Lilah, because of you I have accomplished this great thing and a simple thank you will never be enough.

To all of those silent contributors who have given me their time, patience, expertise, and support: Your words and your actions have left an indelible impact on my life. You helped me do science, laugh, and reminded me that there is joy and friendship everywhere you look.

To all the mice: you are tiny creatures with a mighty impact. Thank you for your contributions to science.

And so we see that I am a sum total of all the people who I have met, loved, and learned from. To all of you, my deepest gratitude forever for helping me accomplish this feat.

Abstract

Psychedelics have been proposed to be highly efficacious in the treatment of various psychiatric conditions with preliminary evidence for efficacy in highly controlled clinical trial populations. Despite this, much is yet unknown about the therapeutic mechanism of these drugs and the time course of their effects. The neuroplastic hypothesis of psychedelic action asserts that these drugs may affect therapeutic benefit through their action as psychoplastogens [1]. Psychoplastogens are drugs that increase the growth, pruning, and reorganization of the brain in a way that is the most optimally efficient, a mechanism referred to as neuroplasticity [2]. This psychedelic induced plasticity has been assessed in many *ex vivo* and *in vitro* experiments in different regions of the brain; however, no one study has examined these changes at the circuit level.

A circuit of particular relevance for understanding the long-term behavioral and therapeutic consequences of psychedelic activity is the one that exists between the ventral hippocampus to the medial prefrontal cortex. This circuit is highly involved in working memory and emotion-based cognitions and plays a large role in the etiology of anxiety-like behaviors both in humans and in rodents [3, 4]. Through my work, I examined changes in plasticity in this pathway *in vivo* in mice using electrophysiology at 4 hours and 24 hours following psychedelic drug administration. I also assessed plasticity at 24 hours in the dorsal hippocampus alone using an *ex vivo* slice model. I found evidence for plasticity in the medial prefrontal cortex and the dorsal hippocampus at 24 hours after drug administration *in vivo* and *ex vivo*, respectively.

To contextualize these results in terms of psychopathology, I assessed changes in an assay of anxiety-like behaviors. The novelty suppressed feeding task measures approach and avoidance behaviors that are highly dependent on the action of the ventral hippocampal to prefrontal cortex circuit [5]. I found evidence in male animals for anxiolysis at 4 hours after psychedelic drug administration, but not at 24 hours. This behavioral result prompted interest in characterizing the time course of the effects of the psychedelic state. Literature suggests that there are acute changes in various domains of biological and physiological responding following psychedelic administration. One such domain is brain oscillatory power, a valuable tool in measuring brain network dynamics [6]. We found that psychedelics caused broadband changes in the hours following psychedelic administration, the peak effect of these changes occurring around 1-2 hours with the effect decaying back to baseline in the 3-4 hours following.

Furthermore, literature suggests that there is an acute increase in stress glucocorticoids following psychedelic administration [7]. I quantified corticosterone levels at various time points following psychedelic administration and found that corticosterone was significantly increased 30 minutes following psychedelic administration. Finally, I assessed the effects of psychedelic administration on a highly validated behavioral proxy of psychedelic drug action, the head twitch response [8]. I found that indeed, after psychedelic administration, the head twitch response increased 5 minutes following and decayed back to baseline from 15-20 min later. Taken together, these data add to the nascent field of psychedelic neuroscience by; 1) providing evidence for the neuroplastic hypothesis at the regional and circuit level; 2) providing evidence for behavioral change following psychedelic administration; 3) and characterizing a time course of the acute network, endocrine, and locomotive effects of the psychedelic state. With these data, I propose a three-phase model of psychedelic action. The first phase is characterized by an increase in HTR

and a rise of the subjective effects, which lead to the rise of the stress response the increase and resolution of which characterize the second phase which includes acute behavioral effects. These behavioral effects may be akin to the afterglow phenomenon observed in humans, reflecting results of a process that regulated the stress response. The final phase of the model represents psychedelic induced plasticity. This plasticity must be taken advantage of in order to lead to robust and long-lasting behavioral benefit.

1. Chapter 1: Introduction

1.1 Prevalence of stress, the Diathesis-Stress Model, and the effects of chronic stress

Despite the advances of the modern world, we find ourselves again plagued by the same uncertainties that line our history books. The occurrence of global wars, national economic crises, increasing racial tensions, and a global pandemic attest to the tumultuous times in which we live. It comes as no shock, then, that stress and stress-related diagnoses are increasing at an alarming rate worldwide, particularly in the United States (U.S.). Indeed, stress is now considered a modern-day national health crisis [9], with 21% of all adults, or approximately 50 million individuals, reporting significant stress-related mental illness, as compared to 16% of all youth, or approximately 2.7 million individuals, who report the same [10]. 55% of those adults and 60% of those youths did not receive care for their mental health issues. Alarmingly, 4.8% of adults, or 12.1 million individuals, reported serious suicidal thoughts and suicide remains the second leading cause of death among youths aged 10-14 [11]. Suicide rates have been steadily increasing for both adult males and females throughout the 21st century, and it is estimated that in 2022-2023, suicide rates reached a historic record high [12].

Undoubtedly, stress plays a significant role in the abysmal mental health outcomes observed in the U.S. and worldwide. The Diathesis-Stress model of psychological disorder etiology asserts that individuals with predispositions, be they biological, psychological, or social, may develop mental health issues when exposed to a significant stressor or chronic stress [13]. This model, though perhaps simplistic in its exposition, highlights the role of chronic stress as a major catalyst for the onset of debilitating disorders such as anxiety, major-depressive disorder

(MDD), post-traumatic stress disorder (PTSD), and even dementia [14-17]. Further, chronic stress can significantly increase the likelihood of engaging in addictive behaviors and other maladaptive coping strategies such as non-suicidal self-injury and suicidal ideation [18-20].

At the biological level, when an individual is faced with a challenging situation with which they cannot effectively cope, the hypothalamic-pituitary axis becomes activated through action of the association cortex, amygdala, and the hippocampus, resulting in a cascade of downstream effects including increased levels of circulating cortisol [21]. The sustained and chronic increase in stress glucocorticoids has been shown to effect virtually all levels of physiology. There is ample evidence demonstrating the ability of stress glucocorticoids to pass the blood brain barrier to directly affect both structural and functional changes in the brain by binding to glucocorticoid and mineralocorticoid receptors on neurons and glial cells [22].

It is important to draw a distinction between chronic stress, which unfolds over the course of weeks to years and is deleterious to long-term health, and acute stress which can last a few minutes to a few hours and is an adaptive process that can enhance memory recall, learning, and performance in a challenging situation [23]. Under acute conditions, stress glucocorticoids can redirect metabolic load and modulate the response of various physiological and neurological systems to promote adaptive cognitive changes in response to the environmental challenge [24]. Evidence suggests that acute stress induces neuroplasticity at excitatory and inhibitory synapses in regions such as the hippocampus and the cortex [25, 26]. Furthermore, evidence suggests that

acute stress induces adaptive modulation of dendritic spine size and morphology in the prefrontal cortex in animal models [27].

1.2 The role of the prefrontal-amygdalar-hippocampal triad and the effects of chronic stress

Key areas in the brain involved in regulation of emotion and cognition include the prefrontal cortex (PFC), the amygdala (AMY), and the hippocampus (HPC). These regions are highly interconnected with abundant afferent and efferent connections and work in tight coordination to maintain homeostatic balance [28, 29]. The PFC is highly involved in higher order functions such as cognitive control, set shifting, and updating of working memory, as well as executive functions such as anticipation, judgement, planning and decision making [30]. The amygdala is vital for emotional processes through its regulation of autonomic and endocrine functions [31]. Similarly important, the hippocampus plays a crucial role in learning, memory formation, and memory recall [32]. These areas share strong bidirectional connections and are integral in processes relevant to stress-induced disorder pathophysiology [33]. This highlights the importance of further investigation into this pathway.

Coordinated action of the AMY-HPC-PFC pathway is highly involved in complex learning and memory formation [34]. In the case of rodent fear conditioning, context/place associations of a shock memory are coded through distinct action of this triad. Neuronal ensembles in the ventral CA1 of the hippocampus encode the spatial information, reactivation of amygdalar neurons form the shock-context association, and action of medial PFC neurons integrates this information to form the complex learning memory [35]. Further, there is evidence that this pathway is highly involved in decision making. Inputs from the hippocampus

contextualize sensory input based on prior experience, enabling the amygdala to form model-based valuations of a decision. This allows the amygdala to coordinate with the PFC to initiate action-based processes for achieving the appropriate outcome. [36].

In understanding the crucial role of these regions in many cognitive processes, we begin to understand that the effects of stress on any one region of this triad can have wide-reaching deleterious consequences. Indeed, the link between chronic stress, cognitive impairment, and mental health outcomes has been demonstrated repeatedly and reliably [37, 38]. MDD has been associated with hypercortisolaemia, reflecting a state of increased cortisol release [39, 40]. Conversely, PTSD is associated with hypocortisolaemia, a state of reduced cortisol circulation [40]. Anxiety disorders are associated with general dysfunction of the HPA axis [41]. Circulating cortisol can directly bind receptors to have notable structural effects. These changes have been evidenced in the hippocampus, finding decreased hippocampal volume, decreased dendritic arborization and spines in CA1 and CA3 respectively, and even a suppression of granule neurogenesis [33, 42]. Conversely, in the amygdala, evidence shows increased spine density, increased excitatory synaptic input, and neuronal hypertrophy, which is associated with increased emotional reactivity [24, 43]. In the PFC, evidence indicates dendritic and global neuronal atrophy [44]. Accompanying these structural changes are a host of functional changes, including decreased plasticity in the hippocampus and weakened functional connectivity of the hippocampus, amygdala, and the prefrontal cortex [24]. These changes lead to highly dysregulated or impaired learning, emotional reactivity, decision making and memory processing, as well as global detriment to cognition [33, 37, 45]. It remains unclear whether these structural changes precede and cause the dysfunction or vice versa. Among the various psychological disorders that can arise from chronic stress, we find that stress significantly increases the

likelihood of anxiety [14, 46]. Thus, it is important to understand the current pharmacological intervention landscape.

1.3 Current state of the treatment landscape for anxiety

An estimated 31% of individuals in the U.S. will experience a significant anxiety disorder in their lifetimes, making it the most common class of psychiatric disorder [47]. There are a host of psychotherapeutic approaches that are employed to treat anxiety with or without a pharmacological adjunct and these have been shown to not only result in symptom reduction, but also in long-term well being [48]. These therapies include approaches such as traditional talk therapy, cognitive behavioral therapy, cognitive restructuring, psychodynamic therapy, and relaxation therapy [48]. This is not an exhaustive list and evidence suggests that cognitive behavioral therapy displays the greatest treatment efficacy with and without pharmacotherapy [48].

Pharmacotherapies for anxiety disorders are comprised of drugs that affect a wide range of neurotransmitter systems, such as serotonin, norepinephrine, and even GABA neurotransmission [49]. The availability of such disparate classes of drugs allows for considerable variability in clinician prescription of pharmacotherapies for anxiety. Despite this, it is estimated that only 60%-85% of individuals respond to their treatment, in that they experience behavioral improvement in some capacity, and approximately half of those individuals people achieve clinically significant recovery from their anxiety [49]. Individuals with anxiety disorders are also highly likely to experience a recurrence of their symptoms, especially if they have comorbid major depressive disorder [50]. The lifetime comorbidity for individuals with social anxiety disorder is

20-70%, for those with generalized anxiety disorder is 43%, for panic disorder is 50%, and for post-traumatic stress disorder (PTSD) is 48% [51]. This makes it considerably more complicated to treat psychopathological symptomatology as it can have a wide range of causes, highlighting the need for a novel treatment approach for both anxiety and depression.

The resurgence of research into hallucinogenic drugs has yielded incredibly promising results in the treatment of stress-induced disorders such as anxiety and depression [52]. Hallucinogenic drugs can be categorized into two classes: classical psychedelics which are active at the serotonin 2A receptor (5-HT_{2A}), and dissociative anesthetics with more variable receptor activity profiles [53]. Such compounds have demonstrated efficacy as rapid and long-lasting treatments for various indices of mental health [54].

1.4 Dissertation Aims

Psychedelics may affect therapeutic benefit through their action at many different biological levels. Of particular note is the hypothesis that psychedelics may do this through their action as psychoplastogens, which are drugs that induce plasticity or metaplasticity in the brain. Plasticity is defined as the capacity of the brain to form, prune, and reorganize both structural and functional connections in a way that is optimally efficient in response to extrinsic stimuli, where metaplasticity refers to the ability to modify subsequent plasticity [55, 56]. In my work, to test the hypothesis that psychedelics act acutely to increase plasticity and metaplasticity of mPFC neurons, I have used electrophysiological techniques in awake mice to measure synaptic plasticity in the HPC-mPFC pathway. Acute and post-acute effects of various serotonergic psychedelics on synaptic strength, and plasticity at HPC-mPFC synapses were measured *in vivo* in rodents.

Further, in an effort to understand the impact of plasticity on stress-induced behaviors, I used behavioral pharmacological techniques to measure improvements in anxiety-like approach and avoidance behaviors in rodents. Finally, to understand the role of stress in these outcomes and characterize a time course of the changes occurring after administration, I quantified circulating cortisol levels at various time points following psychedelic administration, quantified the head-twitch response, and local field potential spectral power.

The following section has been published as:
Banks, M. I., Zahid, Z., Jones, N. T., Sultan, Z. W., & Wenthur, C. J. (2021). Catalysts for change: the cellular neurobiology of psychedelics. *Molecular Biology of the Cell.*

1.5 Catalysts for change: the cellular neurobiology of psychedelics

Matthew I. Banks[a,b], Zarmeen Zahid[b], Nathan T. Jones[c] , Ziyad W. Sultan[a], and Cody J. Wenthur[b,c,d]

[a] Department of Anesthesiology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53706

[b] Neuroscience Training Program

[c] Molecular and Cellular Pharmacology Training Program, University of Wisconsin–Madison, Madison, WI 53706

[d] School of Pharmacy, University of Wisconsin–Madison, Madison, WI 53705

1.5.1. Abstract

The resurgence of interest in the therapeutic potential of psychedelics for treating psychiatric disorders has rekindled efforts to elucidate their mechanism of action. In this Perspective, we focus on the ability of psychedelics to promote neural plasticity, postulated to be central to their therapeutic activity. We begin with a brief overview of the history and behavioral

effects of the classical psychedelics. We then summarize our current understanding of the cellular and subcellular mechanisms underlying these drugs' behavioral effects, their effects on neural plasticity, and the roles of stress and inflammation in the acute and long-term effects of psychedelics. The signaling pathways activated by psychedelics couple to numerous potential mechanisms for producing long-term structural changes in the brain, a complexity that has barely begun to be disentangled. This complexity is mirrored by that of the neural mechanisms underlying psychiatric disorders and the transformations of consciousness, mood, and behavior that psychedelics promote in health and disease. Thus, beyond changes in the brain, psychedelics catalyze changes in our understanding of the neural basis of psychiatric disorders, as well as consciousness and human behavior.

1.5.2. Introduction

Human use of psychedelic drugs traces a remarkable historical arc, extending from the prehistoric realm of myth to the laboratories and clinical treatment rooms of modern day, where these compounds' mysteries are finally yielding to concerted inquiry. And yet, our purpose and that of our ancestors are one and the same: to harness the potential of these compounds for insight and healing. Central to this effort is linking the experience induced by psychedelics, herein defined as psychoactive agonists at the serotonin 5-HT_{2A} receptor in the central nervous system, and their pro-neuroplastic effects. In this review, we lay out the case for psychedelics as catalysts of change, with their neurobiological actions underlying a potential for rapid and long-lasting transformations in mood and behavior when administered within a controlled clinical setting. We focus on the current understanding of the cellular and subcellular mechanisms underlying these drugs' effects on plasticity. As the reader will undoubtedly notice, in spite of hundreds of studies

to date in both human subjects and animal models, many aspects of this understanding are still in their infancy.

1.5.3. History of psychedelics use and research

Evidence for human consumption of psychedelics in traditional medicine and religious ceremonies stretches back into prehistory [57-59]. These naturally occurring psychedelics include ayahuasca, a brew of several plants indigenous to South America containing *N,N*-Dimethyltryptamine (DMT) , 5-MeO-DMT, found in a variety of plant species as well as in the toxic secretions of the Sonoran Desert toad, and psilocybin, found in a myriad of fungus species, mostly of the genus *Psilocybe*. By contrast, lysergic acid diethylamide (LSD), an alkaloid derivative of the fungus ergot, was first synthesized in 1938 and marketed as an adjunct to psychotherapy that could enhance introspection and self-awareness. Indeed, until psychedelics were listed by the United States Drug Enforcement Agency as Schedule I drugs in 1970, thousands of studies were published on the therapeutic potential of psychedelics for psychiatric disorders and on their research potential for understanding psychosis [60]. Over the past two decades, as human subjects research has begun to revive, several small clinical trials have shown the remarkable potential of psychedelics to treat some of the most intractable psychiatric disorders. A parallel research effort has explored the neural mechanisms of psychedelics, for both their profound acute effects on perception and cognition, as well as post-acute effects underlying long-term changes in mental health.

1.5.4. Behavioral and physiological effects of psychedelics in human subjects

The acute behavioral effects of psychedelics (i.e., the 'psychedelic experience') include distorted perception of time, altered sensory perception, heightened emotional response to music, hallucinations, altered sense of self, ego dissolution, and the so-called 'mystical experience'; challenging aspects of the psychedelic experience include strong emotions with negative valence, such as anxiety, fear, and confusion [61, 62]. These challenging aspects are mirrored by an acute stress response shortly after drug administration, as indicated by increases in plasma corticosteroid levels, mild tachycardia, and elevated blood pressure [63, 64]. Although there are concerns of negative long-term consequences with drugs that produce such powerfully altered states of consciousness, there is no evidence for increased incidence of suicidality or psychiatric illnesses in the months following psychedelic experiences; in fact, studies show the opposite [65, 66]. Similarly, in spite of widespread recreational use and characterization of psychedelics as 'drugs of abuse', psychedelics present low addictive potential [67].

Multiple clinical trials over the past 20 years have demonstrated the potential of these agents to treat psychiatric disorders [68], including depression and anxiety [69, 70], substance use [71, 72], and obsessive-compulsive disorders [73]. There is a strong association between quality and intensity of the patient's psychedelic experience and the observed therapeutic benefit [62], suggesting that the phenomenology of the experience, and subsequent integration during adjunct psychotherapy, is critical to psilocybin's therapeutic effects. One or two administered doses result in rapid and prolonged changes in mood and outlook, with symptomatic relief lasting at least 3 - 12 months [74-77]. In healthy subjects, psychedelics promote long-lasting increases in well-being

and positive perspective on life experiences [78, 79]. Thus, psychedelics facilitate long-lasting behavioral changes, presumably mediated by long-lasting structural changes in the brain.

1.5.5. Animal models for investigating mechanisms of psychedelics

Essential for investigating the link between therapeutic effects in patients and underlying neural mechanisms are assays of behavioral effects of these agents in animals [80]. Among the various assays measuring the psychedelic experience in animals, the head twitch response (HTR [8, 81]), consisting of a rapid (<1 sec) shaking motion, reminiscent of a dog shaking itself dry, is the most selective and specific. Compounds such as LSD, psilocybin, and DOI that are psychedelics in human subjects induce HTRs, contrary to closely related non-psychedelic compounds (e.g. lisuride, although sufficient concentrations of serotonin alone can also induce HTRs). However, the HTR has no clear link to the phenomenology of the psychedelic experience in human subjects [82].

Mechanistic studies of drug treatments for psychiatric disorders have relied on rodent models involving behavioral and neural phenotypes induced by acute and/or chronic stressors [83, 84], but studies with psychedelics are in their infancy, and so far have not been definitive [85, 86]. By contrast, there is considerable evidence that psychedelics facilitate learning and memory in animal models. This is likely a consequence of the role in cognitive function of 5-HT_{2A}Rs, whose activation enhances various types of learning and memory [87, 88]. Particularly relevant to animal models of psychiatric disorders, psychedelics enhance both the acquisition and extinction of fear conditioning in rodents [89, 90]. The latter studies are important in two regards: First, they suggest that psychedelics can promote long-term changes in behavior likely in the

absence of self-awareness regarding the induced altered state of consciousness. Second, they demonstrate that psychedelics have the potential to support learning of new behaviors, while also facilitating extinction of older learned behaviors. This suggests a window for enhanced but non-specific plasticity that can be exploited for therapeutic benefit in partnership with mental health professionals.

1.5.6. Pharmacology of psychedelics

Psychedelics have a chemical structure similar to serotonin (5-HT) and are divided into two broad categories that differ in their receptor subtype specificity. The *indolealkylamines* (e.g., LSD, psilocybin, DMT, 5-MeO-DMT) include tryptamine- and lysergic acid-derivatives and □-carbolines and exhibit comparatively less specificity for the 5-HT_{2A}R; in particular, they show significant agonism at 5-HT_{1A}Rs as well as adrenergic and dopaminergic receptors [91, 92]. The *phenylalkylamines* (e.g., mescaline, DOM, DOI) bind more specifically to 5-HT_{2A}Rs as well as 5-HT_{2C}Rs. DOI in particular is commonly used in animal studies, but there are no published studies using this compound in human subjects.

Psilocybin is emerging as the most promising therapeutic for psychiatric disorders and has recently been designated by the FDA as a breakthrough therapy for clinical depression. Psilocybin is a pro-drug converted to the active compound psilocin in the liver via dephosphorylation by alkaline phosphatase [93]. Elements of its appeal as a therapeutic agent include its 4-6 hour duration of action, low toxicity, and large therapeutic window. The LD50 in rats is 293 mg/kg [94, 95], whereas behaviorally effective doses are 1 - 10 mg/kg; an effective dose in humans is 0.3 mg/kg [63, 96]. Psilocin is a high-affinity partial agonist at 5-HT receptor subtypes -1D, -2A,

-2B, -2C, -5, -6, and -7, and has moderate/low affinity for 5-HT -1A, -1B, as well as histamine H1, alpha-adrenergic -2A, -2B, dopamine D3 receptors.

5-HT$_{2A}$ receptor expression

Although psychedelics target a wide range of target receptors, their effects in humans strongly correlate with 5-HT$_{2A}$R occupancy and are almost entirely blocked by 5-HT$_{2A}$ antagonists [97-100], thus motivating our focus on the 5-HT$_{2A}$R. It is important to note, however, that 5-HT$_{2A}$R antagonists are not perfectly selective either, and evidence from animal models suggests the involvement of other receptors in the neurophysiological effects of psychedelics. Furthermore, the effects of psilocybin in human subjects are modulated by the 5-HT$_{1A}$R antagonist buspirone [101]. Thus, the involvement of other receptors in the behavioral and therapeutic effects in humans should not be entirely excluded [91].

Expression of 5-HT$_{2A}$R mRNA and protein in the brain is widespread, with highest density in neocortex, especially the infragranular layers. It is most prevalent in glutamatergic (pyramidal) neurons but is also expressed in inhibitory interneurons [102, 103]. Importantly, mood disorders are associated with increased 5-HT$_{2A}$R density in the brain, especially in the prefrontal cortex (PFC) [104, 105], and antidepressant treatment is associated with decreased density [106, 107], as is the administration of psychedelics [108].

Receptor desensitization and internalization play an important role in regulation of signaling [109] and are independently modifiable. For example, association with PSD-95 inhibits 5-HT$_{2A}$R internalization but does not alter the time course of desensitization [110]. Genetic

deletion of PSD-95 in rodents results in more rapid turnover of 5-HT_{2A}R, reduced dendritic expression, and loss of HTR response to psychedelics [111]. Furthermore, the low addictive potential of psychedelics may be a result of this rapid desensitization and 5-HT_{2A}R receptor internalization (i.e., tachyphylaxis) that occurs in response to single doses of psilocybin and other psychedelics [112, 113].

5-HT_{2A} receptor signaling

The 5-HT_{2A} receptor is a Class A, rhodopsin-like, G-protein-coupled receptor (GPCRs) [114] at which serotonin, the endogenous ligand, is a full agonist with a pKd of 8.9 [115]. Canonically, 5-HT_{2A}Rs couple with the G-protein G_q (Figure 1), catalyzing the production of phospholipase C and inositol triphosphate to mobilize intracellular calcium, activate calcineurin, and inhibit voltage-gated calcium channels (Cav1.2) [116, 117]. In addition, 5-HT_{2A}R coupling with G-proteins from the $G_{\alpha/i}$ family and subsequent inhibition of cAMP formation is also observed [118]. Either of these pathways affords ample opportunity to modify synaptic transmission and plasticity, including through liberation of $G_{\beta\gamma}$ subunits [119, 120]. Depending on physiological context, 5-HT_{2A}R binding can also lead to production of arachidonic acid, 2-AG, and can exert neuromodulatory effects through PLA2, PLD, ERK1/2, NO, CREB, Akt, Fos, TGF-beta, and JAK/STAT [121].

Biased agonism.

Psychedelic agonists at the 5-HT_{2A}R are associated with profound changes in perception and cognition, while other ligands such as serotonin are not. The basis for this difference is

commonly explained using the ternary complex model of receptor activity, which suggests that drug molecules act to shift the equilibrium between a receptor's different conformational states. As each of these receptor states has a different affinity for various downstream binding partners, this can result in a drug exhibiting 'bias', such as preference for activating G-protein-dependent versus β-arrestin dependent signaling [122]. Long-standing evidence indicates that 5-HT_{2A}R signaling efficacy is ligand-dependent, and prone to biased agonism across these pathways [114, 123-127]. Molecular modeling and site-directed mutagenesis initially identified the distinct psychedelic and non-psychedelic ligand binding sites that ultimately lead to these differential functional signaling consequences [128-130]. Most recently, X-ray crystallography studies of the canonical 5-HT2AR-Gq complex bound with psychedelics have pinpointed residues critical for psychedelic agonist activation, such as W335 (6.48), and for coupling to intracellular signaling partners, such as N317 (6.29) and I181 (ICL2) [131-133].

In the case of Gq-dependent signaling, both psychedelic (DOI) and non- psychedelic (lisuride) agonists of the 5-HT2AR activate several share downstream pathways, including pPLC, pPKC, pERK, and pCREB; however, the *magnitude* of these Gq-mediated responses is greater for psychedelic ligands [134]. Non-G_q pathways likely contribute to psychedelic activity as well. For example, in mice lacking G_q expression, the response to DOI is blunted, but not eliminated [135]. Furthermore, psychedelic-dependent phosphoproteomic and transcriptomic signatures are pertussis toxin-sensitive [136, 137], suggesting the involvement of $G_{i/o}$ signaling. Notably, $G_{i/o}$-

dependent outcomes may depend on participation of additional signaling partners, as 5-HT_{2A}R does not appear to participate directly in functional coupling with $G_{i/o}$ [133].

Beyond G-protein-coupled pathways, agonism at 5-HT_{2A}Rs can also activate β-arrestin signaling [138] via PI3K, SRC, and AKT (Figure 1). Our understanding of the relevance of such β-arrestin dependent signaling in promoting psychedelic activity is limited at present. For example, while LSD's ability to promote a β-arrestin biased conformation at 5-HT_{2A}Rs and 5-HT_{2B}Rs is a relevant feature supporting its psychedelic effects [131, 133], N-methyltryptamine psychedelics are functionally insensitive to β-arrestin knockout in mice [139, 140]. Close attention to differences in off-target binding profile [91, 92] and time-dependent evolution in signaling [131] are needed for future studies to yield a more comprehensive picture of β-arrestin effects across the full spectrum of psychedelic ligands. Likewise, such studies should distinguish between β-arrestin *recruitment* and β-arrestin *bias*, as the former is correlated with human recreational dosing of synthetic psychedelics, while the latter is not [138, 141].

Intersection with glutamate signaling.

Glutamate signaling hypofunction is a prominent feature of schizophrenia [142] and major depressive disorder [143], and can be ameliorated by antidepressant administration [144, 145]. Interestingly, psychedelics cause an increase in extracellular glutamate in the PFC [146, 147], resulting in the release of pro-neuroplastic neurotrophic factors [148-150], as well as activation

of ionotropic glutamate receptors (i.e. NMDAR, AMPAR), which contribute to plasticity via NMDAR-dependent processes [151].

Glutamate release also activates metabotropic glutamate receptors (mGluR), which regulate neuronal plasticity [152] and modulate the effects of psychedelic agonism at 5-HT_{2A}Rs. mGluR2 and mGluR2/3 agonists suppress the response to psychedelics, reducing their effects on neuronal activity and cellular signaling and the frequency of HTRs, as does (paradoxically) knocking out mGluR2 receptors [153-156]. Agonism at 5-HT_{2A}R and group II mGluR receptors has reciprocal influences on receptor expression: chronic treatment with the mGluR2 antagonists causes a reduction in 5-HT_{2A}R expression and behavioral and genomic response to LSD [157], while chronic treatment with a 5-HT_{2A}R agonist reduces mGluR2/3 expression and behavioral responses to an mGluR2/3 agonist [158].

This interplay between mGluR2 and 5-HT_{2A}R signaling may arise due to physical (transmembrane) interactions between the receptors [154], which are expressed within close molecular proximity in the mouse PFC [159, 160]. Several studies indicate that 5-HT_{2A} and mGlu2 receptors form heterodimeric complexes that alter their respective downstream signaling, integrating glutamatergic and serotonergic signaling and modulating subsequent G-protein coupling and downstream effects [153, 154, 161]. For example, when cells co-expressing mGluR2 and 5-HT_{2A}R receptors are treated with mGluR2/3 agonists, signaling is propagated through $G_{q/11}$, rather than through the $G_{i/o}$ pathway usually activated by mGluR2. This effect is reduced in 5-HT_{2A}R -/- mice [161], suggesting that 5-HT_{2A}Rs and Group II mGluRs can form heterocomplexes with a distinct signaling profile. Importantly, signaling through this complex may facilitate the rapid antidepressant effects observed with psychedelics, as group II mGluRs

have been implicated in the regulation of PFC, hippocampal, and amygdalar plasticity, as well as fear- and stress-associated learning [162].

1.5.7. Cellular and network-level electrophysiological effects of psychedelics

The signaling pathways activated by psychedelic agonism at the 5-HT_{2A}R provide a basis for structural changes in the brain following the psychedelic experience (Figure 1). How these long-term changes relate to acute effects on brain activity and connectivity is a critical but unresolved question. Psychedelics acutely modulate neural activity and connectivity, and these changes correlate with the phenomenology of the psychedelic experience. For example, psychedelics reduce alpha power in electro- and magnetoencephalographic signals recorded from human subjects [98, 163-165]. The alpha rhythm arises most prominently when subjects have their eyes closed and visual cortical networks are not actively processing sensory information [166]. The reduction in alpha power in subjects administered psychedelics (with eyes closed) correlates with effects on visual imagery [98, 167], suggesting that visual cortical structures are actively processing internally-generated visual information. Similarly, responses to sensory stimulation are suppressed [168-170], consistent with a transition from externally- to internally-driven cortical activity patterns. Psychedelics induce increases in brain signal complexity (an indirect measure of signal diversity and information content), and these changes also correlate with specific aspects of the psychedelic experience [171]. These changes in complexity likely reflect increased information flow along pathways that are not typically engaged in the absence

of the drug. Similarly, synesthesia is a common element of the psychedelic experience [172 8653, 173], thought to rely on existing but dormant connections between unimodal sensory areas [174].

More direct measurements confirm that psychedelics alter connectivity in the brain [175-177], and this plasticity may underlie their therapeutic activity. For example, excessive amygdala reactivity and decreased amygdala and especially amygdala-PFC connectivity are linked to psychiatric disorders [178, 179]. Psychedelics alter connectivity between the amygdala and key brain regions during emotional stimulus processing, effects that may contribute to altered behavioral and neural responses to these stimuli [180, 181]. Psychedelics acutely reduce connectivity within the default mode network (DMN) [182, 183] , a set of brain regions whose activity and connectivity correlate with self-referential mentation in healthy subjects [184] and rumination and depression symptoms in patients [185, 186]. However, longer-term effects of psychedelics on the DMN are inconsistent [54, 187]. More broadly, psychedelics administered to healthy volunteers alter functional connectivity in multiple higher order brain regions [188, 189], and increase between-network connectivity as well as global connectivity [99, 175, 176, 190]. Interestingly, there is some evidence that psychedelics acutely decrease connectivity within canonical resting state networks, suggesting decreased differentiation among brain areas [190, 191]. Thus, there are clear acute changes in connectivity in brain regions that are implicated in

multiple psychiatric disorders, but the mechanisms of these changes and whether these changes persist post-acutely remain unclear.

To study the mechanisms of these changes in connectivity, and how they might relate to long-term pro-neuroplastic effects of psychedelics, we would naturally turn to animal models for more in-depth investigations. However, it is in this context that the complexity of the effects of psychedelics in the brain as well as potentially important inter-species differences come to the fore. For example, it is often asserted that psychedelics have broad excitatory effects in the brain [192, 193], but the evidence is decidedly mixed. Activation of 5-HT_{2A}Rs by psychedelic agonists in acute brain slices induce increase the frequency of spontaneous glutamatergic excitatory post synaptic currents (EPSCs) and NMDAR-dependent network-level excitatory states [194, 195]. Furthermore, the increase in glutamate in PFC mentioned above and the induction of cFos expression [137, 196] are consistent with excitatory effects. By contrast, most studies *in vivo* in animals show that psychedelics suppress neuronal activity or at most have mixed excitatory/inhibitory effects [197-201]. In human subjects, psychedelics consistently induce broadband decreases in brain signal power [163, 165], and any link between these changes and increased excitability is at best indirect [164]. These contradictory observations may arise due to 5-HT_{2A}R-mediated excitation of excitatory versus inhibitor cells in the brain [102, 103], and also due to activation of 5-HT_{1A}R versus 5-HT_{2A}Rs [202, 203], which have opposing effects on membrane excitability [204, 205]. Importantly, differences between effects in human subjects versus animal models may relate as well to species-specific receptor expression patterns [99, 102] and structural differences in the 5-HT_{2A}R [109].

1.5.8. Neural plasticity

The changes in functional connectivity in human subjects described above, along with suppressed adaptation to standard tones and suppressed deviant responses in the auditory oddball paradigm [168], are manifestations of acute effects on neural plasticity. Both acute and long-term effects on neuronal plasticity figure prominently in current models of psychedelic action [193, 206]. Increased expression of neural plasticity, especially in the hippocampus and prefrontal cortex, is associated with symptomatic relief in psychiatric disorders in patients [207] and reversal of behavioral phenotypes associated with chronic stress in animal models [208].

Acute effects of psychedelics on synaptic plasticity have been studied in brain slice preparations. At thalamo-cortical synapses on pyramidal cells in murine mPFC, DOI acts on presynaptic 5-HT_{2A}Rs to facilitate glutamate release and enhance glutamate postsynaptic currents via a mechanism that also relies on presynaptic NMDARs [209]. At the same synapses, DOI also acts on postsynaptic 5-HT_{2A}Rs to cause a decrease in evoked AMPAR-mediated excitatory postsynaptic currents independently of NMDARs [210]. The mechanism for this latter effect overlaps with that of one form of long-term depression (LTD), that induced by low frequency stimulation. However, at these same synapses, DOI also acts presynaptically to facilitate expression of t-LTD, which unlike standard LTD is a form of spike timing-dependent plasticity and is a molecular model of associative learning.

Long-term structural changes induced by psychedelics manifest particularly in spines and neurites of cortical pyramidal neurons and in de-novo neurogenesis in hippocampus. DOI acts on 5-HT_{2A}Rs to increase spine diameter in cultured cortical pyramidal neurons via PAK/Kalirin-7

signaling [211]. Furthermore, psychedelics enhance de-novo neurite formation and spine formation through the TrkB, mTOR, and 5-HT_{2A} pathway [212], all implicated in promoting plastic changes in the PFC [213]. Finally, psychedelics induce neurogenesis in rodent dentate gyrus under the same conditions in which they facilitate learning [90, 214, 215].

These long-term effects of psychedelics are likely mediated at least in part by changes in gene expression [216]. A single administered dose of psilocybin induces changes in plasticity-related gene expression in hippocampus and PFC [217], and 5-MeO-DMT induces large-scale changes in protein expression in human cerebral organoids [218]. Evidence suggests that changes in gene expression manifest in selected subtypes of cortical cells, adding to the specificity as well as complexity of long-term psychedelic effects [219]. Psychedelics interact particularly with signaling by the neuroplasticity-associated protein brain-derived neurotrophic factor (BDNF). DOI induces upregulation of BDNF mRNA via 5-HT_{2A}Rs in neocortex, and a decline in dentate gyrus, likely via an activity-dependent mechanism and CREB signaling [220-222]. DOI increases expression of Arc mRNA via a pathway that depends on glutamate and BDNF signaling [223-225]. Psychedelics trigger increases in plasma levels of BDNF in human subjects [148-150]. Thus, psychedelics are able to mobilize multiple signaling pathways to promote neuroplastic changes in the brain. Given the importance in clinical trials of post-acute psychotherapy sessions, and the extended time course of some of signaling cascades mentioned here, it seems likely that the pro-neuroplastic effects of psychedelics extend beyond the dosing session, i.e. that psychedelics open a window of plasticity that lasts for days or weeks. However, this idea has not yet been tested experimentally.

1.5.9. Stress and Psychedelics

The intersection between psychiatric disorders, stress, and psychedelics is an emerging area of interest [226, 227]. Chronic stress is a major precipitating factor in the etiology of many psychiatric disorders that psychedelics have shown clinical efficacy in treating [228]. Animal models have shown that chronic stress induces behavioral and neural changes that can be reversed by antidepressants [83, 84, 208]. Thus, it is both surprising and intriguing that acute stress may play a role in promoting the neuroplastic effects of psychedelics in the context of treating these same disorders. Like psychedelics, acute stress is pro-neuroplastic [229, 230], and signaling at the 5-HT_{2A}R plays a role in this plasticity [226]. Along with this convergence on plastic mechanisms to modify behavior, psychedelics trigger an acute biochemical stress response consisting of catecholamine and glucocorticoid release [63, 231, 232], and it has been postulated that this stress response is critical for the transformative nature of the psychedelic experience [227].

The mechanism underlying this stress response is unclear, but there are two clear (and not necessarily mutually exclusive) possibilities. First, psychedelics may act directly at 5-HT_{2A}Rs in hypothalamus to induce expression and/or release of corticotrophin releasing hormone (CRH), elevating plasma concentrations of stress-associated glucocorticoids [233, 234], consistent with the established regulatory role of 5-HT in hypothalamic-pituitary-adrenal axis function [235]. Alternatively, the psychedelic-induced altered state of consciousness itself may trigger the acute stress response, as such states frequently include components that are stressful (e.g., fear- or anxiety-provoking). This raises the possibility that the neuroplastic effects of psychedelics depend in part on the acute stress response they induce, but whether the stressful components of the

psychedelic experience are hindrances to or foundational for therapeutic benefit is in dispute [61, 62, 236].

1.5.10. Inflammation and Psychedelics

The therapeutic and pro-neuroplastic effects of psychedelics may also be linked to their anti-inflammatory action [237]. Inflammation and immune system alterations are associated with psychiatric disorders [238, 239], and inflammatory cytokines directly modulate synaptic function and plasticity in the CNS [240, 241]. Importantly, recent work shows that psychedelics have potent inhibitory effects on inflammatory cytokine cascades, which is unsurprising given the primacy of serotonin in immune system function [242]. Psychedelics induce reductions in systemic markers of inflammation [243, 244] and act on 5-HT_{2A}Rs to inhibit peripheral production of proinflammatory cytokines [245, 246]. These data suggest that therapeutic activity of psychedelics may relate to their effects on systemic inflammation, but there may be more direct effects on inflammation in the brain as well. Microglia, the resident immune cells in the brain, express several 5-HT receptor subtypes [247, 248], and respond directly to DOI treatment [248]. This raises the possibility that systemically administered psychedelics could directly modulate microglial function, which becomes aberrant in mood disorders [249] as well as in neurodegenerative disorders [250]. The therapeutic potential for the anti-inflammatory effects of psychedelics are just beginning to be explored, including a phase 1 clinical trial in healthy older volunteers with the goal of using LSD to treat Alzheimer's disease [251].

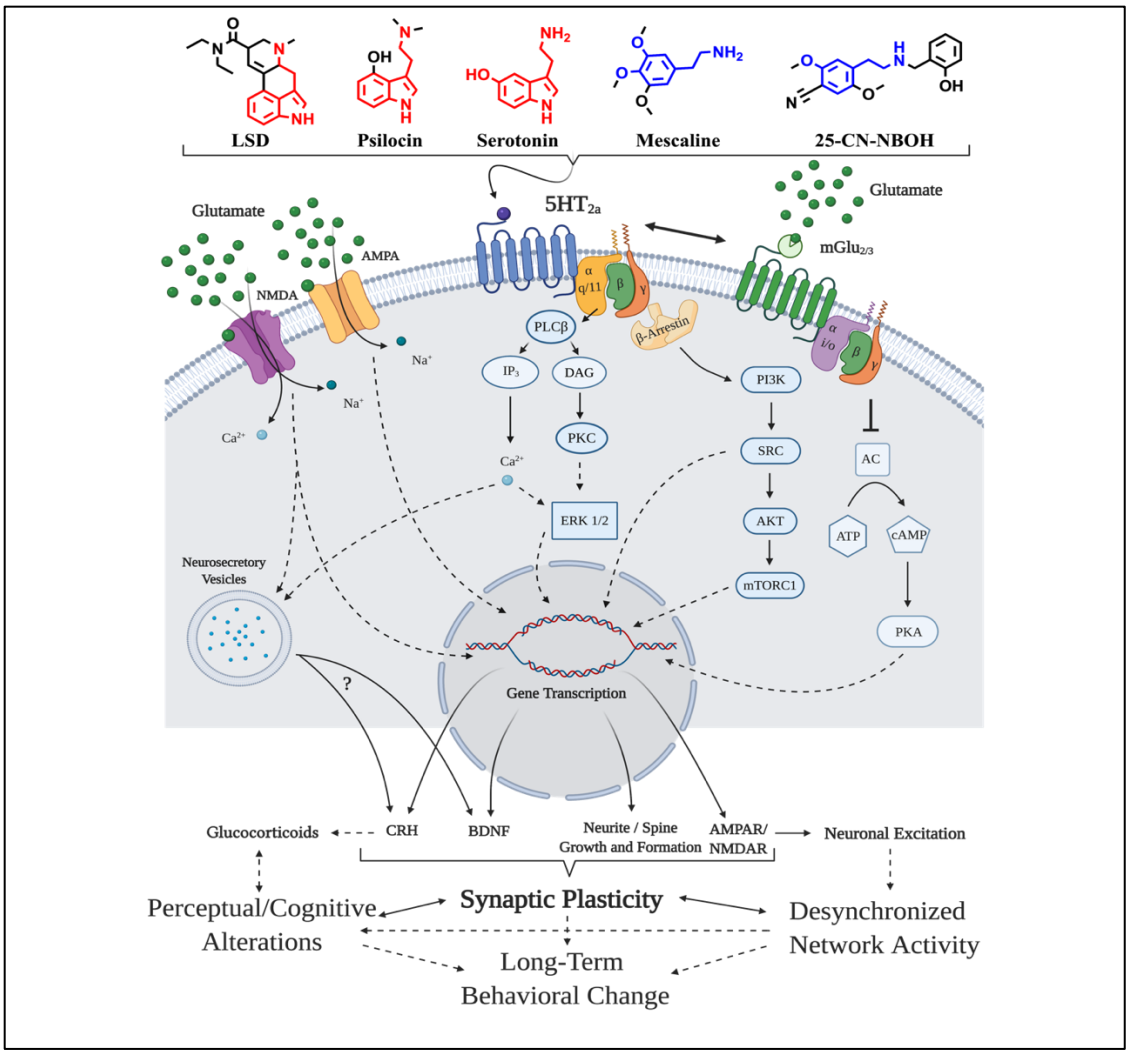
As psychedelic science emerges from the shadows and attracts new resources, further research will deepen our understanding and advance the responsible therapeutic use of these

compounds. Psychedelics induce acute effects on neurotransmitter and neuroendocrine release, neuronal excitability, network connectivity, and perception and cognition, ultimately leading to profound long-term effects on mood and behavior (Figure 1). Elucidating the mechanisms that connect the acute effects of psychedelics to these long-term changes is critical but is complicated by our limited mechanistic understanding of the psychiatric disorders these drugs are effective at treating. Thus, understanding how these drugs work in a clinical setting may also provide needed insight into the neurobiological basis for psychiatric illnesses. Within the context of psychedelics as change agents, an important next step is to identify long-term changes in brain structure that relate to therapeutic activity, which will require support from both clinical and pre-clinical research programs.

Of primary importance are longitudinal clinical studies with sufficient enrollment to identify changes in brain activity and connectivity; these would presumably depend on organizing multi-center trials. These studies should involve both baseline and multiple post-acute neuroimaging scans (structural and functional MRI) to examine the trajectory of changes in the brain, as well as EEG data collection during the dosing session to allow comparisons between these pre/post changes to the neural manifestation of the psychedelic experience. We suggest several fruitful avenues for these imaging studies. Structural and functional connectivity measurements will serve as a foundation for interpreting changes in brain activity and behavior. Task-related functional imaging should be used to identify changes in neural activity that relate to emotional information processing and psychological flexibility, which are important in psychiatric disorders and their treatment. Plasticity can be probed directly in human subjects using transcranial magnetic stimulation combined with either fMRI or EEG [252]. The role of the acute

stress response (measured via plasma corticosteroid levels during the dosing session) and challenging aspects of the psychedelic experience can then be related directly to neural plasticity.

Pre-clinical studies should focus on plasticity, both its locus and acute and long-term mechanisms. Critical unresolved questions include the following. Do psychedelics open a window of plasticity? If so, is this effect non-specific (e.g. across all cortical areas), or focused in regions that are particularly relevant to behavioral phenotypes associated with human psychiatric disorders? How long does the window last? Which signaling pathways are essential for facilitating plasticity? Are there alternative strategies for promoting plasticity that may translate into more effective therapeutic interventions in patients? Despite, or perhaps because of, all the data accumulated so far, we are left with many more questions than we started with. We predict that over the next decade in psychedelic science, our horizons and our minds will be considerably expanded and changed for the better.



1.5.11 Figure 1. Molecular, Cellular, and Systems Support for Psychedelic-Induced Long-Term Changes. Tryptamine and phenethylamine psychedelic compounds, exhibiting structural similarities to serotonin (red) and dopamine (blue) respectively, exhibit high-affinity for serotonin 2A receptors. In mammalian systems, direct (solid arrows) and indirect (dashed arrows) consequences of G-protein and beta-arrestin signaling downstream of receptor activation intersect with enhanced glutamatergic signaling to generate a period of enhanced synaptic plasticity, as supported by neuropeptide synthesis and release, structural changes to neuronal architecture, and altered expression, localization, and phosphorylation of ionotropic glutamate receptors. The consequences of this plasticity are modified by large-scale changes in neuronal network activity and attendant perceptual and cognitive changes that ultimately support long-term changes in behavior.

1.6. Recent advances in the pro-neuroplastic hypothesis of psychedelic action

In recent years, there have been several additions to the field demonstrating the pro-neuroplastic effects of psychedelics both preclinically and clinically. At the transcriptional level, in an *in vivo* rodent model it was found that single administration of psilocybin (2.5 mg/kg) induced increased expression of various plasticity associated proteins [253]. These changes occurred in concert with increased neurogenesis up to 3 days after initial injection and decreases in a depression-like phenotype in these mice [254]. In a similar vein, a study examining the effects psilocybin in a model of rodent fear conditioning found that single administration of psilocybin (0.1 mg/kg, 0.5 mg/kg, or 2.5 mg/kg) 30 minutes prior to fear extinction training induced significant rescue of dendritic arbor complexity, total spine density, and neurogenesis in the hippocampus [255]. These structural changes were accompanied by increases in hippocampal BDNF and mTOR expression and rapid extinction of the fear memory [255].

Another study examining the effects of single administration of psilocybin (0.25 mg/kg, 0.5 mg/kg, 1 mg/kg, and 2 mg/kg) found significantly increased spine density through induction of spine formation in the mPFC 24 hours after administration; some of these changes persisted up to 1 month post dosing [256]. Interestingly, this increase in structural plasticity was not ablated with ketanserin, suggesting receptor subtypes other than the 5-HT_{2A} receptor may be contributing to the neuroplastic effects [256]. Nevertheless, this structural plasticity was accompanied by increased excitatory transmission in the PFC as seen through mini excitatory post-synaptic potentials in layer 5 pyramidal neurons, indicating an increase in functional plasticity as well [256]. Structural plasticity in the mPFC has been found to persist for up to 1 month post single

administration [257]. Taken together, these data strengthen the hypothesis that there is significant enhancement of regional plasticity following psychedelic administration.

In examining the effects on metaplasticity, authors found that 2 weeks after administration of a serotonergic psychedelic, functional metaplasticity was enhanced in whole-cell voltage clamp recordings of the nucleus accumbens [258]. Further, studies have shown clear behavioral effects in pre-clinical models coinciding with the time course of these plastic changes. One paper found significant enhancement of prosocial behaviors in rodents after administration of LSD [259] and another found evidence for the reopening of a critical social reward period in rodents [260].

It is interesting to note that though some studies directly implicate the actions of serotonin receptor pathways in this increased plasticity or metaplasticity, not all studies find evidence for serotonin receptor involvement. It is now vital to fully characterize the contributions of other receptor systems in these neuroplastic effects. There is literature suggesting that psychedelic administration results in an acute increase of stress glucocorticoids in both human and rodent models [7, 63, 261]. The role of these elevated glucocorticoid levels in the mechanism of action of these drugs and psychedelic-associated plasticity is unclear. However, considering the role of acute stress glucocorticoids in promoting adaptive plasticity, it is possible that psychedelics and stress share pro-neuroplastic mechanisms. In this thesis, I will characterize the time course of stress glucocorticoids while searching for evidence for functional and behavioral plasticity in regions implicated in stress-induced disorder pathophysiology after a single administration of psychedelics. I hypothesize that there will be increased synaptic plasticity and metaplasticity in the hippocampus and in the hippocampus to medial prefrontal cortex pathway. I also hypothesize that the acute release of corticosterone in response to psychedelic administration is necessary to

open a critical period for psychedelic-associated increases in synaptic plasticity and behavioral change. Consequently, the time course of the stress response will be in line with the time course of plastic and behavioral change. I have served as primary author for chapters 2-4. I have conducted all data analysis, and write up for the work in chapter 2, and have conducted data collection, analysis, and write up for the works in chapters 3-4.

The following section has been published as:

Zahid, Z, Sultan, Z. W., Krause, B., Wenthur, C. J., Pearce, R., Banks, M. I (2024), Divergent Effects of Ketamine and the Serotonergic Psychedelic DOI on Hippocampal Plasticity and Metaplasticity. *Psychedelic Medicine*

2. Chapter 2: Divergent Effects of Ketamine and the Serotoninergic Psychedelic 2,5-Dimethoxy-4-Iodoamphetamine on Hippocampal Plasticity and Metaplasticity

Zarmeen Zahid*,[1–3], Ziyad W. Sultan*,[1], Bryan M. Krause,[1], Cody J. Wenthur,[2–4], Robert A. Pearce,[1,3], and Matthew I. Banks,[1,3,4]

[1] Department of Anesthesiology, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA.

[2] School of Pharmacy, University of Wisconsin, Madison, Wisconsin, USA.

[3] Neuroscience Training Program, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA.

[4] Transdisciplinary Center for Research in Psychoactive Substances, University of Wisconsin, Madison, Wisconsin, USA.

* These authors contributed equally.

2.1. Abstract

Introduction: Serotonergic psychedelics and ketamine produce rapid and long-lasting symptomatic relief in multiple psychiatric disorders. Evidence suggests that despite having distinct molecular targets, both drugs may exert therapeutic benefit via their pro-neuroplastic effects. Following treatment with ketamine or serotonergic psychedelics, patients are reported to be more open to behavioral change, which is leveraged for psychotherapy-assisted reframing of narratives of the self. This period of enhanced behavioral change is postulated to be supported by a post-treatment window of enhanced neural plasticity, but evidence for such ‘‘metaplastic’’ effects is limited. In this study, we tested for neural plasticity and metaplasticity in murine hippocampus.

Methods: Brain slices were obtained from C57BL/6J mice 24 h after treatment (intraperitoneal injection) with saline, ketamine, or the serotonergic psychedelic 2,5-dimethoxy-4 iodoamphetamine (DOI). Extracellular fiber volleys (FVs) and field excitatory postsynaptic potentials (fEPSPs) were recorded in stratum radiatum of CA1 in response to stimulation of Schaffer collateral fibers before and after induction of short-term potentiation (STP) and long-term potentiation (LTP).

Results: Before LTP induction, responses differed across treatment groups ($F_{2,67} = 5.407$, $p = 0.00665$), with fEPSPs enhanced in slices from DOI-treated animals ($p = 0.0182$), but not in ketamine-treated animals ($p = 0.9786$), compared with saline. There were no treatment effects on LTP ($F_{2,56} = 0.6$, $p = 0.516$), but there were on STP ($F_{2,56} = 4.409$, $p = 0.0167$), with enhanced STP in DOI-treated animals ($p = 0.0352$), but not in ketamine-treated ($p = 0.9999$) animals, compared with saline. A presynaptic component to the mechanism for the DOI effects was suggested by (1) significantly enhanced FV amplitudes ($F_{2,61} = 3.17$, $p = 0.049$) in DOI-treated

animals ($p = 0.0457$), but not in ketamine-treated animals, compared with saline ($p = 0.8677$); and (2) enhanced paired pulse ratios ($F_{2,61} = 3.581$, $p = 0.0339$) in slices from DOI treated animals ($p = 0.0257$), but not in ketamine-treated animals ($p = 0.4845$), compared with saline.

Conclusions: DOI, but not ketamine, induced significant neuroplastic and metaplastic effects at hippocampal CA1 synapses 24 h after treatment, likely in part via a presynaptic mechanism.

2.2. Introduction

Ketamine and serotonergic psychedelics ameliorate symptoms of multiple psychiatric disorders in patient populations [68, 262] and modify behavioral phenotypes in rodent tasks dependent on neuronal circuitry relevant to these disorders. [208, 259, 263, 264]. In patients administered these drugs, improvements in symptoms are linked to enhanced openness and psychological flexibility. [265-270]. Unlike existing medical therapies, the effects of these treatments are both rapid and persistent, continuing well beyond their plasma exposure time-course; [271] the therapeutic effects of serotonergic psychedelics last several-fold longer than those of ketamine. [75, 76, 272]. The underlying neural mechanisms of action remain incompletely characterized. Ketamine is a dissociative anesthetic that blocks both N-Methyl-D-Aspartate (NMDA) receptors and hyperpolarization-activated cyclic nucleotide-gated (*HCN*) *channels.* [273]. Serotonergic psychedelics exert their behavioral effects in humans primarily via agonism at 5-HT_{2A} receptors. [97, 99]. Because ketamine and serotonergic psychedelics have distinct molecular targets in the brain and produce distinct effects on perception and cognition, the mechanisms underlying their anti-depressant activity action likely overlap only in part. [274-280].

Long-term behavioral effects in human subjects are presumed to reflect neuroplastic changes. [1, 281, 282]. There is considerable evidence from preclinical models for rapidly induced, persistent neuroplastic effects of ketamine and serotonergic psychedelics in medial prefrontal cortex and hippocampus, and that this plasticity underlies changes in behavioral phenotypes. These neuroplastic changes include increases in dendritic spine density and size, [208, 212, 218, 258, 283-288] enhanced neurogenesis in the hippocampus, [90, 214 , 289-291] and increased expression of plasticity-associated genes such as *cfos* and Sgk1 in neocortex and hippocampus. [217, 292-297]. Plasticity has also been observed in the nucleus accumbens. [298]. These drug-induced structural and functional changes represent clear evidence of acutely induced, long-lasting neural plasticity. Furthermore, these changes associate with modifications in behavioral phenotype in tasks assessing psychiatric disease-relevant processes. [208, 283, 298, 299].

In psychedelic-assisted psychotherapy, post-dosing integration sessions are designed to leverage improved cognitive flexibility and new openness to change in perspectives and self-narratives in the days and weeks following treatment. [300]. This enhanced behavioral plasticity is postulated to arise when serotonergic psychedelics and ketamine open a long-lasting window of neuroplasticity that outlasts its clearance from the brain. [298, 301, 302]. In this model, the neural basis for this enhanced plasticity would be acutely induced and persistent neural changes that manifest post-acutely as lowered threshold for, or enhanced magnitude of, synaptic plasticity in neural circuits following treatment. This phenomenon is referred to as ‘metaplasticity’, [56, 303] and is distinct from, though possibly dependent on, neuroplastic changes summarized above. To date, support for this model has been demonstrated in mPFC and nucleus accumbens, [258, 298] but not yet in hippocampus.

We investigated the effects of ketamine and 2,5-Dimethoxy-4-iodoamphetamine (DOI) on neural plasticity and metaplasticity in the hippocampus of mice. DOI is a serotonergic psychedelic that is more selective for the 5-HT_{2A} receptor in comparison to tryptamine psychedelics (e.g., psilocybin) and is known to result in increased glutamate neurotransmission, similar to other serotonergic psychedelics. [153, 304, 305]. Evidence suggests that signaling deficits in hippocampus, mPFC, and amygdala associate with symptoms of depression and anxiety. [306-308]. In particular, an overarching psychological framework for the mechanisms of these drugs' therapeutic effects is grounded in an altered narrative of the self, which would likely involve autobiographical memory formation and retrieval *via* the hippocampus. [309]. Consistent with this model, previous studies have shown that both serotonergic psychedelics and ketamine strengthen hippocampal CA1 synapses in rodent models. [283, 310]. In this study, we investigated synaptic plasticity and metaplasticity in the hippocampus 24 hours after administration of DOI and ketamine.

2.3. Methods

All experimental protocols conformed to American Physiological Society/National Institutes of Health guidelines and were approved by the University of Wisconsin Animal Care and Use Committee.

Animals

Male and female C57BL/6J mice (n = 55 mice; 31 females; after exclusion: n = 51; 28 females), 12-20 weeks old, were bred in house (F2 generation) or ordered from Jackson Labs.

Mice were maintained on a 12:12 light-dark cycle (light on at 06:00) with *ad libitum* food and water. All animals were housed in groups until drug administration.

Drugs

Aliquots of (±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride (DOI; Millipore Sigma) and ketamine hydrochloride (Pfizer, 100 mg/mL < 0.10 mg/mL benzethonium chloride added as a preservative) diluted in 0.9% sodium chloride were filtered through a 0.22 μm pore membrane and stored at –80°C. Pairs of animals were randomly assigned to a drug treatment, and the experimenter was blinded to the treatment condition during the experiment and analysis. Intraperitoneal injections (DOI: 1 mg/kg, Ketamine: 10 mg/kg; DOI, ketamine, 0.9% saline: 5 ml/kg total volume) were administered approximately 24 hours in advance of dissections, and animals were placed into a new cage after drug administration. The chosen dosage of DOI has been shown to elicit a robust head twitch response, a behavioral marker for hallucinogenic effects, [311, 312] and consistent behavioral effects.[313, 314]. For ketamine, 10 mg/kg is commonly used in preclinical models of psychiatric disorders and has demonstrated robust and long-lasting neural and behavioral changes at this dose.[315]. At higher doses, ketamine begins to have anesthetic effects, [316] which have been suggested to arise from mechanisms distinct from those supporting the antidepressant effects seen at lower doses. [317].

Brain Slice Preparation

Approximately 24 hours after drug administration, dorsal hippocampal slices were prepared as previously described. [318]. Briefly, animals were deeply anesthetized with 3%

isoflurane then decapitated. Brains were quickly extracted from the skull and placed into ice-cold "cutting artificial cerebrospinal fluid" (cutting ACSF; see below for details) saturated with carbogen (95% O_2/5% CO_2). The cerebellum was cut off at ~15-degree caudo-rostral angle to create an "off coronal" cutting plane for slicing. The brain was then glued caudal side down onto a metal stage, mounted into the vibratome (Model 7000smz-2 vibratome, Campden Instruments; 80 Hz; 0.11 mm/s), and submerged in ice-cold cutting ACSF. Coronal slices (400 μm) containing hippocampus were hemisected and incubated at 33°C for 30 minutes in carbogen-saturated 'recording' ACSF. Slices were then allowed to come to room temperature (approximately 24°C) for 60 minutes before transfer to recording chambers for electrophysiology. Cutting ACSF consisted of (in mM): 127 NaCl, 1.88 KCl, 1.21 KH_2PO_4, 26 $NaHCO_3$, 10 glucose, 2.5 sodium ascorbate, 5 kynurenic acid, 1.44 $MgSO_4$, 11 $MgCl_2$, and 2.17 $CaCl_2$. Recording ACSF consisted of (in mM): 127 NaCl, 1.88 KCl, 1.21 KH_2PO_4, 26 $NaHCO_3$, 10 glucose, 1.44 $MgSO_4$, and 2.17 $CaCl_2$. All solutions were buffered to pH 7.3-7.4 when saturated with 95% O_2/5% CO_2 and had a recorded osmolality between 294-297 mOsm/kgH_2O.

Data collection

Brain slices were placed in a submersion-style recording chamber with carbogen-saturated ACSF flowing at a rate of 3.0 ml/min (Minipuls 3, Gilson Inc, Middleton, WI), maintained at 30 °C (TC-344C Automatic Temperature Controller, Warner Instruments, 234 Hamden, CT). Slices were placed upon an elevated mesh netting to allow for perfusion of both surfaces, with nylon-strung platinum harps to anchor slices onto the netting. To measure fiber volleys (FVs) and field excitatory postsynaptic potentials (fEPSPs), tungsten recording electrodes (100 KΩ, World Precision Instruments, Sarasota, FL, USA) were inserted at a depth of 70-150 μm into the *stratum*

radiatum of hippocampus. Concentric bipolar stimulating electrodes (200 KΩ, World Precision Instruments, Sarasota, FL, USA) were placed ~1 mm away in *stratum radiatum* to stimulate Schaffer collateral inputs to CA1 pyramidal cells.

fEPSP stimulus-response (S-R) curves were obtained to determine the intensity that elicited the half-maximal fEPSP slope. Stimulation values ranged from 40-500 uA. Bipolar stimuli (0.2 ms per polarity, separated by 0.1 ms) were delivered via constant-current stimulus isolator units (Multi Channel Systems STG 4004, MCS, Reutlingin, Germany). The intensity producing the half-maximal fEPSP slope was chosen for LTP protocols.

During LTP-induction protocols, test stimuli were delivered at 0.05 Hz until a stable 30-minute baseline period was achieved (defined as a <10% shift in fEPSP slope). The theta-burst stimulation (TBS) protocol to induce LTP consisted of 10 trains stimuli delivered at 5 Hz, with each train consisting of 4 stimulus pulses delivered at 100 Hz. Responses were then recorded for 60 additional minutes following TBS.

Recordings were obtained using pClamp 10 software (Molecular Devices, San Jose, CA). Data were amplified ×1000, filtered between 1 Hz and 20 kHz using a Microelectrode AC Amplifier (Model 1800, A-M Systems, Everett, WA), and digitized at 40 kHz (National Instruments, Austin, TX).

2.4. Data Analysis

Data preprocessing

Evoked responses from each slice (n = 89 slices from 55 animals) were visually inspected for the following exclusion criteria: FV amplitude >30 % of the fEPSP amplitude at the half-maximal intensity or peak fEPSP amplitude <0.5 mV. Additional criteria for exclusion from the LTP analysis were emergence of a population spike during the 30-minute baseline recording and baseline instability, which may have indicated poor tissue health or temperature or mechanical fluctuations in the bath. In total, n = 72 slices from 51 animals (38 slices from female animals) were used in the SR analysis and n = 60 slices from 44 animals (29 slices from female animals) for the LTP analysis. The peak amplitude (mV) of the presynaptic FV and the maximum rising slope of the fEPSP (mV/ms) were calculated in Clampfit software using the *analyze/statistics* function with optional smoothing (5 samples, 0.3 ms).

Stimulus Response Curves

Sigmoidal model fits were applied to fEPSP S-R data obtained from each slice using the function *fit()* in MATLAB, with the following function $R = R_{Max}/\left[1 + e^{-M(S-S_{50\%})}\right]$, where R_{Max} is the asymptotic response, M is the slope, and $S_{50\%}$ is the stimulus intensity eliciting half maximal response. Hypothesis testing of stimulus-response data was performed using one-way ANOVA. Effects of treatment (Saline, DOI, and Ketamine) were assessed on the fit parameters ($R_{Max,}$ $M,$ and $S_{50\%}$). Once significance was established, Tukey's HSD test was used for post hoc comparisons.

FV S-R data were not well-fit by sigmoid curves. Instead, maximum fiber volley amplitudes from each slice were compared across drug groups in a one-way analysis of variance (ANOVA) in R (v4.2.2; R Core Team, 2022). Significant differences were subsequently tested with Tukey's HSD for post hoc comparisons.

Measures of short- and long-term plasticity

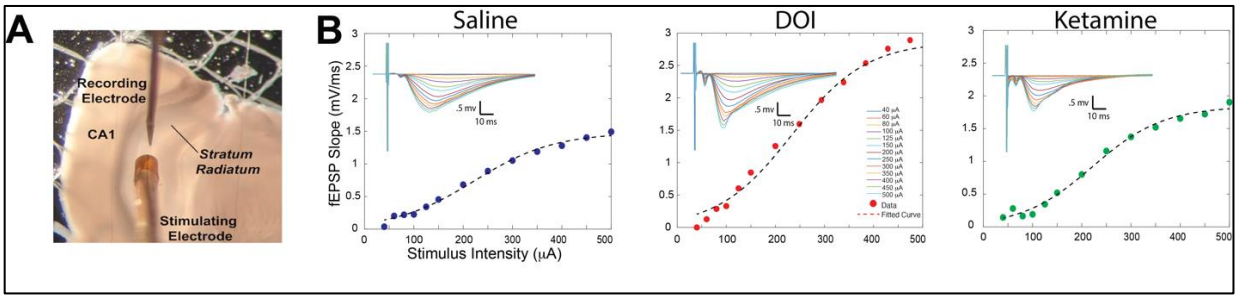
Two measures were used to characterize short-term plasticity (STP). First, the magnitude of the normalized fEPSP slope was measured during the period t = 0 – 5 minutes and normalized by the average of the last 10 minutes of the baseline period. Second, the decay of the fEPSP slope time series from t = 0 to 60 minutes was measured by applying a bi-exponential fit using the *fit()* function in MATLAB. Because only the overall time course of this decay was of interest, and because data from a small number (7%) of experiments were better fit by mono-exponential decays, the time course of decay was characterized as the weighted time constant $\tau_{Wt} = (a_1\tau_1 + a_2\tau_2)/(a_1 + a_2)$, where a_i and τ_i are the amplitudes and time constants of the two exponential components. The magnitude of LTP was measured as the fEPSP slope averaged over the time window t = 50 – 60 minutes post-TBS, normalized by the average of the last 10 minutes of the baseline period.

Hypothesis testing for STP and LTP was performed using one-way analysis of variance in *R* (v4.2.2; R Core Team, 2022). We compared the effect of drug condition (Saline, DOI, and Ketamine) on the mean fEPSP slope in the first five minutes post-TBS and on τ_{Wt} (for short-term plasticity), and on mean fEPSP slope over the last 10 minutes post-TBS (for LTP).

Analysis of paired pulse ratio

To investigate the contributions of presynaptic mechanisms to observed treatment effects, paired pulse ratios (PPR) of fEPSPs were assessed [319]. PPRs were assessed from the first two stimuli of the first train of the TBS inducing LTP (interstimulus interval = 10 ms), calculated as the ratio of the second to the first fEPSP slope. Thus, a PPR > 1 indicates paired pulse facilitation. The data were compared across drug groups in a one-way analysis of variance (ANOVA) in R (v4.2.2; R Core Team, 2022). Significant differences were subsequently tested with Tukey's HSD for post hoc comparisons.

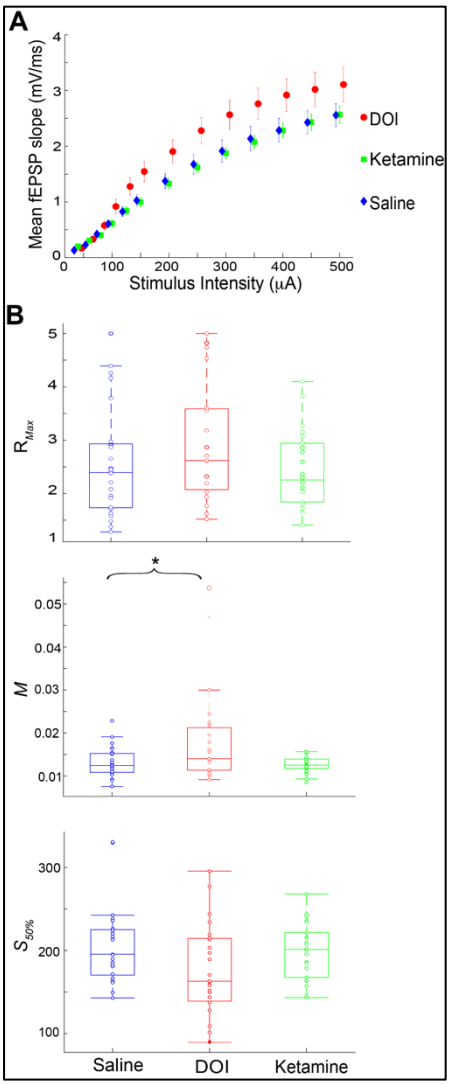
2.5. Results



2.5.1. Figure 1. Picture of slice and example fEPSP S-R curves and fEPSP traces for each treatment group. Panel **A** is an image of the dorsal hippocampal slices, electrodes and areas are marked. The figures in panel **B** show single slice examples of S-R curves recorded in *stratum radiatum* in CA1 in response to Schaffer collateral stimulation at varying stimulation intensities (40-500 uA) before LTP induction. Sigmoidal curve fits are superimposed. Averaged traces for each experiment are shown as insets.

Pre-treatment with DOI increases excitability of Hippocampal CA1 synapses

Stimulation of Schaffer collateral inputs to *stratum radiatum* in CA1 evoked FVs and fEPSPs with amplitudes and time courses typical of this experimental preparation [318] (Figure 1). Example fEPSP S-R curves recorded in slices obtained from saline-, DOI-, and ketamine-treated animals are shown in Figure 1, and averaged results for each condition are shown in Figure



2A. fEPSP S-R curves obtained in slices from DOI-treated animals were markedly different compared to those obtained from saline- or ketamine-treated animals. The slope (*M*) of fEPSP S-R curves exhibited a significant treatment effect (Figure 2B, $F_{1,2} = 5.407$, $p = 0.00665$). In post-hoc testing, *M* for DOI was significantly different from saline ($p = 0.0182$) and ketamine ($p = 0.0136$), whereas ketamine was not significantly different from saline ($p = 0.979$). No significant treatment effects were observed for R_{Max} or $S_{50\%}$ (R_{Max}: $F_{1,2} = 1.55$, $p = 0.222$; $S_{50\%}$: $F_{1,2} = 2.76$, $p = 0.0706$). Thus, slices from DOI-treated animals exhibited greater excitability compared to ketamine- or saline-treated animals. These data indicate that DOI had acutely induced a form of synaptic plasticity in hippocampus that persists for at least 24 hrs.

2.5.2. Figure 2. Slices from DOI-treated animals exhibited increased excitability compared to ketamine and saline. A. Averaged fEPSP S-R curves across all slices for each drug condition before TBS induction. **B.** fEPSP S-R curve fit parameters for each drug condition pre-TBS. Each symbol is one individual slice. Boxplots show median, upper and lower quartile ranges, and data range excluding outliers. R_{Max}: asymptotic response; *M*: slope; $S_{50\%}$: stimulus intensity eliciting half maximal response. DOI-treated animals had slopes that were significantly greater compared to saline ($p = 0.0182$) and ketamine ($p = 0.0136$). There were no differences observed between treatment groups for R_{Max} or $S_{50\%}$ (R_{Max}: $F_{2,67} = 1.55$, $p = 0.222$; $S_{50\%}$: $F_{2,67} = 2.76$, $p = 0.0706$).

Pre-treatment with either DOI or ketamine does not alter LTP

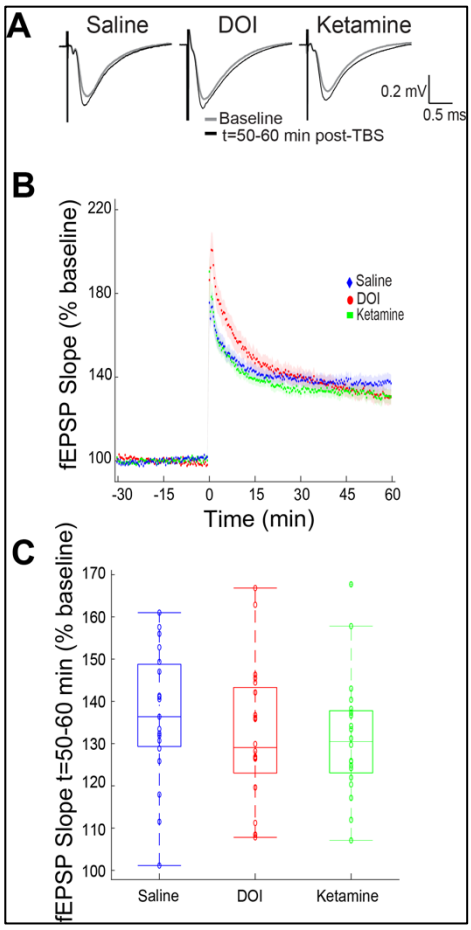
Having observed evidence for an acute, persistent change in excitability in response to DOI, we next asked whether induction and maintenance of LTP itself is different 24 hours after administration of DOI or ketamine. S-R curves were obtained, and the

stimulus intensity that produced an approximately 50% maximal response was selected for the LTP protocol. After a 30-minute baseline period, TBS-induced LTP, defined as potentiation that lasted at least 60 minutes, was readily observed (Figure 4A, B). However, no difference was observed between treatment groups in the magnitude of LTP at t = 50-60 minutes (Figure 4C; $F_{1,2}$ = 0.67, p = 0.516). Thus, neither DOI nor ketamine affected the magnitude of LTP 24 hours after treatment.

Pre-treatment with DOI enhances short-term plasticity

Although the magnitude of LTP was not different between treatment groups, fEPSP slopes in slices from DOI-treated animals were considerably larger than those from saline- or ketamine-treated animals immediately after TBS (Figure 4B), indicating that in addition to strengthening synapses, treatment with DOI also increases STP (Figure 5A, B; $F_{1,2} = 4.41$, p = 0.0167). Post-hoc tests showed that responses from the DOI group were significantly enhanced compared to saline (p = 0.0352) and ketamine (p = 0.0332), while ketamine was not different from saline (p = 0.999). These data indicate that 24 hrs after treatment, DOI, but not ketamine, induced changes in plasticity in hippocampus. By contrast, no differences across treatment groups were observed in the time course of decay (τ_{Wt}) of the fEPSP slope to its steady-state baseline following TBS (Figure 5C; $F_{1,2} = 0.607$, p = 0.549).

The effects of DOI are presynaptic

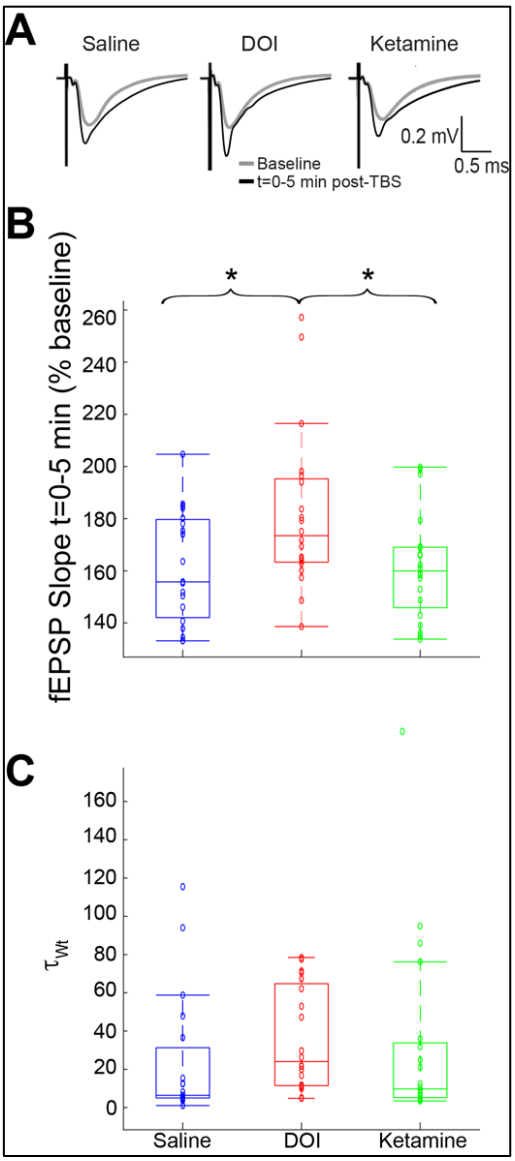


2.5.3. Figure 3. Summary of LTP across treatment groups. A. Example pre- and post-TBS fEPSP traces averaged from t=50-60 mins from each treatment group. **B**. fEPSP time series data for the three treatment groups from baseline to 60 minutes post-TBS. Symbols are mean +/- standard error of the mean. **C.** fEPSP slope averaged between t=50-60 minutes post-TBS. Each symbol is one experiment. Boxplots show median, upper and lower quartile ranges, and data range excluding outliers. Groups were not significantly different ($F_{2,56} = 0.67$, $p = 0516$).

FV amplitudes represent excitability of the stimulated afferent fibers. FV amplitudes were examined to evaluate presynaptic contributions to the results of Figure 2 and 4. FV S-R curves showed a clear difference between DOI-treated animals compared to ketamine- or saline-treated animals (Figure 5). Maximal FV amplitudes exhibited a significant treatment effect ($F = 3.17$, $p = 0.0490$), and post-hoc testing indicated that FV amplitudes were significantly greater in DOI-treated animals compared to the other groups ($p = 0.0458$), while no difference was observed between saline and ketamine ($p = 0.868$). This suggests that the increased excitability in slices from DOI-treated animals shown in Figure 2 was at least partly due to presynaptic effects of DOI. To determine the extent of this presynaptic contribution, we compared presynaptic and postsynaptic responses directly by plotting fEPSP slopes as a function of FV amplitudes (Figure 6). Here, we reasoned that if the effect of DOI was entirely presynaptic, then there should be no difference between treatment groups when fEPSP slopes are considered directly as a function of FV amplitude, since a given presynaptic input will elicit the same postsynaptic response if there are no postsynaptic changes induced by DOI. Indeed, the data from the three treatment groups

largely overlaps in Figure 6A, consistent with this hypothesis. To test this quantitatively, we log transformed the data and applied a linear mixed effects model with random slopes and intercepts (Figure 6B). We found no significant difference in fitted slopes (Saline: 0.608; DOI: 0.583; Ketamine: 0.588) or intercepts (Saline: 0.423; DOI: 0.429; Ketamine: 0.382) between treatment groups (all p values > 0.226).

The paired pulse ratio (PPR; i.e., the ratio of the second to the first synaptic response to two stimuli presented at short interstimulus interval) is commonly used to evaluate presynaptic short term plasticity [320]. To further elucidate presynaptic contributions to the observed effects of DOI, we evaluated the PPR for fEPSPs evoked by the first two stimuli (separated by 10 msec) in the LTP-inducing protocol. We found that there was a significant difference between the groups ($F_{2,60} = 3.581$, $p = 0.0339$; Figure 7), with PPR in DOI-treated animals significantly greater to saline-treated animals ($p = 0.0257$), but PPR in ketamine-treated animals no different from saline ($p = 0.484$). These results support a presynaptic component to the mechanism underlying the observed neuroplastic and metaplastic effects of DOI in hippocampus.



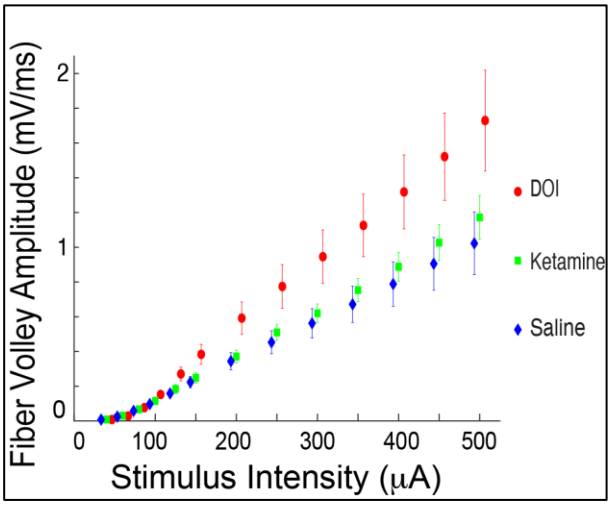
2.5.4. Figure 4. Summary of STP across treatment groups. A. Example average pre- and post-TBS fEPSP traces from each treatment group. 5 mins from each treatment group. **B.** Average fEPSP slope from t=0-5 mins post-TBS. Groups were significantly different ($F_{2,56}$ = 4.409, p = 0.0167). Responses from the DOI group were significantly enhanced compared to saline (p = 0.0352) and ketamine (p = 0.0332), while ketamine was not different from saline (p = 0.999). **C.** Weighted time constant τ_{Wt} across treatment groups. No significant differences were observed across groups ($F_{2,56}$ = 0.607, p = 0.549). In both A and B, each symbol is one experiment. Boxplots show median, upper and lower quartile ranges, and data range excluding outliers.

2.6. Discussion

The 1–2-week period following treatment with ketamine and serotonergic psychedelics is called the 'after-glow' period, during which subjects exhibit dramatic changes in mood and perspective, and openness to new experiences, ideas, and narratives. [321, 322]. The pro-neuroplastic properties of ketamine and serotonergic psychedelics, demonstrated most convincingly in pre-clinical models, likely contribute to these effects. [278]. The psychedelic experience itself has been described as transformative, transgressing normative boundaries of lived experience so profoundly that many subjects are reminded that there are many possible stories they can construct about themselves and their relationship to the world, and thus change their actions accordingly. [309]. Neuroscience researchers have taken this a step farther, however, proposing that the transformative effects of psychedelics may be mediated by 'metaplastic' neural changes, such as altered thresholds for and magnitudes of synaptic plasticity, lasting for days and weeks after the drugs have left the CNS. [56, 302, 303]. This

suggestion is based in part on the importance of 5-HT_{2A} receptor signaling during the critical period of neuroplasticity that arises during neurodevelopment. [301]. Indeed, the integration sessions that follow psychedelic dosing in standard models of psychedelic-assisted psychotherapy rely implicitly on a post-exposure enhancement of capacity for behavioral change.

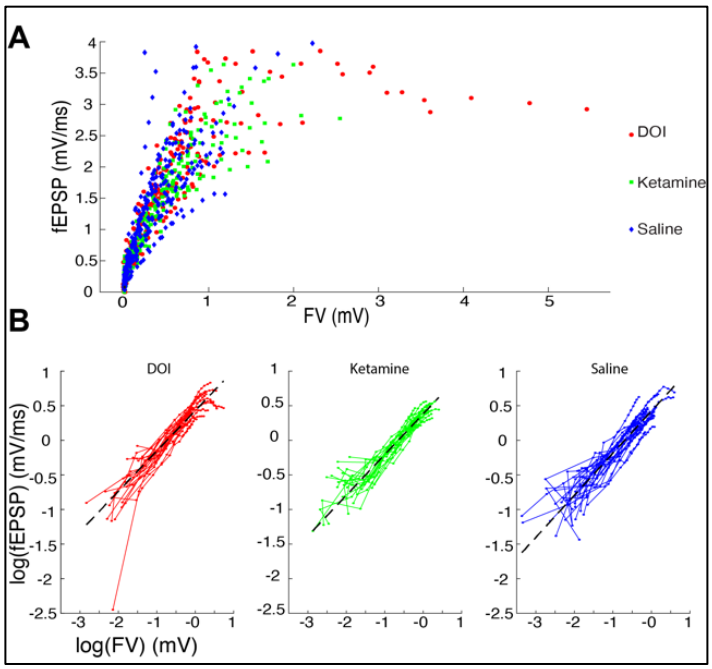
Here, we evaluated the effects of a serotonergic psychedelic (DOI) and ketamine on plasticity and metaplasticity at Schaffer collateral synapses in CA1 of murine hippocampal brain slices, a widely accepted *in vitro* molecular model of memory formation. [323]. We observed an increase in the slope of fEPSP S-R curves at these synapses 24 hours after administration of DOI, but not ketamine, suggesting an increase in neuronal excitability. Previous results indicate that a 1 mg/kg IP dose of DOI is fully cleared from the CNS of mice by 24 hours, indicating that these changes persist beyond the presence of the drug at these synapses. [324]. These results for DOI are consistent with previous studies showing direct, rapid, and persistent neuroplastic effects of psychedelics. Changes in neurite outgrowth and spine and synaptic density have been observed following brief application of LSD in cultured rat cortical neurons [325] and following a single administration of psilocybin *in vivo* in murine mPFC. [286, 299]. In addition, enhanced synaptic responses have been observed in hippocampal brain slices



2.5.5. Figure 5. Summary of Fiber Volley (FV) stimulus-response (S-R) curves. Averaged FV S-Rs across all slices for each drug condition. DOI was significantly different compared to saline ($p = 0.0458$) where Ketamine was not ($p = 0.868$).

three days after administration of psilocybin *in vivo* in a mouse model of stress-induced phenotypes. [283].

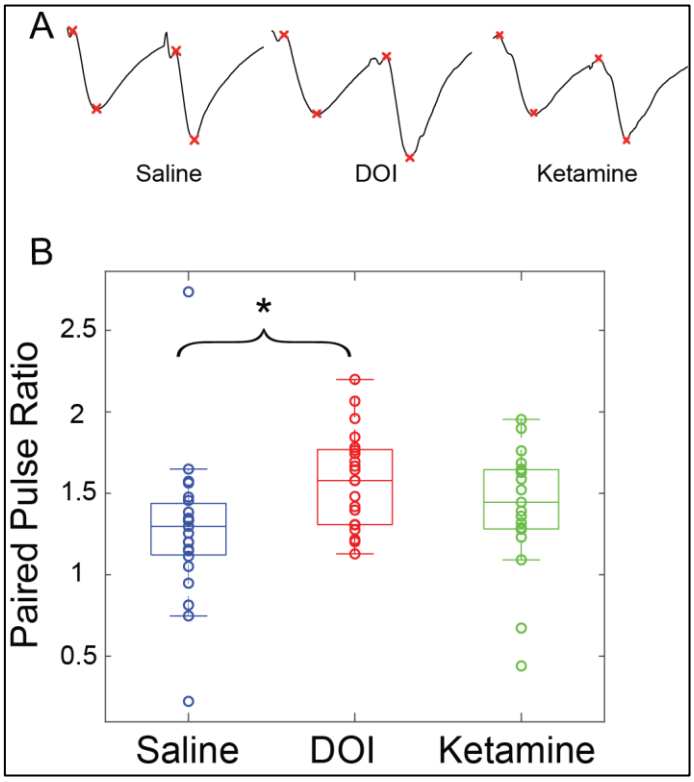
The absence of neuroplastic effects of ketamine at hippocampal synapses in this study is surprising, as previous studies have shown rapid and persistent neuroplastic effects of ketamine in cultured neurons *in vitro* and in mPFC *in vivo.* [208, 285, 325, 326]. Ketamine has also been shown to induce LTP in hippocampal slices when applied directly, [326, 327] but the post-acute effects reported at the 24-hour time point are sex-specific and may depend on pre-existing stress exposure. [310]. In addition, neuroplastic changes in response to ketamine have been reported to occur after multiple exposures to the drug *in vivo* followed by exposure *ex vitro*, and on different time scales in different regions of the brain, which may also contribute to distinct outcomes in hippocampus and mPFC. [326, 328].



2.5.6. Figure 6. No effect of treatment on fEPSPs when plotted versus FV amplitude. A. Stimulus-response data from all slices and all treatment groups showing overlap of data when plotted as a function of FV amplitude. **B.** Data from **A** separated by treatment group and plotted on a log-log scale to demonstrate suitability for linear regression analysis. We found no significant difference in fitted slopes (Saline: 0.608; DOI: 0.583; Ketamine: 0.588) or intercepts (Saline: 0.423; DOI: 0.429; Ketamine: 0.382) between treatment groups (all p values > 0.226).

Importantly, we observed evidence that DOI induced metaplastic effects, i.e., changes in neuroplasticity, at Schaffer collateral synapses in CA1, consistent with effects observed previously in

mPFC 24-hours after administration of DOI. [299]. Although the magnitude of LTP in response to TBS stimulation was indistinguishable between treatment groups, the magnitude of STP was significantly enhanced for animals previously treated with DOI compared to those previously receiving ketamine or saline. STP is defined as the initial, transient increase in response amplitude immediately following TBS. This early phase of plasticity is mechanistically distinct from the later phase of LTP at Schaffer collateral synapses in CA1, [329] and likely relies on changes in presynaptic signaling. [330]. Further support for a presynaptic mechanism underlying the effects of DOI comes from the observations that FV amplitudes were increased following DOI pre-treatment (Figure 5) and from analysis of PPRs (Figure 7). The observed increases in FV amplitudes may arise due to increased excitability of axonal fibers, or increased numbers of fibers secondary to increased numbers of synaptic release sites. The latter is consistent with previous observations of increased numbers and size of dendritic spines in mPFC following administration of serotonergic psychedelics in mice. [258, 286]. The observation that PPRs in slices from DOI-treated animals were enhanced compared to



2.5.7. Figure 7. Increased paired pulse ratio (PPR) for DOI treated slices. A. Representative mean paired-pulse responses from single slice experiments. The pairs of x's denote measurements of baseline and peak amplitude values. **B.** PPR was measured as the slope of the second fEPSP divided by the first. The groups were significantly different ($F_{2, 60} = 3.581$, $p = 0.0339$) with DOI treated slices having significantly higher ratios compared to saline ($p = 0.0257$). Ketamine was not significantly different from saline ($p = 0.485$).

saline suggests that these new release sites have smaller release probability compared to pre-treatment release sites. Differences in release probability at newly established release sites following acute neuroplasticity has been shown previously at hippocampal synapses. [331]. Thus, our observations that STP, FV amplitudes, and PPRs were significantly enhanced in DOI-treated animals are consistent with a presynaptic component to the mechanism for the neuroplastic and metaplastic effects of DOI, but postsynaptic changes may contribute as well.

DOI-induced changes in STP are likely to be functionally significant, even though, compared to LTP, STP occurs on a much shorter timescale, does not involve the transcription of plasticity associated genes, and is less durable. [330]. STP can have significant effects on neural information transfer [332, 333] and can modulate neural responses to transient inputs [334] and network responses to sustained inputs, [335] as well as induce state switching in neurons (up and down states). [336]. Interestingly, STP has been observed to depend on presynaptic NMDA receptors (NMDARs), [329, 337] and this may underlie the effects of DOI on both STP and S-R curves (excitability) observed here. DOI binds with high affinity at 5-HT_{2A} receptors, and evidence suggests that 5-HT_{2A} receptors and NMDARs are closely linked. For example, activation of 5-HT_{2A} receptors enhances NMDAR-dependent transmission and plasticity at thalamo-cortical synapses via a presynaptic mechanism. [338]. Further, in a study assessing the effects of the agonist DOB, which, like DOI, has similarly strong affinity for both 5-HT_{2A} and 5-HT_{2C} receptors, activation of 5-HT_{2A} receptors was necessary for the NMDAR-induced changes to EPSPs and resting membrane potential through a presynaptic mechanism. [339].

Serotonergic psychedelics, including DOI, have been evidenced to increase extracellular glutamate in cortical areas through activation of 5-HT_{2A} receptors on cortical pyramidal cells.

[340, 341]. The interplay between these 5-HT_{2A} receptors and glutamate receptors may be due to the close proximity with which they exist on the transmembrane domain and also due to the formation of heterodimeric complex between these receptors. [1]. Thus, it is possible that the observed effects of DOI on both S-R curves and STP arise due to enhanced glutamatergic neurotransmission through NMDARs downstream of presynaptic 5-HT_{2A} receptor activation. Indeed, literature suggests that DOI induced metaplasticity may be dependent on NMDA receptor activation and so this proposed mechanism may also provide context for the absence of an effect on excitability or STP with ketamine, due to its direct antagonism of NMDARs. [342].

Under the definition first put forward by Abraham and Bear (1996), – "Metaplasticity is manifest as a change in the ability to induce subsequent synaptic plasticity" – DOI's effects on subsequent STP are metaplastic. While effects on subsequent LTP or LTD have more commonly been provided as examples of such metaplasticity, [258, 298] the DOI-induced change in STP at 24 hours following administration is clearly also reflective of long-lasting changes to functional neuronal dynamics. In all, our work provides evidence for neuroplastic and metaplastic effects at CA1 Schaffer collateral synapses at 24 hours following serotonergic psychedelic administration, but not ketamine administration. Our findings provide evidence for a presynaptic mechanism for metaplasticity and a mechanistic basis for change in network level activity in response to psychedelic drug administration.

Future Directions

As the data in this study are limited, future experiments should be conducted to elucidate the results here. First, our experiments were focused in dorsal hippocampus, and we did not

investigate regional heterogeneity in the observed effects of DOI. For example, dorsal and ventral hippocampus are anatomically distinct, with different patterns of afferent and efferent connections. [343]. Functionally, the dorsal portion plays a direct role in memory processes where the ventral portion plays a more direct role in stress responses and emotionally motivated behaviors, such as approach and avoidance. [343]. Future experiments will elucidate whether the effects reported here generalize to ventral hippocampus. The use of additional psychedelics such as psilocybin can be helpful as positive controls along with the addition of an NMDA receptor antagonist pretreatment with DOI and other psychedelics to discern the receptor level contributions to the observed mechanism. The identity of the subtypes of 5-HT receptors involved also remains an open question. Data suggest that DOI also has high affinity as a partial agonist of the 5-HT_{2C} receptor. [344-346]. It has also been shown that the 5-HT_{2C} receptor, in part, facilitates 5-HT_{2A} receptor induced effects on locomotion, suggesting that DOI may exert behavioral effects through coordinated action at both receptor subtypes. [347]. Future experiments with specific receptor antagonists will help resolve this question. Further clinical and preclinical work is needed to better understand the mechanism and role of plasticity and metaplasticity in the brain following serotonergic psychedelic administration. Considering the enhanced excitability seen here, as well as the proposed evolutionary role of metaplasticity in 'tuning' synaptic signaling to prevent saturation that impairs learning, investigations into metaplastic effects of DOI on LTD may be of substantial mechanistic importance. Further assessment of these outcomes across alternative brain regions, time-courses, and species will also be key experiments regarding the translational impact of this finding on optimization of psychedelic-assisted psychotherapy protocols.

Author Contributions: *Ziyad Sultan contributed to the acquisition of the data*, and *Zarmeen Zahid and Ziyad Sultan contributed to the analysis, and interpretation of data, and drafting the*

manuscript. Robert Pearce contributed to the acquisition and interpretation of data and drafting the manuscript. Bryan Krause contributed to the analysis of data and drafting the manuscript. Cody Wenthur contributed to the interpretation of data and drafting the manuscript. Matthew Banks contributed to the conception of the work, analysis and interpretation of data, and drafting the manuscript.

Funding: *Funding was provided by the UW Department of Anesthesiology, UW Office of the Vice Chancellor for Research and Graduate Education, the National Institute of Mental Health (R01MH122742 to CJW), the National Institute of General Medicine (R01GM118801 to RAP) and the National Institute of Neurological Disorders and Stroke (T32NS105602 to ZZ).*

Competing Interests: *Matthew I. Banks is a paid consultant for VCENNA, Inc. No research support for this work was provided by VCENNA. Cody J. Wenthur receives research funding from Psilera Inc. No research support for this work was provided by Psilera Inc. Zarmeen Zahid, Ziyad W. Sultan, Robert A. Pearce, and Bryan M. Krause declare no competing interests.*

3. Chapter 3: Psychedelic Induced Plasticity in the Ventral Hippocampal to Medial Prefrontal-Cortical Circuit and the Intersection with Stress and Approach and Avoidance Behaviors *In Vivo*

Zarmeen Zahid[1,2,4], Aidan J. Zeimet[1], Jack L. Zheng[1], Mallory A. Kuppe[1], Sean M. Grady[1], Andrew Lee[1], Leonardo M. Suarez[1], Daleep S. Sandhu[1], Michael H. Sutherland[1], Olivia N. Moroney[1], Bianca Schnarr[1], Nate T. Jones[2], Cody J. Wenthur[2,3,4], Matthew I. Banks[1,3,4]

[1]Department of Anesthesiology, University of Wisconsin School of Medicine and Public Health, Madison, WI, 53706

[2]School of Pharmacy, University of Wisconsin, Madison, WI, 53705

[3]Transdisciplinary Center for Research in Psychoactive Substances, University of Wisconsin, Madison, WI, 53705

[4]Neuroscience Training Program, University of Wisconsin School of Medicine and Public Health, Madison, WI, 53706

3.1 Abstract

Introduction: Psychedelic drugs have shown efficacy as treatments for psychiatric conditions. One postulated mechanism involves increasing neuroplasticity in the brain. Though there is considerable data suggesting regional brain changes in plasticity, no study has examined plasticity at the circuit level. The ventral hippocampal to prefrontal cortex circuit is highly involved in

anxiety-like behaviors and cognitions and is a good candidate circuit in which we can assess plasticity. It is also important to put these changes in context of behavioral outcomes. In this study, we tested plasticity in the ventral hippocampal to prefrontal cortex circuit and we tested behavioral change through the novelty suppressed feeding task, a behavioral assay of anxiety-like behaviors.

Methods: C57BL/6 mice were chronically implanted with a bipolar stimulating electrode in the ventral hippocampus and a twisted wire recording electrode in the prefrontal cortex. These animals were stimulated at varying amplitudes to record local field potential responses and then injected with either psilocybin (1 or 3 mg/kg), 4-acetoxy-N,N-dimethyltryptamine (4-AcO-DMT), 6-fluoro-N,N-diethyltryptamine (6-FDET), or saline (5 mL/kg). These animals were then stimulated again at 4 hours and 24 hours to measure change in evoked response. Further, in another cohort of animals, the novelty suppressed feeding task was conducted. In this task, animals are food deprived for a period of 12 hours and then injected with either psilocybin, 4-AcO-DMT, 6-FDET, N,N-tetramethylenetryptamine (pyr-T), 5-methoxy-N,N-tetramethylenetryptamine (5-MeO-pyr-T), 5-methoxy-N,N-diethyltryptamine (5-MeO-DET), 5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MiPT), or *5,6-di*methoxy-N-methyl-N-isopropyltryptamine (5,6-Di-MeO-MiPT). At either 4 hour or 24 hours after drug administration animals are then placed within an open field with a sucrose-soaked food pellet in the center. Time in center and latency to feed measures were collected.

Results: In examining the difference of the maximum evoked response from baseline to the post drug period, we found that fractional change from baseline was not significantly different for any drug group after 4 hours ($F_{4,35} = 1.908$, $p = 0.1310$). However, fractional change was significantly different at 24 hours ($F_{4,39} = 6.042$, $p = 0.0007$) for 4-AcO-DMT ($p = 0.0014$) as compared to

saline. In the NSF, we found that at 4 hours neither male nor female animals were significantly different in the latency to feed; however, when we examined total time spent in the center ($p = 0.0005$), we find that males in the 4-AcO-DMT and Pyr-T group spent significantly more time in center ($p = 0.0282$, and $p = 0.0380$ respectively). Females are not significantly different at 4 hours ($p = 0.0960$). At 24 hours, there was no significant difference between males ($F_{5,49} = 1.596$, $p = 0.1788$) or females ($F_{7,54} = 1.772$, $p = 0.1120$).

Conclusion: In conclusion, this paper finds evidence for plasticity in the prefrontal cortex 24 hours after psychedelic administration and for a decrease in anxiety-like behaviors in the novelty suppressed feeding task at 4 hours after psychedelic administration.

3.2. Introduction

Serotonergic psychedelics are drugs that induce profound alterations to the conscious experience and produce visual, auditory, and somatosensory perturbations to the user's perception [348, 349]. These drugs are agonists at the 5-HT_{2A} receptor and have shown efficacy as novel therapeutics in the treatment of various psychiatric disorders such as major depressive disorder, anxiety, and addiction [52, 54, 350, 351]. Indeed, in various clinical trials these drugs have demonstrated remarkable improvements to long-term wellbeing even outside of the context of psychiatric burden [78, 79, 352, 353]. Despite the host of literature supporting their therapeutic potential and applications in wellbeing, the mechanism by which these behavioral changes are

affected is unclear. A major hypothesis in the field posits that psychedelics may be exerting their therapeutic effects through action as psychoplastogens, or drugs that induce neuroplasticity [1].

Neuroplasticity refers to the capacity of the brain to form, prune, and reorganize in a way that is optimally efficient [55]. Plasticity is a vital mechanism by which individual organisms are able to regulate cognition and behavior in response to environmental stimuli [354]. This plasticity is significantly impaired in cases of psychiatric burden or neurological deficit, [354]. There is evidence that psychedelics may increase plasticity in various ways, from the transcriptional level to the cytoarchitectural level [295, 355]. Upregulation of BDNF, PSD-95, GLUA-1 protein, CREB, and various kinases have been observed after single administration of psychedelics in various *in vitro* studies [255, 256, 356]. This transcriptional plasticity may contribute to the various structural changes that are also observed in the brain following psychedelic administration. Indeed, there have been studies demonstrating structural plasticity at acute time points, 4 hours to 48 hours, and even post-acute time points up to 7 and even 30 days post-dosing. In an *in vitro* model examining the effect of single administration of N,N-DMT and LSD to cortical rat neurons for a period of 24 hours, significant dendritogenesis and increased complexity to dendritic arbor was observed [212, 357]. This effect is purported to be 5-HT_{2A} dependent as it was completely blocked by ketanserin, a 5-HT_{2A} receptor antagonist [212]. Similarly, a study examining the effects of N,N-DMT *in vitro* found significant dendritogenesis and spinogenesis up to 24 hours after single administration [212]. In the same vein, a study using an *in vivo* swine model to observe synaptic density found that after single administration of a serotonergic psychedelic there was significantly increased synaptogenesis up to 7 days later in PFC and

hippocampus [358]. Such structural changes have been shown to persist for up to a month after dosing [256].

Along with this evidence of structural plasticity, there is evidence for other forms of plasticity following psychedelic administration such as increased phosphorylation of protein kinase Akt and mTOR to lead to potentiation of AMPA receptors [259]. This increase in functional plasticity was reflected in an increase in behavioral plasticity through enhancement of prosocial behaviors in rodents [259]. In another paper, authors found that 2 weeks after administration of a serotonergic psychedelic, functional metaplasticity was enhanced in whole-cell voltage clamp recordings of the nucleus accumbens. This increase in functional plasticity coincides with the reopening of a critical social reward period in rodents demonstrating behavioral plasticity [260]. Finally, our recent study demonstrated plasticity 24 hours after DOI administration in an increased response to SR stimulation, and metaplasticity as evidenced through enhanced short term plasticity [359].

It is important to note that there is considerable variation in the regions implicated in studies of psychedelic plasticity. With results ranging from subcortical to neocortical in various *in vitro* models, it is unclear whether circuit level or local regional changes in plasticity are driving behavioral effects. It is also important to consider that dysfunction in plasticity may have a role in the etiology and symptomatology of psychiatric disease, and this dysfunction can occur both regionally and at the circuit level. Of note is the ventral hippocampal to prefrontal cortical (vHPC-PFC) circuit. This circuit is integral to higher order cognition, emotional regulation, and memory processing due to its reciprocal connections with memory and executive functioning areas [360].

This bidirectional pathway has many afferents originating at either region to form monosynaptic or polysynaptic connections along this pathway [361, 362].

In cases of psychiatric burden, such as major depressive disorder or schizophrenia, there is significant dysregulation of this circuit in humans and in rodent models of stress induced phenotypes [5, 360]. This reliable dysregulation makes this an ideal candidate circuit to experimentally assess the potential effects of psychedelic induced plasticity *in vivo*. Further, various anxiety related behaviors, such as approach and avoidance, rely heavily on the actions of this circuit [363]. These behaviors are vital for survival and require efficient threat valuation and higher order decision making. In cases of anxiety disorder, these approach and avoidance behaviors, like decision making and other cognitions, have been evidence to be highly dysregulated and thus may serve as potent indicators for anxiety-induced behaviors [364]. The approach-avoidance conflict is also highly relevant for understanding stress-induced deficits in behavior, because it has translational implications to analogous human behaviors [365]. Increased avoidance behaviors are analogous to loss of resilience, which is characteristic of depressive symptomatology in humans, while increased approach behaviors may be analogous to risk seeking behaviors [366]. Efficient threat valuation is a vital adaptive mechanism, and inappropriately shifting the balance between approach and avoidance is highly characteristic of anxiety disorders [367].

In support of this, evidence suggests that there is overlap in the circuits implicated in anxiety-induced behavioral deficits and in the balance of approach and avoidance behavior. Two distinct populations of neurons, a deep and superficial layer of pyramidal neurons originating in the ventral CA1 of the HPC and terminating in the PFC, are responsible for shifting approach and

avoidance behaviors [363, 368]. These parallel circuits maintain a crucial balance in that the deep layer of neurons preferentially activates PFC pyramidal cells fast spiking interneurons to promote avoidance, while the superficial later activates PFC inhibitory interneurons to promote approach behaviors [363]. Thus, a task that probes approach and avoidance behaviors would be highly relevant to understand both circuit level implications of pharmacological intervention as well as in examining the behavioral effects of any such intervention.

It is interesting to note that despite the evidence for psychedelic-induced plasticity and metaplasticity, it is not yet clear whether changes in plasticity occur specifically due to the hallucinogenic effects at the 5-HT_{2A} receptor. Vital questions about an exact mechanism for psychedelic induced plasticity remains unanswered. Herein, we look for changes in plasticity along the vHPC-mPFC pathway after serotonergic psychedelic administration and for the corresponding behavioral effects of the drugs.

3.3. Methods

All experimental protocols conformed to American Physiological Society/National Institutes of Health guidelines and were approved by the University of Wisconsin Animal Care and Use Committee.

Animals

Male and female C57BL/6 mice (*in vivo* electrophysiology*:* males n = 14; *ex vivo* corticosterone measurements: males n = 36, females n = 64; *in vivo* behavior: 4-hour experiments: males n = 90, females n = 90; 24 hour experiments: males n = 50, females n =59) 6-8 weeks old,

were bred in house (F2 generation) or ordered from Jackson Labs. Mice were maintained on a 12:12 light dark reversed schedule (lights on at 9:00 pm), and all experiments were performed during their scheduled dark period. Mice had *ad libitum* access to food and water unless being food restricted as per experimental protocol, at which point they only had *ad libitum* access to water. Animals used in the corticosterone measurements and the behavioral experiments were housed in groups until time of experimentation which was terminal following completion. Animals used in electrophysiological experimentation were housed in groups until surgical electrode implantation, after which they were housed individually.

Drugs

All controlled substances were handled by authorized users on the Schedule I and Schedule II–V DEA research licenses and WI Special Use Authorizations held by Dr. Matthew Banks or Dr. Cody Wenthur. Psilocybin powder (Usona Institute; Madison, WI) was diluted in 0.9% sterile saline, then acidified to a pH of 1–2 with 1 M HCl, sonicated for 30–60 s, and brought to a pH of 6–7 using 1 M NaOH. This material was passed through a 0.2 μm filter and administered intraperitoneally (IP) at either 1 mg/kg or 3 mg/kg. Aliquots of 4-acetoxy-N,N-dimethyltryptamine (4-AcO-DMT), 6-fluoro-N,N-diethyltryptamine (6-FDET), N,N-tetramethylenetryptamine (pyr-T), 5-methoxy-N,N-tetramethylenetryptamine (5-MeO-pyr-T), 5-methoxy-N,N-diethyltryptamine (5-MeO-DET), 5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MiPT), and *5,6-**di***methoxy-N-methyl-N-isopropyltryptamine (5,6-Di-MeO-MiPT) (Usona Institute; Madison, WI) were diluted in 0.9% saline, filtered through a 0.2 μm pore membrane, and stored at –80 °C until they were ready for use. Aliquots were then thawed and administered right away IP at 1 mg/kg for each drug.

In vivo electrophysiology

Surgical Implantation

To assess electrophysiological changes and detect local field potentials (LFPs), electrode implantation surgery was conducted. Animals were induced for surgery using 4% isoflurane anesthesia and after an initial induction period of 4-5 minutes, were maintained under 2% or lower anesthesia with periodic respiration and consciousness checks. After shaving of the fur from the skull, injection of the local anesthetic bupivacaine, and injection of the systemic slow-release analgesic buprenorphine-SR, a vertical midline incision was placed to visualize the skull bones. After scraping of the skull to remove connective tissue and scoring with a scalpel blade to create abrasions for cement adhesion, careful measurements of Bregma and Lambda were taken with the Neurostar stereotactic StereoDrive software digital atlas (StereoDrive, Neuostar, Germany). After adjusting for any possible tilt or yaw, insertion coordinates were obtained using the digital atlas, marked on the skull, and drilled into for complete craniotomy.

A tungsten twisted-wire (California Fine Wire, Grover Beach, CA) recording electrode was placed in the prelimbic or cingulate cortices of the mPFC (AP: 1.98–2.1 mm, ML: 0.3–0.4 mm, DV: 1.9-2.3), as this area receives dense afferents from the ventral hippocampus and is part of the vHPC-mPFC pathway [369]. Each animal was also implanted with a custom bipolar stimulating electrode (MicroProbes for Life Science, Gaithersburg, MD) in the right hemisphere in the CA1 region of the ventral hippocampus (AP: -3.6 to -3.8 mm, ML: 3.2–3.3 mm, DV: 3.8–4.3 mm) [369]. Bilateral skull screw electrodes were then inserted through the occipital plate and tied together to serve as a ground. All insertions were conducted with the stereo drive for

ultraprecise insertion. Dental cement (Fusio A3; Pentron; Orange, CA) was then used to affix the electrodes in place and build a headcap around the electrode interface board (EIB-16; Neuralynx, Bozeman, MT). Animals were given a minimum of 5 days to recover from surgical procedures before the first recording session.

Electrophysiological Data Acquisition

All recordings were performed during the animals' dark (i.e. active) period using equipment and software from Tucker-Davis Technologies (TDT; Alachua, FL). Individual animals were placed in a clear plastic beaker (6" diameter) within a dark sound-attenuation chamber. The animal was then attached to a headstage (ZC16) on flexible tether to allow for free movement about the chamber. Recordings were obtained using a PZ5 preamplifier and RZ5D amplifier hardware and Synapse software (filtered at 0.4-457 Hz, digitized at 1017 Hz). Upon collection, these data were stored for offline processing and analyses.

Electrophysiological Experimental Protocol

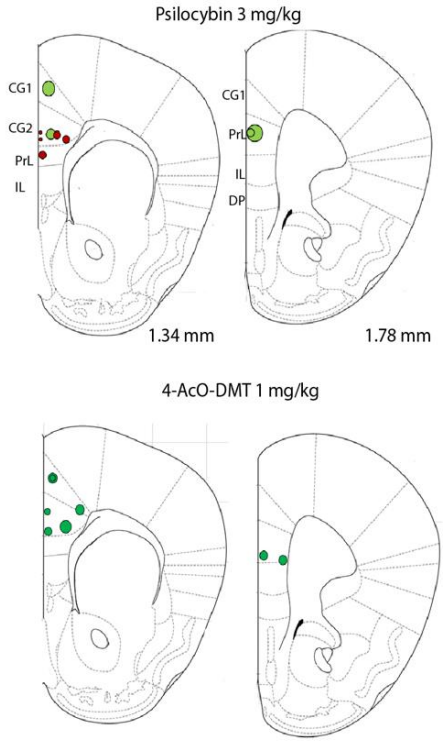
To measure changes in synaptic strength and plasticity, we recorded from animals at various time points; Baseline Day: 1 day prior to drug exposure; Recording Day 1: day of drug administration; Recording Day 2-7: every day following drug administration. On Baseline Day, stimulus-response curves were obtained for each animal in which stimulation intensities ranging from 0 μA up to 800 μA were applied. On Recording Day 1, a stimulus response curve was obtained, and then the animal was administered either a serotonergic psychedelic or saline (5 mL/kg). Spontaneous LFP activity was recorded for the duration of a 4-hour acute exposure

period; these data will be reported in a separate publication. The end of the 4-hour acute exposure period was punctuated with a stimulus response curve to measure change in synaptic strength and plasticity. The plasticity assay protocol was then repeated at 24 hours and every day for 7 days following. Animals were injected with more than one drug in random assignment with at least 7-14 days in between subsequent drug injection.

Data Analysis

Data were preprocessed using custom MATLAB scripts (Mathworks, Natick, MA). Data was visually assessed for a stimulation artifact and 60 cycle noise. Stimulus evoked data was constrained to 0.02 seconds before the stimulus artifact and 0.2 seconds after. The peak period was manually identified upon visual inspection and the program then quantified the peak by averaging over a window of 1 x 10^{-3} seconds around the manually indicated peak location. Time information was also collected. Traces that did not show a stimulus artifact were excluded, as well as data that had significant noise that a notch filter could not eliminate, and data in which the electrodes looked to be damaged which was determined by the presence of floating channels. The maximum peak amplitude at every stimulation intensity in a given stimulus response curve was then compared to the maximum evoked response from the baseline stimulus response curve to obtain a fractional change value:

$$\frac{Maximum\ Response_{Post-Administraion} - Maximum\ Response_{Baseline}}{Maximum\ Response_{Baseline}}$$



3.3.1. Figure 1. Final electrode locations. Final electrode locations as determined by histology denoted by green or red circles. The green denotes an increase in response following psychedelic injection where red shows a decrease. Size of the circle corresponds to amplitude of response. Electrode final locations were in a range from the cingulate cortex to the prelimbic cortex.

Histology

The locations of the stimulating and recording electrodes were verified histologically. Upon completion of experimental protocols animals were stimulated at 1000 **µ**A for 30 s to effectively lesion the stimulation site. The animal was then perfused with saline and fixed with formalin to prepare brain tissue for sectioning. Brain tissue was transferred to sucrose and promptly upon sinking was fixed onto a plate for cryotome sectioning. The brain was sectioning into 20 µm thick slices and were placed within a sucrose well and manipulated carefully using paintbrushes to be affixed to glass slides for nissl staining. Nissl staining procedures followed standard protocol, and upon completion, slices were observed under microscope magnification to determine final electrode location (Paxinos and Franklin; Figure 1).

Behavioral Measurement

Novelty Suppressed Feeding

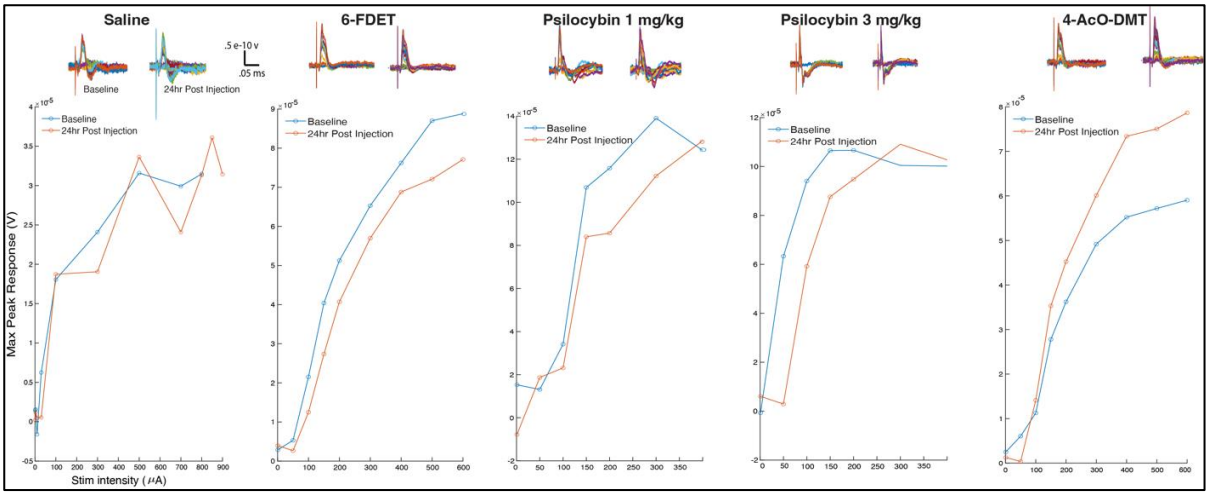
The novelty suppressed feeding task (NSF) was used to assess changes in approach and avoidance behaviors. The NSF is a valuable measure of outcomes where a positive valence construct (reward) is placed in opposition to a negative valence construct (acute threat). In this assay, animals will need to overcome a level of stress and anxiety to achieve the food pellet reward. Animals were placed on food restriction for a total period of 12 hours. For experiments conducted at 4 hours post drug administration, animals were injected at hour 8 of their food restriction period. For experiments conducted at 24 hours post drug administration, animals were food restricted 12 hours after their injection and tested at 24 hours after the injection. For the tests, the animals were placed within a brightly lit (900 lux in the center, 500 lux in the periphery) novel environment (60x40x40) in which there was a food pellet soaked in sucrose available for their consumption in the center point (9 cm radius) of the apparatus. Separate cohorts of animals were tested for each drug at either 4 hours, or 24 hours after administration. No animal was reused across drug groups.

Data Analysis

Behavioral data were manually scored by a blinded observer for latency to feed and time in center was quantified by a behavioral software ANY-maze (Wood Dale, IL). All statistical analyses were performed using GraphPad Prism, version 10 (San Diego, CA). Non-parametric

tests such as the Mann-Whitney U test and a Kruskal-Wallis U test were used to analyze these data.

3.4. Results

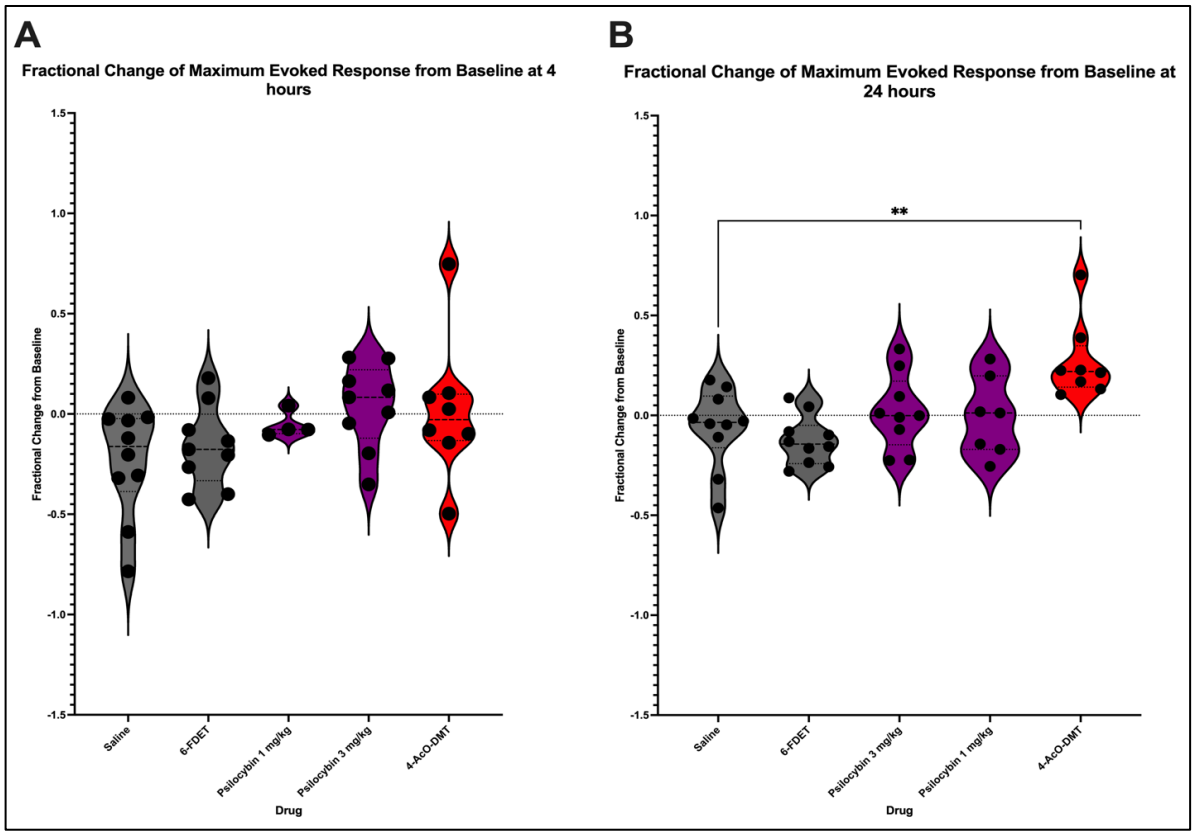


3.4.1. Figure 2. Representative raw traces and stimulus response curves at baseline and 24 hours after injection. Representative raw traces from different animals at the baseline and 24 hours after drug injection. Stimulus response curve plots display the maximum peak amplitude at every stimulation intensity.

Increased maximum evoked response 24 hours after psychedelic administration

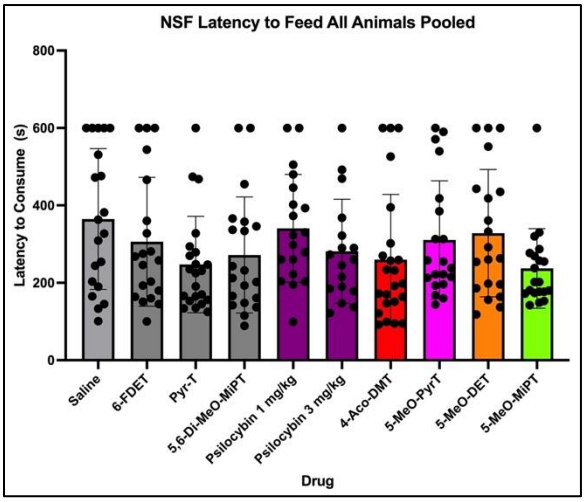
In an assessment of plasticity along the vHPC-mPFC pathway, we found that fractional change from baseline (Figure 3, A) was not significantly different for any group after 4 hours ($F_{4,35}$ = 1.908, p = 0.1310). However, fractional change was significantly different at 24 hours ($F_{4,39}$= 6.042, p = 0.0007; Figure 3, B) for 4-AcO-DMT (p = 0.0014) as compared to saline. We do not see a significance for either dose of psilocybin; this dissociation between the hallucinogenic drugs must be investigated further as evidence suggests that at the same dose, psilocybin results in 10-25% greater concentration in the brain at 15 minutes post administration

compared to 4-AcO-DMT [370]. These data necessitate a dose response to assess full neuroplastic effects.



3.4.3. Figure 3. Fractional change in the maximum evoked response from stimulus response curves at both 4 hours and 24 hours after drug administration. The maximum response from the stimulus response curves from 24 hours after drug injection were compared to peak responses from the baseline period to obtain a fractional change score. **A.** There was no significant difference among groups at 4 hours ($F_{4,35} = 1.908$, $p = 0.1310$) **B.** At 24 hours ($F_{4,39} = 6.042$, $p = 0.0007$) 4-AcO-DMT injected animals showed a significantly increased maximum peak response compared to saline ($p = 0.0014$).

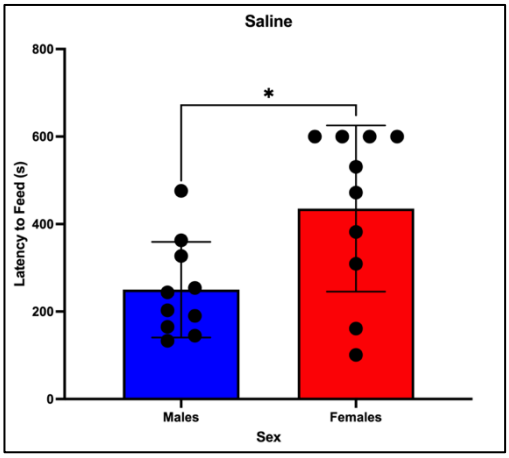
Increased time in center for male animals but not females 4 hours after psychedelic administration but not at 24 hours



3.4.4. Figure 4. Latency to feed for all animals in the NSF assay 4 hours after drug injection. Groups are not significantly different with both sexes combined in a Kruskal-Wallis test for significance H(10) = 14.74, p = 0.0984.

In the NSF, with both sexes combined, we find that no groups are significantly different when examined for the latency to feed measure in a Kruskal Wallis test for significance (H(10) = 14.74, p = 0.0984; Figure 4). we examined males and females separately in their behavioral outcomes as the values for latency to feed in the saline group were significantly different for males (Mdn: 223.5) and females (Mdn: 501.5) in a Mann-Whitney test ($U(N_{males} = 10, N_{females} = 10) = 23$, p = 0.0402; Figure 5). However, we find that at 4 hours, neither male nor female animals were significantly different in the latency to feed. Though males were initially significant, the result did not survive multiple comparisons (Males: H(10) = 19.36, p = 0.0223; Females: H(10) = 14.82, p = 0.0960; Figure 6, A,B). However, when we examined total time spent in the center, males spent significantly more time in center (H(10) = 29.63 , p = 0.0005) when under the experience of 4-AcO-DMT (p = 0.0282) or Pyr-T (p = 0.0380; Figure 6, C). Females were not significantly different at 4 hours (H(10) = 14.28, p = 0.1128). At 24 hours, there was no difference between the male (Mdn: 314.0) and female (Mdn: 381.0) saline latency to feed scores in a Mann-Whitney test ($U(N_{males} = 10, N_{females} = 11$; Figure 7) nor was there any difference in any groups in a two-way ANOVA ($F_{(5, 41)} = 1.546$, p = 0.1971). Similarly, we find that animals combined were not significantly different (H(8) = 13.74, p =

0.0560) in a Kruskal-Wallis test. There was no significant difference between males ($F_{(5,49)}$ = 1.596, p = 0.1788; Figure 7, C) or females ($F_{(7,54)}$ = 1.772, p = 0.1120; Figure 7, C) separately.

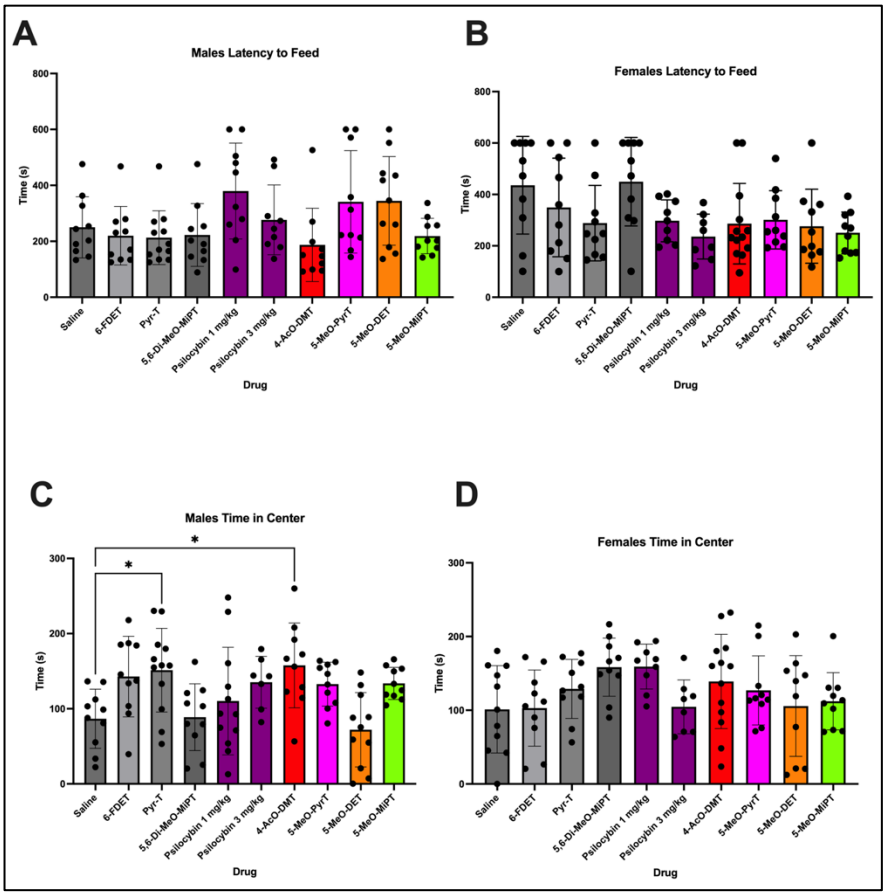


3.4.4. Figure 5. Latency to feed for males and females in the NSF assay 4 hours after saline injection. Males (Mdn: 223.5) and females (Mdn: 501.5) show significantly different scores in a Mann-Whitney test for significance in the latency to feed 4 hours after saline injection U(N_{males} = 10, $N_{females}$ = 10) = 23, p = 0.0402.

3.5. Discussion

The main findings of this paper include evidence for plasticity at 24 hours after administration of 1 mg/kg of the serotonergic psychedelic 4-AcO-DMT and 5-HT_{2A} agonist Pyr-T *in vivo* along the vHPC-mPFC pathway. Further, there is also evidence for decreased time in center 4 hours after injection of 4-AcO-DMT and Pyr-T for males in a test of approach and avoidance, the novelty suppressed feeding task, suggesting that these drugs induce anxiolysis at an acute time period following administration. In current literature, psychedelics have been shown to be highly efficacious treatments for various psychiatric disorders, yet the mechanism by which they affect their change is unknown. Neuroplasticity is enhanced after psychedelic administration in various *in vivo* and *ex vivo* models looking in isolated regions. The vHPC-mPFC pathway has been shown to be highly involved in important cognitions and functions such as threat valuation and memory related mechanisms [371-374]. This circuit is also consistently dysregulated in cases of psychiatric condition and this deficit can be recapitulated in animal models of stress-induced phenotypes [5, 364]. In studies looking at structural plasticity, increased spine number and spine size was evidenced in the PFC following single administration of psychedelics [325], though whether these synapses arose from hippocampal projections is unclear. In a previous study from

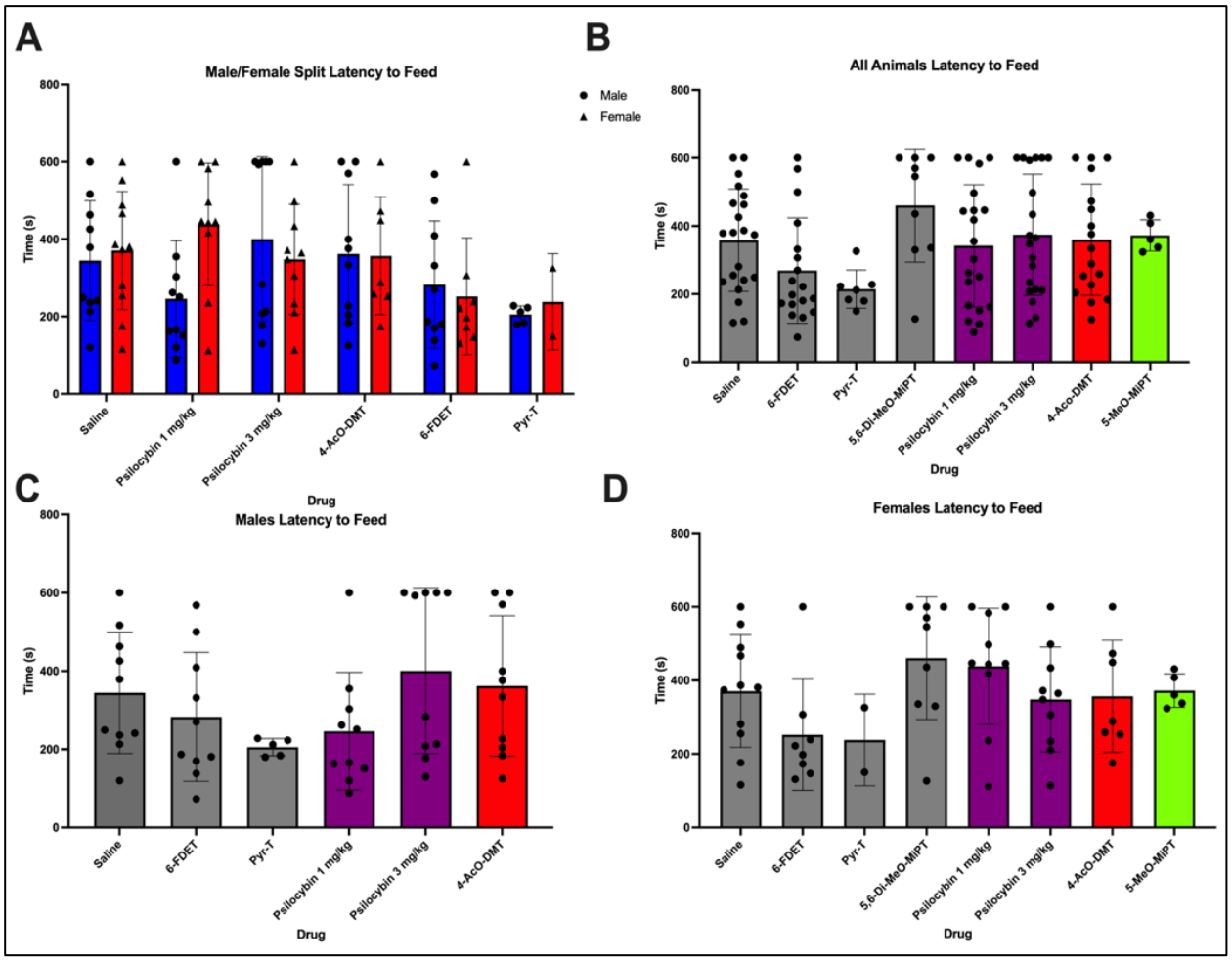
our own lab, we found evidence for plasticity and metaplasticity in the hippocampus, but we did not distinguish specific populations of fibers projecting to the mPFC.



3.4.5. Figure 6. Scores of latency to feed from pellet and amount of time spent in the center zone for both males and females in the novelty suppressed feeding task 4 hours after psychedelic administration. Psilocybin was administered at either 1 or 3 mg/kg, saline was administered at 5 mL/kg and all other compounds were administered at 1 mg/kg **A.** In a Kruskal Wallis test for significance, males were significantly different $H(10) = 19.36$, $p = 0.0223$ but this did not survive multiple comparisons. **B.** Females were not significantly different in the latency to feed ($H(10) = 14.82$, $p = 0.0960$). **C.** When measuring time spent in center, we find that males were significantly different $H(10) = 29.63$, $p = 0.0005$ for animals injected with Pyr-T ($p = 0.0380$) or 4-AcO-DMT (0.0282). **C.** Females were not significantly different ($H(10) = 14.28$, $p = 0.1128$).

No study thus far has investigated changes in plasticity along the vHPC-mPFC pathway *in vivo* or *in vitro*. We assessed changes in neuroplasticity along the vHPC-mPFC pathway, dysfunction of which is highly implicated in the etiology psychiatric conditions, in a *in vivo* model. In this work, we find evidence for plasticity at 24 hours after psychedelic administration along this pathway only with 4-AcO-DMT and not psilocybin. 4-AcO-DMT is a pro-drug for psilocin [375] but has about 20-fold lower potency at the 5-HT_{2A} receptor *in vivo* in mice as compared to psilocybin [370, 376]. So it may be that contributions from another receptor, such as

the 5-HT_{2C}, are the cause for this enhanced plasticity [305]. Or, perhaps, there may be an optimal dose at which circuit level plasticity is potentiated, one that is lower than the given 1 mg/kg of psilocybin. This necessitates future experiments with a full dose response to understand plasticity in the vHPC-mPFC pathway.



3.4.6. Figure 7. Scores of latency to feed from pellet for both males and females in the novelty suppressed feeding task 24 hours after psychedelic administration. **A.** Latency to feed from pellet for each drug by males and females. Groups were not significantly different in a two-way ANOVA ($F_{(5, 41)} = 1.546$, $p = 0.1971$). **B.** All animals combined. Groups were not significantly different in a Kruskal-Wallis test ($H(8) = 13.74$, $p = 0.0560$), but this did not survive multiple comparisons. **C.** Males were not significantly different ($F_{(5,49)} = 1.596$, $p = 0.1788$) **D**. Females were also not significantly different ($F_{(7,54)} = 1.772$, $p = 0.1120$).

In studies of behavioral effects of psychedelic administration, authors have found that single administration of psychedelics results in decreased stress-induced anxiety-like behaviors, such as increased time in the open field, or increased social behaviors. More specifically, a paper examining the effects of psilocin and the serotonergic psychedelic DOI in the NSF, found that psilocin decreased latency to feed, but DOI did not [377]. In our behavioral data we find that there was significant anxiolysis in a test of approach and avoidance behaviors for serotonergic psychedelic 4-AcO-DMT and non-hallucinogen Pyr-T but not for psilocybin or the other hallucinogenic analogs.

It is interesting to note that 4-AcO-DMT results in behavioral changes in a test of approach and avoidance at 4 hours post administration and also results in plasticity at 24 hours along a circuit that is highly involved in these behaviors, yet we do not see these results with the other psychedelics. We see a behavioral change at 4 hours but not a corresponding change in plasticity. This suggests that there may be a region involved in these behaviors, such as the amygdala, that may be the locus for more immediately plastic change. We see a similar result in behavior with Pyr-T which is non-hallucinogenic, suggesting that these outcomes may not necessarily rely on the action of the 5-HT_{2A} receptor. In all it is clear that results of psychedelics that are known to act in the same receptor system seem to have differential outcomes in the same assays.

It has been found that stressful or corticosterone inducing situations promote negative valence behaviors in rodents, suggesting that perhaps a clearer and more consistent behavioral signal would be seen in an assay of positive valence behaviors. Another paper similarly found

that in the NSF, rodents display increased avoidance behaviors after chronic corticosterone exposure. It follows then, that situations in which there is an increase in basal corticosterone levels, such as when the animals are being food deprived, may result in impaired measurements of anxiety-like approach and avoidance behaviors [378]. In the future it is vital that assays of positive valence, such as those that leverage reward valuation, be utilized in tests of psychedelic behavioral efficacy. Further, the sex specificity of the behavioral effect is to be noted. There may be an effect of endogenous hormonal cycles on such behavioral outcomes. There is some anecdotal evidence to suggest that psychedelics may disrupt normal hormonal rhythms in females [379]. In all, this work invites further investigation into the circuit level neurobiological and behavioral effects of psychedelics.

4. Chapter 4: Characterizing the Time Course of the Psychedelic Response: Assessing Changes in Spectral Power, the Head-Twitch Response, and the Stress Response

Zarmeen Zahid[1,2,4], Jack L. Zheng[1], Sean M. Grady[1], Aidan J. Zeimet[1], Mallory A. Kuppe[1], Andrew Lee[1], Leonardo M. Suarez[1], Daleep S. Sandhu[1], Michael H. Sutherland[1], Olivia N. Moroney[1], Nate T. Jones[2], Cody J. Wenthur[2,3,4], Matthew I. Banks[1,3,4]

[1]Department of Anesthesiology, University of Wisconsin School of Medicine and Public Health, Madison, WI, 53706

[2]School of Pharmacy, University of Wisconsin, Madison, WI, 53705

[3]Transdisciplinary Center for Research in Psychoactive Substances, University of Wisconsin, Madison, WI, 53705

[4]Neuroscience Training Program, University of Wisconsin School of Medicine and Public Health, Madison, WI, 53706

4.1. Abstract

Introduction: The psychedelic state induces a range of biological and physiological changes. There is evidence that there are broadband changes in brain oscillatory power following psychedelic administration. Along with this, there is evidence for changes in HPA axis function in an induction of the cortisol/corticosterone stress response. Further, a measure of drug on boarding, the head twitch response, has been shown to be significantly increased after psychedelic

administration. To more fully characterize the psychedelic state I will use *in vivo* electrophysiology, ELISA assay, and magnetometer recordings to characterize the effects on spectral power, corticosterone level, and the head twitch response.

Methods: To record local field potentials, C57BL/6 mice were chronically implanted with a twisted tungsten wire electrode. Animals were then injected with either psilocybin (1 or 3 mg/kg), 4-acetoxy-N,N-dimethyltryptamine (4-AcO-DMT; 1 mg/kg), or 6-fluoro-N,N-diethyltryptamine (6-FDET; 1 mg/kg) and were recorded for the 4 hours following administration. To quantify changes in corticosterone, animals were injected with the chosen drug at various time points and anesthetized for blood collection from a retro-orbital bed of capillaries. This blood was processed and analyzed in an ELISA assay. Finally, to measure the time course of the head twitch response, animals were implanted with a cranial magnet. They were then injected with the chosen drug and placed within a magnetometer to measure changes in the magnet signal. This signal was then processed, and head twitch response count was quantified.

Results: In examining changes to spectral power, we find that within the first 2 hours following drug administration, there seem to be variable outcomes across drug groups that then decay over the subsequent 3-4 hours. These results being: psilocybin 3 mg/kg showed an increase in delta power and theta power while displaying a modest suppression in alpha power and beta power. 4-AcO-DMT showed an increase in delta power, while displaying suppression of all other bands. In an analysis of HTR, we found that HTR increased for serotonergic psychedelics peaking at 5-10 minutes and decaying after 15-20 minutes. Finally, when analyzing stress glucocorticoids, we found that corticosterone was significantly increased after psychedelic administration ($F_{(4,70)} = 40.77$, $p < 0.0001$). In post hoc tests we find that the AUC for psilocybin is significantly increased

as compared to saline ($p < 0.0001$), 6-FDET is significantly increased as compared to saline ($p = 0.0160$), and saline is significantly different from no-injection ($p < 0.0001$).

Conclusion: In conclusion, the time course of psychedelic drug effects is variable across the different domains of neuro-oscillation, limbic system activation, and behavioral responding.

4.2. Introduction

In various clinical trials, serotonergic psychedelics have demonstrated efficacy as rapid and long-lasting treatments for psychiatric disorders such as major depressive disorder, anxiety, and even disorders with more elusive treatment profiles such as chronic pain and eating disorders [52, 54, 74, 380, 381]. Despite this clear demonstration of therapeutic impact, the mechanism of action by which these drugs affect benefit is yet unclear. Psychological and physiological changes that occur during the acute psychedelic experience likely play a role in therapeutic outcomes. It then becomes important to characterize the time course of these various changes in an effort to understand the mechanistic underpinnings of long-term behavioral outcomes.

Assessing changes in brain oscillatory power using extracellular recording methods has long been an valued tool for understanding neurophysiology and behavior [382]. These oscillatory networks are thought to be the critical link between single-neuron activity to cognition and behavior [383]. Neural oscillations can be described as a subset of the larger power spectrum that stand out amongst the background activity and can be separated out into specific frequency bands [382]. The different frequency bands include delta (1-4 Hz); theta (4-8 Hz); alpha (8-13 Hz); beta (13-30 Hz); and gamma (30-80 Hz) and the functional significance of these bands have been associated with changes in cognition, behavior, and even disease states [382].

In clinical and pre-clinical trials examining the effects of psychedelics, many studies have found evidence for changes in spectral power following administration [6, 164, 165, 384-387]. In all these studies, there are broadband decreases in oscillatory power after psychedelic administration, however, most consistently seen is a decrease in alpha power. This may be one of the most robust biological signatures of psychedelic administration to date. Alpha waves are associated with a state of relaxed wakefulness when the eyes are closed and may reflect the degree to which visual brain activity is being subject to inhibition [388]. It has been postulated that a decrease in alpha power may be occurring due to the visual aspect of psychedelic hallucination and an increase in global connectivity and disinhibition. To fully characterize the time course of these changes, we conduct recordings of local field potentials (LFPs) in response to drug administration.

Attenuation of neural oscillations may occur due limbic system activation and output. Evidence suggests that following psychedelic administration, there is an increase in the level of circulating stress hormones, likely due to increased activation of the hypothalamic pituitary axis (HPA) [7]. Once activated by an external or internal stimulus, the anterior hypothalamus releases corticotropin releasing hormone (CRH) which then stimulates the release of adrenocorticotropic hormone (ACTH), acting in the adrenal gland to release cortisol or corticosterone [389]. This cascade is tightly regulated via a negative feedback loop and works to maintain an important homeostatic balance [389]. The hypothalamus, which is a central region in this stress-response axis, contains many receptors activated by serotonergic psychedelics such as the 5-HT_{2A}, 5-HT_{1A}, 5-HT_{2c}, sigma-1, and dopamine receptors [390-392].

Evidence in both clinical and preclinical models indicates that serotonergic psychedelics induce an acute biochemical stress response. Administration of DOI, a serotonergic psychedelic, induced a 5-HT_{2A} receptor-dependent release of ACTH and corticosterone in rats [233]. By contrast, chronic administration of DOI decreased 5-HT_{2A} binding in the paraventricular nucleus and ACTH release [393], suggesting that the stress response is mitigated by receptor internalization. Previously, we observed an acute increase in serum corticosterone in mice 15 minutes after psilocybin administration - a time point corresponding to peak induction of head-twitch response (HTR) behavior [7]. To fully characterize this stress response, we administered serotonergic psychedelics psilocybin and 4-AcO-DMT, and a non-hallucinogenic 5-HT_{2A} agonist 6-FDET at varying time points from 5-1440 minutes.

It is yet unclear whether the stress response is initiated in an emotional response of fear and anxiety to the subjective experience of psychedelics, a biological consequence of drug binding, or both. In preclinical trials using rodent models, it is understandably difficult to assess the subjective experience of these drugs, however, one proxy measure of psychedelic drug onboarding has been validated. The head twitch response (HTR) has been used as a measure of psychedelic drug entry into the central nervous system (CNS) and is evidenced to be dependent on 5-HT_{2A} receptor binding [347]. Interestingly it has been evidenced that the potency of the HTR in rodents correlates very strongly with the dose and intensity of the subjective experience in humans [81]. Herein, we use various techniques to characterize the time course of the psychedelic state in spectral power, the corticosterone response, and the head twitch response.

4.3. Methods

Animals

Male and female C57BL/6 mice (Electrophysiology: n = 15 males; Corticosterone Assay: n = 30 males, n = 64 females; Head Twitch Response Assay: n = 36 males), 8-12 weeks old, were obtained from Jackson Labs or the UW Madison Breeding Core (The Jackson Laboratory, ME, USA). Mice were maintained on a 12:12 reversed light-dark cycle (light on at 9:00) with *ad libitum* food and water. All animals were housed in groups of 4 until drug administration.

Drugs

All controlled substances were handled by authorized users on the Schedule I and Schedule II–V DEA research licenses and WI Special Use Authorizations held by Dr. Matthew Banks or Dr. Cody Wenthur. Psilocybin powder (Usona Institute; Madison, WI) was diluted in 0.9% sterile saline, then acidified to a pH of 1–2 with 1 M HCl, sonicated for 30–60 s, and brought to a pH of 6–7 using 1 M NaOH. This material was passed through a 0.2 μm filter and administered intraperitoneally (IP) at either 1 mg/kg or 3 mg/kg. Aliquots of 4-acetoxy-N,N-dimethyltryptamine (4-AcO-DMT) and 6-fluoro-N,N-diethyltryptamine (6-FDET) were diluted in 0.9% saline (5 mL/kg), filtered through a 0.2 μm pore membrane, and stored at –80 °C until they were ready for use. Aliquots were then thawed and administered right away IP at 1 mg/kg for each drug. Animals in electrophysiology experiments were used in multiple treatment groups with at least a 7 day wash out period between drugs.

In vivo electrophysiology

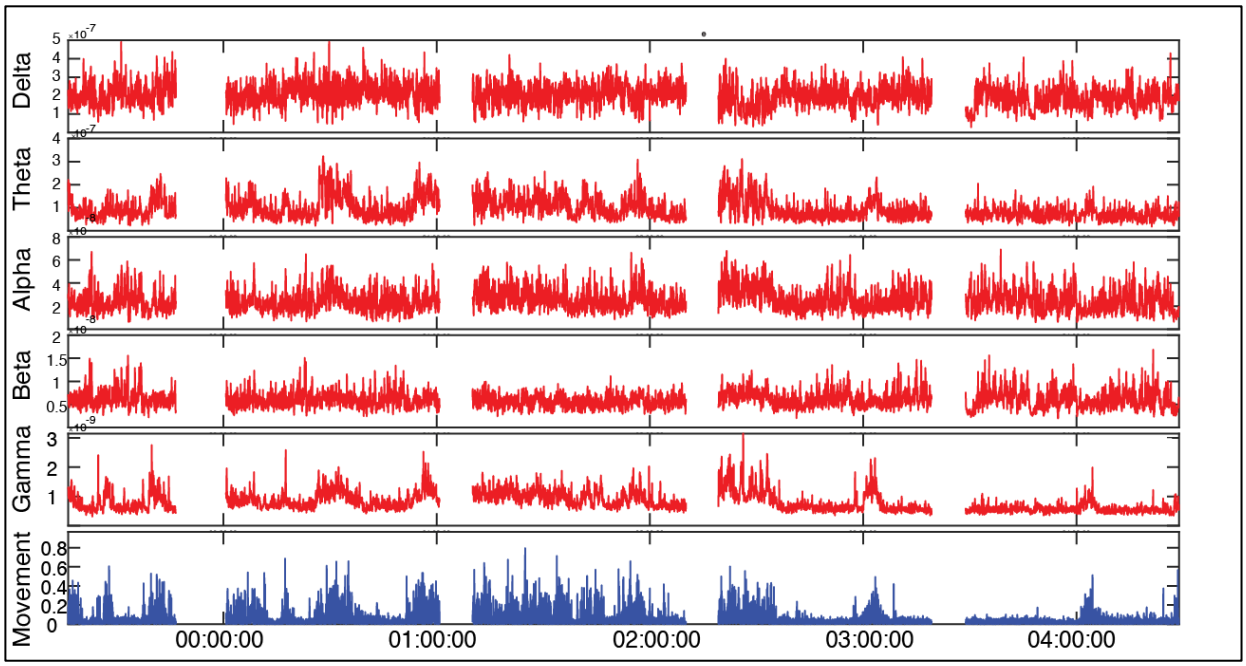
Surgical Implantation

In an effort to assess electrophysiological changes and detect extracellular local field potentials (LFPs), electrode implantation surgery was conducted. Animals were induced for surgery using 4% isoflurane anesthesia and after an initial induction period of 4-5 minutes, were maintained under 2% or lower anesthesia with periodic respiration and consciousness checks. After shaving of the fur from the skull and injection of a local anesthetic, bupivacaine, and injection of systemic slow-release anesthetic, buprenorphine, a vertical midline incision was placed to visualize the skull bones. After scraping of the skull to remove connective tissue and scraping the skull with a scalpel blade to create abrasions for dental cement adhesion, careful measurements of Bregma and Lambda were taken with the Neurostar stereotactic stereodrive system digital atlas (StereoDrive; Neurostar; Germany). After adjusting for any possible tilt or yaw, insertion coordinates were obtained using the digital atlas and craniotomies were drilled into the marked locations. A tungsten twisted-wire (California Fine Wire Company, Grover Beach, CA) recording electrode was placed in the prelimbic or cingulate cortices of the mPFC (AP: 1.98–2.1 mm, ML: 0.3–0.4 mm, DV: 1.9-2.3), as this area is highly involved in higher order cognition as well as self-referential thinking, which are both highly impacted under the acute psychedelic state [394]. Bilateral skull screw electrodes were then inserted through the occipital plate and tied together to serve as a ground. All insertions were conducted with the stereo drive for ultraprecise insertion. Dental cement (Fusio A3; Pentron; Orange, CA) was then used to affix the electrodes in place and build a headcap around the electrode interface board (EIB-16; Neuralynx, Bozeman, MT). A magnet was also affixed onto the headcap for use in head twitch response assay to confirm

time course of drug onboarding. Animals were given a minimum of 5 days' time to recover from surgical procedures before the first recording session.

Electrophysiological Recordings

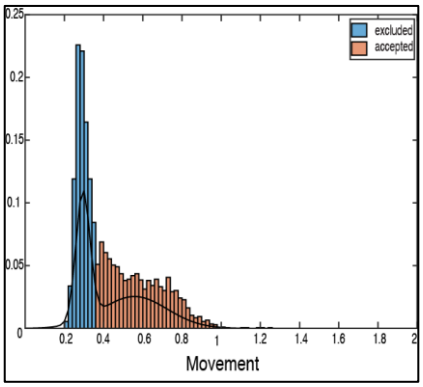
All recordings were performed during the animals' dark (i.e. active) period using equipment and software from Tucker-Davis Technologies (TDT; Alachua, FL). Individual animals were placed in a clear plastic beaker (6" diameter) within a dark sound attenuation chamber. The animal was then attached to a headstage (ZC16) on a flexible tether to allow for free movement about the chamber. Recordings were obtained using a PZ5 preamplifier and RZ5D amplifier hardware and Synapse software (filtered at 0.4-457 Hz, digitized at 1017 Hz). To measure changes in spectral power, we recorded from freely behaving animals 20 minutes prior to drug administration and then for the 4 hours following. Upon collection, these data were stored for offline processing and analyses.



4.3.1. Figure 1. Raw LFP spectral data and movement data from saline injected animal. Raw data from an animal injected with saline. The effect of movement is visibly evident thus prompting a movement correction in the final analysis.

Data Pre-Processing

LFP activity was analyzed using custom written MATLAB scripts (Mathworks; Natick, MA). LFP data were segmented into 4-second time bins, and the magnitude of spectral bands were calculated in each time bin, as follows: delta (1-4 Hz); theta (4-8 Hz); alpha (8-13 Hz); beta (13-30 Hz); and gamma (30-80 Hz). Once spectra were calculated, a movement correction was applied to the data by fitting two gaussian functions to the movement data to determine periods of high and low movement in order to correct for the effects of movement. This is a necessary step because movement can not only cause significant motion artifacts in the data, but it has a signature in and of itself [395]. A visual inspection of the raw data displaying differing traces during periods of low vs high movement (Figure 1) revealed the necessity of such a step. Once the movement data were fit, a Boolean from the accepted fit was applied to the spectral data which was then averaged into 5-minute bins and then averaged across animal treatment groups. Data from the gamma band we excluded due to high levels of variability across animals.



4.3.2. Figure 2. Example of gaussian fit clustering. This is a visual representation of fitting two gaussians to the movement data to cluster into high and low movement data streams where the periods of low movement are used for final analyses.

Corticosterone Measurement

Retro-Orbital Bleeds

Collection of circulating blood was done using retro-orbital (RO) bleeds. Animals were placed under 2% isoflurane anesthesia using a nose cone. Upon confirmation of loss of consciousness, a capillary tube was lowered into the RO space to target a bed of capillaries. Upon draw, blood was spun down in a centrifuge at 10,000 rpm (11,292*g*) for 10 min at 4 °C (Eppendorf-Centrifuge 5430 R; Eppendorf; Hamburg, Germany). The serum fraction was separated and stored at −80 °C. Upon thawing, the plasma corticosterone concentration was assessed using a colorimetric ELISA analysis (Corticosterone ELISA Kit; Enzo-Life Sciences; Farmingdale, NY) following the provided protocol. All independent biological samples were run in technical duplicate or triplicate, and calculated concentrations were corrected for assay dilution (40×).

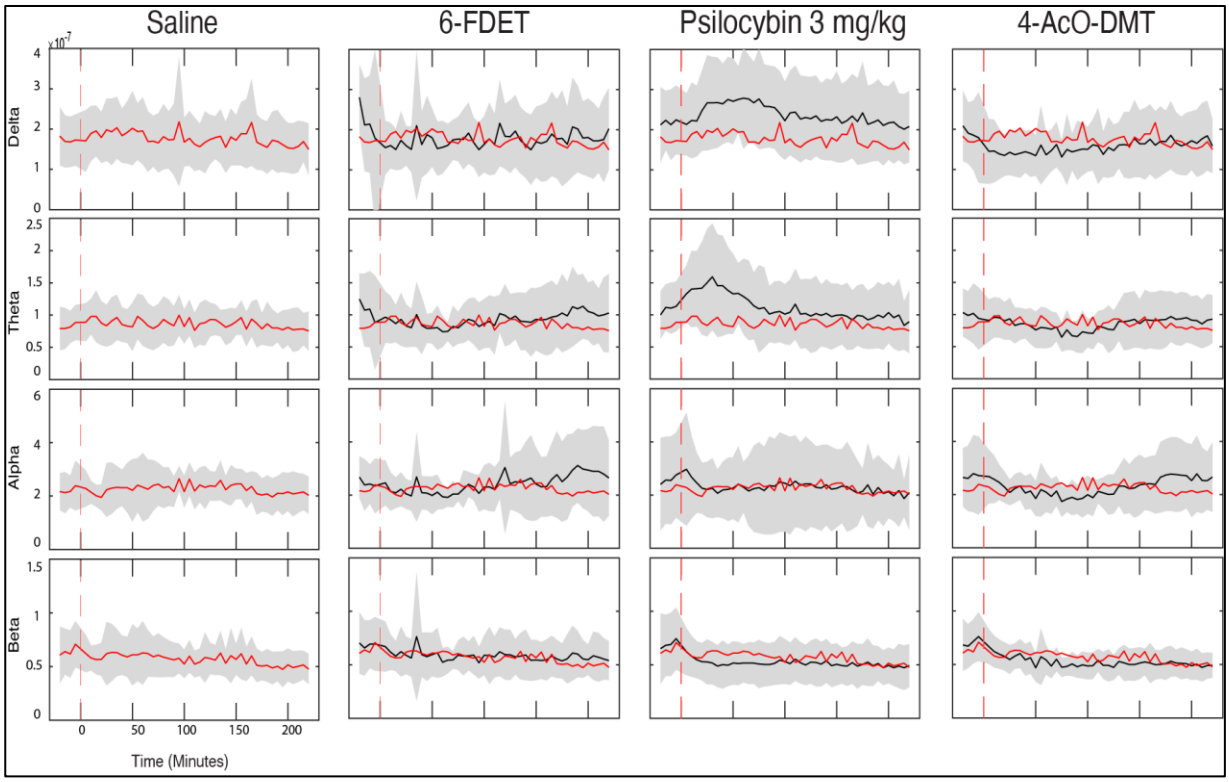
Statistics

Statistical analyses were performed using GraphPad Prism, version 10 (San Diego, CA). Area under the curve analysis were conducted using prism as well as an ordinary one-way ANOVA and a two-way ANOVA corrected using Dunnett's multiple comparisons test.

Head Twitch Response

To ascertain the time course of psychedelic drug entry, action in the nervous system, and time course of 5-HT_{2A} receptor occupation, we used the head-twitch response (HTR). Animals

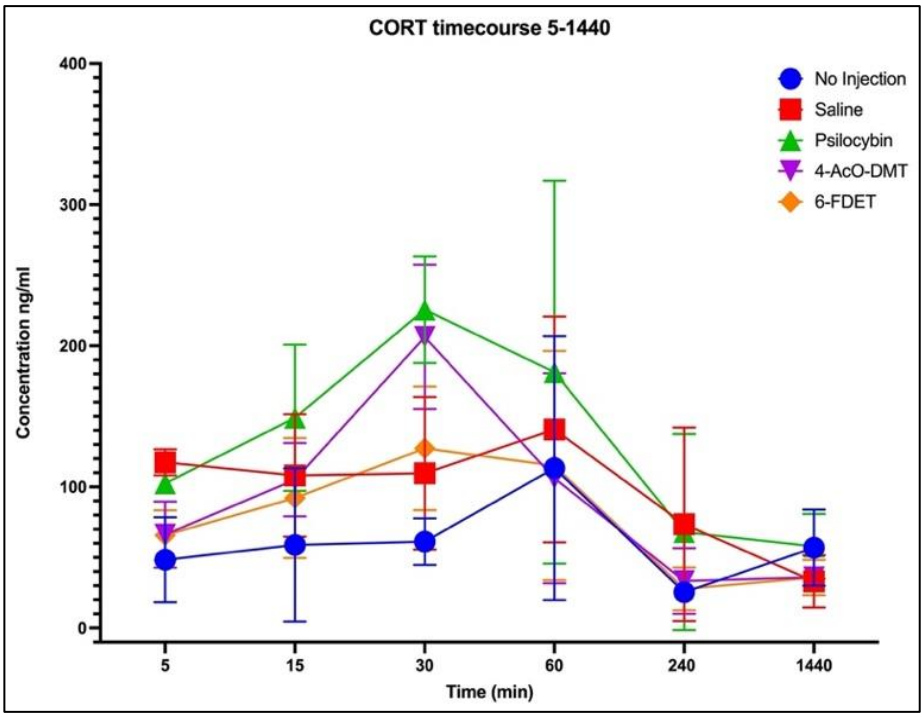
were fitted with a cranial magnet at time of electrode insertion surgery and HTRs were measured using a lab-made copper wire (California Fine Wire Company, Gorver Beach, CA) magnetometer capable of detecting the high frequency head movements that occur during HTRs [8]. Animals recorded 20 minutes of a baseline signal after which they were injected and recorded for up to 4 hours after initial administration. Head twitches were quantitatively assessed using a custom MATLAB (Mathworks, Natick, MA) script written to detect movements within a high frequency range.



4.4.1. Figure 3. Time course of changes in spectral power 4 hours following drug administration. Individual spectra data has been averaged in 5-minute bins with a 20-minute baseline and 240-minute post injection period. Injection time is denoted by a dashed red line at time 0 and saline spectra have been reproduced in each plot denoted by a solid red line. Saline n = 10, 6-FDET n = 12, Psilocybin 3 mg/kg n = 17, and 4-AcO-DMT n = 12. 6-FDET seems to show a slight suppression in the delta and theta band following drug administration. In 3 mg/kg psilocybin we see a clear increase in delta, in theta and a modest suppression of alpha and beta. 4-AcO-DMT shows a clear suppression of delta, theta, and quite noticeably alpha and beta.

Data pre-processing

Recordings were obtained using a PZ5 preamplifier and RZ5D amplifier hardware and Synapse software. These movement data were filtered into two streams: 1) a high frequency stream for HTR event detection, and 2) a low frequency stream (0.1 - 40Hz) for analysis of bounds. The higher frequency movement stream was further processed by manual validation of HTR occurrence using custom MatLab scripts. The lower frequency movement stream was processed so that baseline periods of stillness and activity set the bounds for quiescence and active wakefulness.

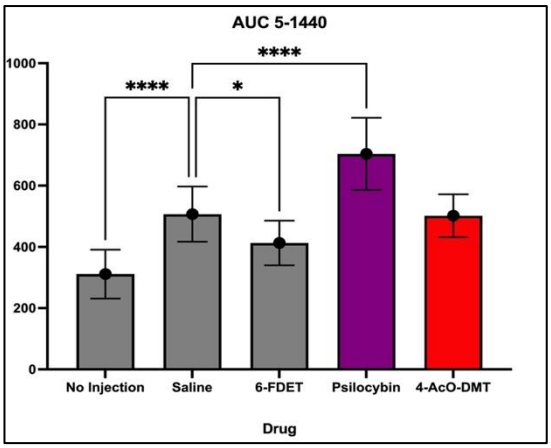


4.4.2. Figure 4. Time course of serum corticosterone levels at from 5 to 1440 minutes. Psilocybin was administered at 3 mg/kg, saline at 5 mL/kg, and all other injected compounds at 1 mg/kg. In a two-way ANOVA corrected for repeated measures using Dunnett's multiple comparison test, there is a time ($p < 0.0001$) and drug ($p = 0.0082$) interaction.

4.4. Results

Variable changes in spectral power across drugs

In an analysis of changes to spectral power (Figure 3) we find a few different outcomes in the time series data. The saline data remain relatively stable from baseline to the post injection period, as expected. When we examine psilocybin at a dose of 3 mg/kg, we see a marked increase in delta power and in theta power immediately following psychedelic administration. Along with this, we see a modest suppression of alpha and beta as compared to the baseline period. Conversely, 4-AcO-DMT shows a clear suppression of delta, theta, and quite noticeably alpha and beta immediately following psychedelic administration. 6-FDET shows a modest suppression in the delta and theta band compared to the baseline period following drug administration. These changes seem to decay back to baseline around 150 minutes post administration. The data are considerably variable across the various drugs.



4.4.3. Figure 5. Area under the curve from 5-1440 minutes for all groups. In a one-way ANOVA, groups are significantly different from each other ($F_{4,70} = 40.77$, $p < 0.0001$). In post hoc tests we find that the AUC for psilocybin is significantly different as compared to saline ($p < 0.0001$), 6-FDET is significantly different from saline ($p = 0.0160$) and saline is significantly different from no-injection ($p < 0.0001$).

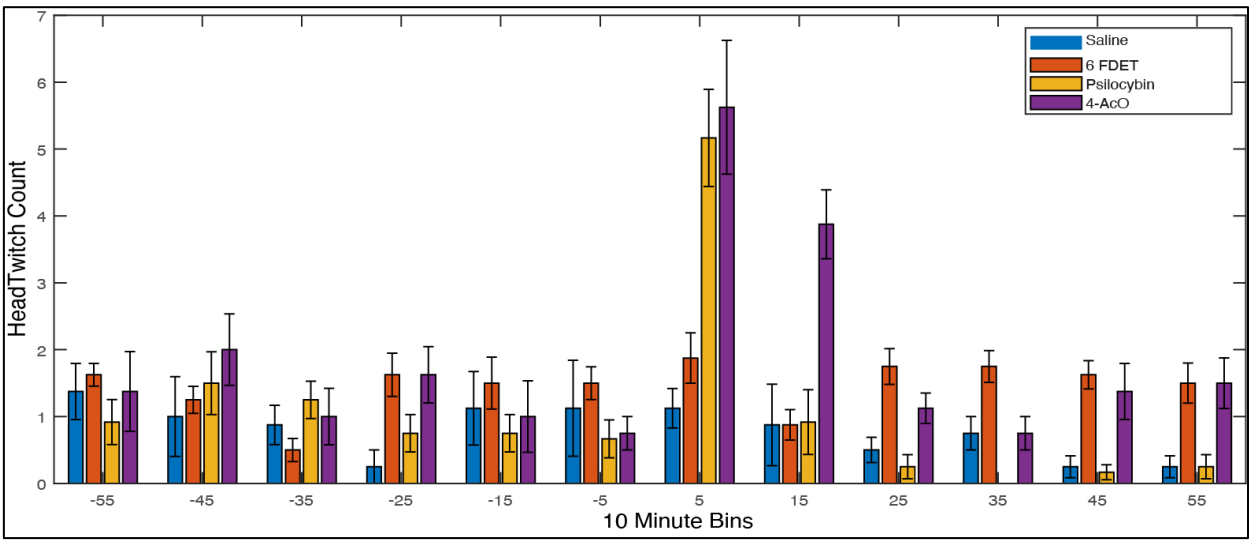
Increased corticosterone after drug administration

In an examination of the time course of corticosterone concentration from 5-1440 minutes after drug administration (Figure 4), we find that there is a significant time ($F_{(5,70)} = 9.526$, $p <$

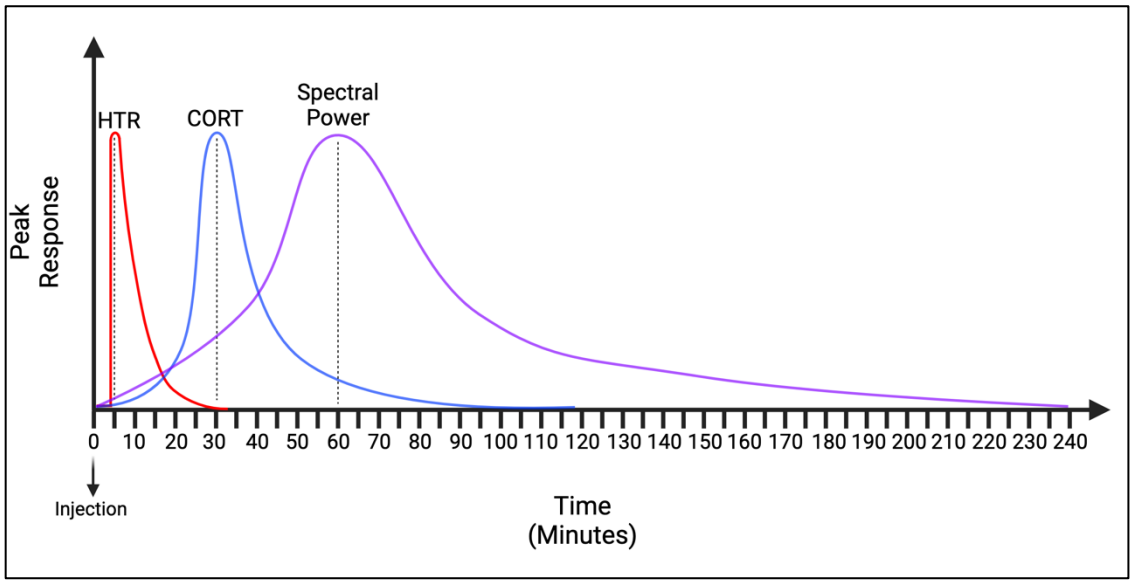
0.0001) and drug interaction ($F_{(4,70)} = 3.726$, $p = 0.0082$) in a two-way repeated measures ANOVA. When corrected for multiple comparisons, psilocybin is significantly different from saline ($p = 0.0424$). These results suggest that there is a clear increase in corticosterone and an activation of the HPA axis following psychedelic drug administration, but not after a non-hallucinogenic substance. Further, in an analysis of area under the curve (AUC) of corticosterone in a one-way ANOVA, we find that the groups are significantly different ($F_{(4,70)} = 40.77$, $p < 0.0001$). Upon conducting post-hoc testing, we find that AUC for the psilocybin group is significantly different as compared to saline ($p < 0.0001$; Figure 5) displaying an increase in AUC. 6-FDET is also significantly different as compared to saline ($p = 0.0160$) displaying a decrease in AUC.

Increase in the head twitch response following drug administration

In an analysis of the HTR (Figure 6), a proxy measure of drug onboarding and 5-HT_{2A} receptor occupation, we find that both serotonergic psychedelics, psilocybin and 4-AcO-DMT display an immediate acute increase in number of HTRs as compared to saline. This response peaked 5-10 minutes after administration and decayed back to baseline after 15-20 minutes. No such increases are seen in the 6-FDET group.



4.4.4. Figure 6. Time course of the head twitch response in 10-minute bins following psychedelic administration. After psychedelic administration, both psilocybin 3 mg/kg and 4-AcO-DMT 1 mg/kg show an immediate increase in the rate of head twitches. This increase tapers off back to baseline by 15-20 minutes after injection. Saline n = 8, 6-FDET n = 8, Psilocybin n = 12, 4-AcO-DMT n = 8. All animals were males.



4.4.5. Figure 7. Visual representation of the time course of the changes in head twitch response, corticosterone level, and spectral power. Curves are not drawn to scale, this is an approximation of the time course of the effects on head twitch response, which peaks at 5 minutes after psychedelic administration and decays around 15-20 minutes later; corticosterone level which peaks at 30 minutes post psychedelic administration and decays in the time following; and spectral power which peaks in the hour to 2 hours following psychedelic administration and decays in the subsequent hours. This figure was made on biorender.com.

4.5. Discussion

Despite the host of clinical research outlining the therapeutic utility of psychedelics in the treatment of various psychiatric conditions, the mechanism by which this behavioral change is imparted remains unknown [396-398]. Psychedelics are evidenced to induce acute changes to consciousness in various domains as well as induce plasticity in various regions and along the vHPC-mPFC circuit. It is likely that the various acute biological and physiological effects of the psychedelic state play a role in the long-term outcomes. To date, there has not been a full characterization of the psychedelic state in pre-clinical models and a comparison of the time course of these changes. Herein, we characterized changes to spectral power, HPA axis function, and locomotive behavioral change.

In our study, we found that following the administration of the serotonergic psychedelic psilocybin at 3 mg/kg, there is a marked increase in delta power and theta power, and a modest suppression of alpha power and beta power. Low frequency oscillation, or delta power, is canonically associated with cortical down states and loss of consciousness, though there are some pharmacological exceptions to this that result in the maintenance of the conscious experience while increasing delta power [399]. Serotonergic psychedelics happen to be one such exception. Various clinical studies have evidenced an increase in delta power following N,N-DMT administration; This increase in power was associated with increases in global connectivity and positively correlated with the intensity of the subjective experience in humans [165, 400]. Studies assessing the effects of 5-MeO-DMT, a DMT analog, and psilocin, the active metabolite of

psilocybin, have also found an increase in delta power pre-clinically [202, 387, 401]. Our data fall in line with current literature and suggest that this increase in delta power is a robust characteristic of the psychedelic state. Interestingly, evidence shows that an increase in delta has also been associated with inward-directed cognition or mentation, so it may be that under the acute psychedelic state, there is global cortical deafferentation and reorientation of hierarchical processing to top-down integrally generated sensory experiences [402].

This idea is supported by the result with theta oscillations. These oscillations in the PFC have been known to become synchronized with hippocampal theta when engaging in spatial working memory tasks and have also been associated with tasks requiring hypervigilance or high awareness [403, 404]. It may be that the observed decreased in theta is reflective of impaired memory function; as mice highly rely on thigmotaxis and spatial navigation through various sensory systems, this may suggest that there is a shift to more top-down processes, or a reweighting of relevant stimuli from externally-generated to the precedence of internally-generated stimuli.

Further, alpha power has been associated with relaxed wakefulness states, such as when the mind wanders during eyes closed rest and is generally thought to have a role in maintaining necessary inhibition of incoming stimuli [405, 406]. In clinical studies of psychedelic induced changes to electroencephalography signals (EEG), authors found suppression of alpha power following administration, and this was significantly associated with profound alterations in consciousness, intense ego dissolution, and was predictive of the intensity of visual hallucinations [191]. Taken together, these results suggest that there may be a decrease in inhibitory mechanisms

coupled with an impaired working memory, specifically, spatial and navigational memory to again increase top-down mechanisms to override normal sensory processing.

Finally, beta oscillations have also been associated with several vital functions such as cognitive control, decision making, memory, and even movement [407]. This broad association with both perceptual and motor processes makes it difficult to understand the implications of these oscillations in isolation; however, beta oscillations have been associated with the clearing of working memory and in filtering of stimuli and information in the working set [408]. A decrease in this band again suggests that there may suppression of filtering, inhibitory, and reductive mechanisms in the brain following psychedelic administration. Similarly, though we see a decrease in delta for 4-AcO-DMT as opposed to an increase, there is marked suppression of theta, alpha, and beta following psychedelic administration. There may be some effect of dose here as 4-AcO-DMT is given at 1 mg/kg compared to psilocybin 3 mg/kg. Most interestingly we find that non-hallucinogenic serotonergic N,N-DMT analog, 6-FDET seems to display a modest suppression of delta and theta, with no clear changes in the other band. Suggesting that the effects on alpha and beta may in part be specific to the psychedelic state.

Indeed, when we examine the time course of the HTR we find that only psilocybin at 3 mg/kg and 4-AcO-DMT at 1 mg/kg showed an increase in the number of head twitches following drug administration, and we do not see this with 6-FDET. The HTR has been associated with 5-HT_{2A} receptor activation and more importantly, has been associated with the hallucinogenic experience [8]. This response overlaps in occurrence with the acute stress response.

In an analysis of area under the curve of corticosterone concentration after administration of serotonergic psychedelics we find that the groups are significantly different from each other ($F_{4,70} = 40.77$, $p < 0.0001$). Specifically, psilocybin at 3 mg/kg shows significantly increased area under the curve as compared to saline. Interestingly, we see a decrease in AUC for 6-FDET ($p < 0.0001$). There is an interesting dissociation again with 4-AcO-DMT and psilocybin suggesting that there are some important differences between the time course of these drugs and perhaps and effect of dose.

Taken together, we find that HTR increases for serotonergic psychedelics peaking at 5-10 minutes and decaying after 15-20 minutes. We find that corticosterone significantly increased after administration only for psilocybin at 3 mg/kg, peaking at 30 minutes, and actually decreased for 6-FDET at 1 mg/kg, and we do not see any differences with 4-AcO-DMT at 1 mg.kg. Finally, in examining the spectral power we find that within the hour following drug administration, there seem to be variable outcomes across drug groups that then decay over the subsequent 3 hours. These results being: psilocybin 3 mg/kg showed an increase delta power and theta power while displaying a modest suppression in alpha power and beta power. 4-AcO-DMT showed an increase in delta, while displaying suppression of all other bands. Here we notice that there are opposite results in the delta and theta band for psilocybin and 4-AcO-DMT and our data suggests that there may be a role of corticosterone in the physiological effects of these drugs. Indeed, one study found that acute boluses of corticosterone, such as those that would have been released upon psilocybin administration, resulted in enhanced EEG delta power and so spectral power may be linked to the stress response [409]. Finally, we found that 6-FDET displayed modest suppression of delta power and theta power. It is of particular note that we see significant changes in all measures of this experiment with 6-FDET compared with saline, but that it is in the opposite direction of that

of the serotonergic psychedelics. The time-course of these changes seem to overlap but are considerably variable even across drugs within the same class (Figure 7). With a current interest in psychedelics or 5-HT_{2A} receptor agonists with minimum anxiety inducing characteristics, 6-FDET may have impactful contributions.

5. Chapter 5: Discussion

5.1. Summary of Findings

Psychedelics have been shown to be highly efficacious treatments for various psychiatric conditions. Despite the growing body of literature outlining the utility of these drugs as novel therapeutics, there still remain some vital questions about the mechanistic underpinnings of these behavioral effects. Of particular note is the neuroplastic hypothesis of psychedelic action which posits that psychedelics exert their therapeutic benefit as psychoplastogens, drugs that induce plasticity. Indeed, various preclinical and clinical studies have found evidence for changes in plasticity associated genes, structural changes to cellular anatomy such as spinogenesis and dendritogenesis, and increased metaplasticity as evidenced through enhancement of long-term potentiation and short-term potentiation. Despite this, no study to date has conducted an *in vivo* analysis at the circuit level in behaving animals. Further, along with neuroplasticity, there is evidence for changes in stress-induced disorder-phenotypic behavior, limbic system activation, and network level brain oscillations. To date, there has not been a full characterization of these measures and a comparison of their time course during the psychedelic state preclinically. Herein, I presented data in an effort to fill these vital gaps.

In **chapter 2,** to provide evidence for the neuroplastic hypothesis, I conducted an *ex vivo* investigation in hippocampal slices to observe changes in plasticity in the Schaffer collaterals of the ventral CA1. This study found enhanced plasticity at 24 hours after serotonergic psychedelic DOI administration, as evidenced through an increase in the maximum evoked response obtained in stimulus response curves. This suggests that cells in the hippocampus are more primed and

excitable 24 hours after psychedelic administration. Furthermore, after application of an LTP protocol I found evidence for metaplasticity. The short-term component, aptly named STP, was significantly enhanced compared to saline and ketamine. Changes in STP suggest presynaptic mechanisms, so we analyzed fiber volleys to determine presynaptic fiber contribution and found this to be significantly increased for the psychedelic group as compared to saline and ketamine. To take this a step further, I conducted a paired-pulse ratio analysis and found that, interestingly, the ratio was increased for the DOI condition as compared to the other groups. This suggests that, in response to the first stimulation, all synaptic vesicles may have released their contents as they were excitable and already primed to do so. The response to the second stimulation involved contribution from newly formed synapses and synapses that would not normally fire but were now firing due to the observed increase in cell excitability. Given that the hippocampus is vital for higher order cognition and adaptive regulation of memory processes and plasticity , these data provide strong evidence for a role of plasticity in the actions of serotonergic psychedelics.

In **chapter 3,** I furthered this investigation by examining changes in plasticity along the ventral hippocampal to medial prefrontal cortex pathway *in vivo* in behaving animals. Such work has not been previously published and understanding circuit level effects of these drugs provides a more nuanced look into mechanistic underpinnings of psychedelic action and is translationally relevant. I found no change in plasticity at 4 hours after psychedelic administration. However, at 24 hours after administration, the serotonergic psychedelic 4-AcO-DMT showed significantly increased maximum evoked responses in stimulus response curves. This is evidence for plasticity along a circuit that is highly implicated in psychiatric disorder pathophysiology. Further, in an effort to connect these changes in plasticity to behavioral changes in an anxiety-like phenotype, I examined changes in latency to feed and time in center in the novelty suppressed feeding task - a

task of negative valence assessing approach and avoidance behaviors. Such behaviors are also evidenced to rely heavily on the vHPC-mPFC pathway, and so this task was particularly well-suited for this investigation. Contrary to my initial hypothesis, I did not find significant changes in behavior at the same time course as the changes in plasticity. Rather, I found that males displayed an increased amount of time spent in the center zone 4 hours after 4-AcO-DMT, and Pyr-T administration, and no significant changes in behavior in either sex at 24 hours. 4-AcO-DMT is a serotonergic drug that converges on psilocin in the body, whereas Pyr-T is a 5-HT_{2A} agonist N,N-DMT analog drug. This dissociation between behavior and electrophysiology is compelling and suggestive of two main ideas: 1) our plasticity results are not seen across all psychedelics tested, and this may reflect a need for more regional precision and specificity. The vHPC-mPFC is known to be part of a triad with the amygdala and so perhaps the pronounced changes are occurring in another part of this bidirectional circuit; 2) the negative valence of the assay and the acute stress we know is induced with psychedelic administration may not result in observable behavioral effects. As in humans, it may be necessary to leverage a positive valence assay or perhaps a reward to potentiate a behavioral signal imparted by psychedelic administration.

In looking outside of the effects on plasticity, we find that there are changes in various domains under the acute psychedelic state. One such being the acute stress response. Stress has been known to be acutely plastic and adaptive, so it may be that there is some convergence of this stress-induced plasticity with that psychedelic-induced plasticity; or perhaps psychedelics actually leverage the plasticity induced by the acute stress response. In an effort to unravel this idea, I compared the time course of the stress response along with some other key measures of cognition and behavior as outlined in **chapter 4**. One such key measure is the head twitch (HTR)

response which should inform us as to psychedelic drug onboarding and 5-HT_{2A} receptor occupation. Similarly, it was important to compare these measures with a measure of network level change: spectral power. Broadband changes in spectral power have been evidenced both preclinically and clinically and indeed my data are in agreement with the current literature.

In the 1-4 hours following psilocybin 3 mg/kg administration there was an increase in delta power and theta power, and a modest decrease in alpha power and beta power. For 4-AcO-DMT we found broadband suppression of all bands, delta, theta, alpha, and beta. Several studies have evidenced suppression of alpha power after psychedelic administration and so these results support current consensus in literature. In 6-FDET we found a modest suppression of delta and theta. However, we must address the interesting dissociation that exists for the delta band, in that the psilocybin group displayed enhancement of delta, whereas the 4-AcO-DMT and 6-FDET group displayed suppression of delta. It has been evidenced that acute boluses of corticosterone, such as those that may be released during an acute stress response, enhance delta power.

To assess the stress response, I quantified circulating blood and found that 30 minutes following drug administration, AUC of corticosterone level for psilocybin 3 mg/kg showed a significant increase in corticosterone levels, and 6-FDET actually showed a significant *decrease* in corticosterone levels. These results suggest that there may indeed be a role of the stress response in our LFP spectral findings and provide a strong basis for a pivotal role of the stress response in the physiological effects of serotonergic psychedelics, though they do not fully capture the depth of the effects as 6-FDET does not show an increase in stress rather a decrease. Literature has evidenced that delta power increases in cases of inward directed mentation and in the case of psychedelics, we understand self-referential thinking and hierarchical processing is highly

effected. It may be that though 6-FDET does not induce a stress response or changes in plasticity, there may be a 5-HT_{2A} dependent mechanism for increase in top-down processing that is being reflected here. Indeed, research suggests that 5-HT_{2A} receptor activation may play a large role in processes related to the self and may be the cause of this change in delta power [88, 410]. This idea further suggests that with 4-AcO-DMT, there may contributions from a non-5-HT_{2A} receptor that modulates its effects on the brain.

In all, these data suggest that the time course of the psychedelic state in these different domains is variable and does not line up one to one preclinically. Changes to locomotive behavior, the HTR, occur within 5 minutes of psychedelic administration. Changes in the HPA axis activation are seen in an increase in stress glucocorticoids 30 minutes after psychedelic administration, and changes in spectral power develop over the 1-2 hours following psychedelic administration. This variability in response is notable and makes clear the complex nature of the psychedelic state.

5.2. Limitations

Though these data add a considerable deal of evidence to the field, there are limitations to these approaches and methods that must be improved upon. In **chapter 2** we were limited by the drugs that we were able to utilize in our investigation. It is vital that we assess other serotonergic psychedelics and different doses to determine hippocampal effects. Further, there are some natural limitations that exist in *ex vivo* slice preparations. These experiments are conducted in a highly controlled cellular environment and are devoid of vital connections to other regions in the brain. Therefore, it becomes challenging to translate and generalize these results separately from the

conditions of this experiment. Further, stimulation and recording in one region may not comprehensively display the full host of changes being affected by drug administration thereby limiting our resulting signal.

In **chapter 3** and **chapter 4** all electrophysiological experimentation required a major survival surgery. Though the animals that were utilized displayed acceptable responses and were themselves healthy, there is a risk that the animal was permanently altered from the surgery in a way that may affect the results. There is also a risk that despite using a digital atlas and a stereotactic arm to insert electrodes, they were not placed in the exact and appropriate coordinates. Final electrode location was confirmed by histology, and though I had achieved an acceptable range around my coordinates, neither the stimulating electrode nor the recording electrode was in the exact same coordinates across animals. Optimizing these procedures is of vital import considering the necessity for precise insertion.

Further, in the process of insertion I damaged the tissue I had traveled through to reach my final target. Therefore, deficits and changes in behavior may exist and be imperceptible yet important to our outcomes. Moreover, for ease of interpretation, all of these experiments were performed with male animals. This provides a very narrow view of psychedelic effects as evidence suggests that hormonal cycles may play a role in the biological response to these drugs [379]. In the case of all the experiments contained herein, we were limited by the doses of serotonergic psychedelics applied. It is necessary to conduct a dose response in all of the assays to fully encapsulate psychedelic effects.

5.3. Future Directions

The results herein prompt further inquiry into the various aspects of the psychedelic response. Firstly, it is vital that we improve upon the methods and address the limitations above such as inclusion of additional doses and drugs for these experiments as well as the inclusion of female animals. In the investigation of *in vivo* plasticity, the inclusion of the amygdala (AMY) as a triad in the HPC-PFC pathway is of vital import, as the amygdala plays a significant role in emotional regulation and regulates many homeostatic behaviors in the brain through reciprocal connections with the HPC and the PFC [31, 35]. Examining the AMY-HPC-PFC circuit will provide insight into the contributions of other areas and will shed light onto the locus of plastic change. In considering the behavioral results, it is important that an assay of positive valence be used to determine behavioral effects of psychedelics. As in humans, animals may respond better in assays in which we leverage a reward for behavioral outcome.

When considering the changes in spectral power it becomes important that we also obtain neural connectivity and signal complexity information. These measures will provide a more in-depth picture of changes to brain network oscillatory dynamics that may contribute to the therapeutic response. Similarly, a more complete time course of the stress response must be obtained in an effort to associate changes in stress to changes in plasticity and neural oscillations. It would be interesting to see selective inhibition of the corticosterone response with psychedelic administration and its effect on spectral power, connectivity, and complexity. There remain yet many more questions to answer in this field.

5.4. Conclusion

In conclusion, the data contained in this thesis provide strong evidence for plasticity and metaplasticity following psychedelic administration both *ex vivo* and *in vivo*. Taken together, my data suggest extensive biological and physiological effects of psychedelic administration. Including acute effects such as changes to spectral power, HTR, and the stress response as well as post-acute enhancement of plasticity and metaplasitcity at 24 hours in the HPC and the PFC. With this, I propose a three-phase model of psychedelic action. Phase 1 is characterized by the development of the subjective effects, as characterized by an increase in HTR. These subjective effects persist past the acute HTR period and peak within this phase. Shortly following the peak of these effects is the onset of the second phase of these effects. This phase is characterized by the activation of the HPA axis and an acute stress response. The resolution of this acute stress response leads to acute behavioral effects due to the activation of inhibitory stress regulatory pathways, such as those involving the activation of inhibitory $5HT_{1A}$ receptors. The final phase of this model is characterized by psychedelic induced plasticity. This phase begin acutely in transcriptional and structural plasticity changes and post acutely results in increased plasticity and metaplasticity in regions relevant to long-term behavioral change. I hypothesize that long-term behavioral change will only be affected if this post-acute psychedelic induced plasticity is exploited in some phenomenologically meaningful way. There must be some level of integration for these biological changes to take hold. I conclude that psychological integration is a necessary aspect of psychedelic induced behavioral benefit. This work adds data to a nascent field and provides a basis for further exploration of the neuroplastic hypothesis of psychedelic action. It is with great hope I present these data and urge the field forward into a more nuanced and rigorous inquiry of psychedelic action and application.

6. Appendix I: Examining the Role of Experience and Conscious Awareness for Psychedelic-Induced Psychological Change: Study Protocol for a Double-Blind, Placebo-Controlled, Four Arm Trial of Midazolam in Combination with Psilocybin in Healthy Adults

Zarmeen Zahid[2,4], Christopher R. Nicholas[1,8], Matthew I. Banks[2,3,8], Richard L. Lennertz[2,8], Cody J. Wenthur[4,8], Bryan M. Krause[2], Paul R. Hutson[4,8], Christina J. Sauder[6,7], John D. Dunne[5,8], Charles L. Raison[6,7,8]

[1] Department of Family Medicine and Community Health, University of Wisconsin, Madison, WI 53706

[2] Department of Anesthesiology, University of Wisconsin, Madison, WI 53706

[3] Department of Neuroscience, University of Wisconsin, Madison, WI 53706

[4] School of Pharmacy, University of Wisconsin, Madison, WI 53706

[5] Department of Asian Languages and Cultures, University of Wisconsin, Madison, WI 53706

[6] Department of Psychiatry, University of Wisconsin, Madison, WI 53706

[7] School of Human Ecology, University of Wisconsin, Madison, WI 53706

[8]Transdisciplinary Center for Research in Psychoactive Substances, University of Wisconsin, Madison, WI 53706

6.1. Abstract

Serotonergic psychedelics rapidly and durably enhance wellbeing in both patients and healthy participant, but the underlying mechanisms are unclear. These drugs have two prominent acute effects: A) profound yet transient alterations in perception and cognition and B) induction of neuroplasticity, though this has been demonstrated directly only in preclinical models. Previous studies in humans have shown an association between the subjective quality of the psychedelic experience and therapeutic outcomes. However, because psychedelics likely induce neuroplasticity as well, it is unclear as to whether this association is causal. This study has two overarching aims. First, we will test for psilocybin-induced neural and behavioral plasticity in human participants. Second, we will test the role of neuroplasticity in the long-term behavioral effects of psilocybin. Using an innovative approach, we will measure responses to direct stimulation of the brain using TMS-EEG, bypassing sensory and motor pathways that may themselves be altered by psychedelics. Furthermore, we will co-administer psilocybin with midazolam, a benzodiazepine with amnestic and anti-neuroplastic activity. Our pilot study showed that midazolam does not suppress the subjective quality of the acute psychedelic experience when co-administered with psilocybin. However, memory of the experience is impaired, and long-term behavioral effects that are associated with therapeutic benefit are blunted.

6.2. Introduction

Serotonergic psychedelics, acting as agonists at the serotonin 2A receptor, rapidly enhance wellbeing in both patients [69-73, 411-415] patients and healthy participants [78, 79] in a robust

and long lasting manner [416]. Some improvements include enhanced cognitive flexibility, improved affect, and pronounced decreases in psychopathological symptomatology [417]. Though such behavioral improvements are well established, the biological basis for these effects remains unclear. Current research efforts have focused on two prominent acute effects of these drugs: 1) Serotonergic psychedelics produce profound yet transient alterations on perception and cognition and 2) Serotonergic psychedelics are pro-neuroplastic compounds. Gaining an improved understanding of the relationship between these two effects and their impacts on long-term outcomes is important when considering implementation models for psychedelic-assisted therapy approaches.

Psychedelic-induced long-term changes in wellbeing: The role of the psychedelic experience

Serotonergic psychedelics are known to acutely alter consciousness in a profound way. This acute effect is termed the 'psychedelic experience' and can include a heightened sense of connection, enhanced meaning,, alterations in perception resulting in complex visual imagery, changes to auditory processing, synesthesia, and changes in cognition [418]. Beyond these measurable changes, psychedelic experiences may also effect more abstract processes such as awe, self-transcendence, and beliefs regarding the nature of consciousness [418]. Along with this, phenomena characterized as 'mystical' in nature are often reported, and these are evidenced to lead to breakthrough or insight-type moments for the individual. The occurrence of these phenomena and the extensive subjective effects that can be experienced have been found to be highly predictive of therapeutic outcomes, and some argue, maybe even necessary for such long-

term benefit [419]. Despite this, the causal significance of the acute psychedelic experience in long-term therapeutic benefit has not been directly tested.

Psychedelic-induced long-term changes in wellbeing: The role of neuroplasticity

Neuroplasticity refers to the brain's capacity to grow, prune, and reorganize in a way that is optimally efficient. In cases of stress-associated psychiatric disorders such as major depressive disorder, neuroplasticity is markedly decreased and even linked to symptom severity [420-423]. Blunted neuroplasticity is a presumed biological substrate of being "stuck" in a constricted world view with very rigid beliefs, often being unable to modify behaviors to meet environmental demand, demonstrating psychological inflexibility. These deficits in neuroplasticity are recapitulated in preclinical models of stress induced disorders [208, 282, 283] and application of serotonergic psychedelics in these models has been evidenced to induce rapid changes within hours that are robust and long lasting up to several weeks [258, 286, 325]. Indeed, in various *ex vivo*, *in vitro,* and *in vivo* experiments, application of serotonergic psychedelics such as psilocybin resulted in rapid growth of new neurites, synapses, and enhanced synaptically mediated intercellular communication [258, 286, 325]. These structural and functional changes parallel the time course of symptomatic relief in patients [76, 396] and so we posit that acutely, these drugs may alter prefrontal cortical circuitry involved in emotional processing and cognition such as

psychological flexibility to facilitate subsequent behavioral reorientation during the post-dosing psychotherapeutic "integration" sessions that are standard practice in psychedelic clinical trials.

Psychedelic-induced long-term changes in wellbeing: Detangling the role of the psychedelic experience and neuroplasticity

Having identified the salience of both the subjective experience and plasticity in the effects of psychedelic administration, it is yet unclear which phenomenon is responsible for long-term behavioral change. Until now, it has not been possible with current clinical paradigms to distinguish the contributions of both. In this study, we leverage the plasticity-blocking properties of midazolam to do so [45,46,56]. Though preclinical animal models have been useful in providing insight, no work to date has examined the functional role of neural plasticity in the therapeutic effects of psychedelics in human participants.

There is surprisingly little evidence that neuroplasticity occurs in human participants in response to psychedelic administration as is seen in preclinical animal models. There is even some disconnect between molecular site of action between preclinical and clinical studies. In human participants, multiple lines of evidence support the role of 5-HT_{2A} receptors in the biological and phenomenological effects of serotonergic psychedelics [283, 286] , however in animal models there is evidence that the pro-neuroplastic effects may be completely independent of these receptors [27, 29] Though some long-term changes in functional connectivity and task-related activity have been observed in humans [43,44,62,63] results are inconsistent between studies and do not fit within a unified overarching theoretical framework. The barriers here have been

primarily technical as the most common and validated approaches in examining plasticity preclinically are not appropriate for use in human participants.

Aims

In our study, we will directly test for neural plasticity in the human brain in response to serotonergic psychedelic administration using an innovative approach. We will measure responses to direct stimulation of the brain using TMS-EEG. TMS-EEG directly probes cortical responses, bypassing sensory and motor pathways that may themselves be altered by psychedelic administrations. Prefrontal and occipital sites will be chosen for evaluation. This direct demonstration of neural plasticity in human participants after psychedelic administration will have significance beyond the therapeutic context as there have been few demonstrations of neuronal plasticity in a clinical context [70]. These results will have implications for understanding and treating a wide range of medical conditions ranging from psychopathology to neurodegenerative disorder, stroke, and even traumatic brain injury. The hypothesis being tested in this study are as follows: 1) Psilocybin will induce behavioral and neural plasticity that is specific to the prefrontal cortex, 2) Midazolam will block memory for the psychedelic experience

while maintaining its subjective quality, and 3) Midazolam will block psilocybin-induced plasticity and psilocybin's long-term effects on wellbeing.

6.3. Methods

This is a single site, 4 week, double-blind, placebo-controlled randomized trial in a cohort of healthy volunteers being administered psilocybin +/- midazolam with psychological support, with all procedures taking place at the University of Wisconsin-Madison (Figure 1). In this parallel group design, medically healthy adults (n = 110, 18-65 years old; 10% attrition expected, yielding 100 evaluable participants) with no major DSM-5 axis I diagnosis but who do have a modest decrement in emotional wellbeing (indexed by a WEMWBS score ≤ the population median of 51 [48]) will be randomized to one of four treatment groups: 1) psilocybin (25 mg) with intravenous (IV) midazolam (n = 22), 2) psilocybin (10mg or 25mg) with IV saline which

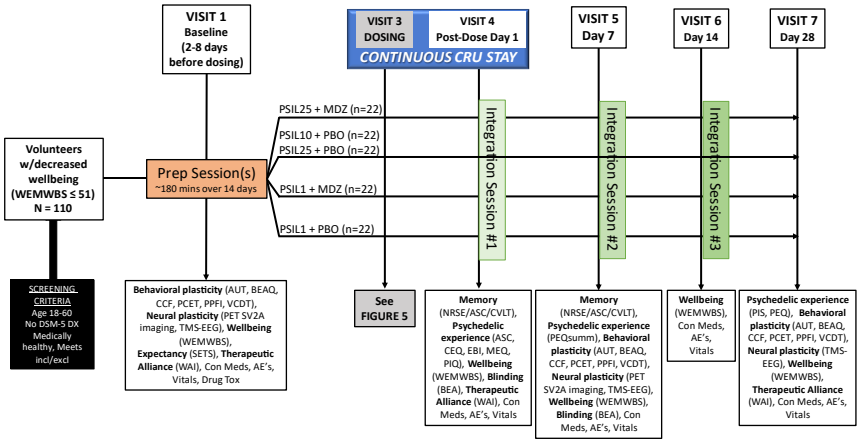


Figure 1. Experimental schedule. AE = adverse event; ASC = Altered States of Consciousness questionnaire; AUT = Alternative Use Task; BEA = Blinding Efficacy Assessment; BEAQ = Brief Experiential Avoidance Questionnaire; CCF = Cognitive Control and Flexibility questionnaire; CEQ = Challenging Experiences Questionnaire; Con Med = concomitant meds; Drug tox = urine drug screen; EBI = Emotional Breakthrough Inventory; MDZ = midazolam; MEQ = Mystical Experiences Questionnaire; NRSE = Narrative Report of Subjective Experience; PBO: placebo; PCET = Penn Conditional Exclusion Test; PEQ = Persisting Effects Questionnaire, PET = positron emission tomography; PIQ = Psychological Insight Questionnaire; PIS = Psychological Insight Survey; PPFI = Personalized Psychological Flexibility Index; PSIL(X) = psilocybin Xmg; SETS = Stanford Expectations of Treatment Scale; TMS: Transcranial Magnetic Stimulation; VCDT = Visual Contrast Detection Threshold; Vitals = blood pressure, heart rate, pO_2; WAI = Working Alliance Inventory; WEMWBS = Warwick Edinburgh Mental Wellbeing Scale;

will act as the placebo for midazolam (n = 44), 3) psilocybin (1 mg) which will act as placebo for the medium- and high-dose psilocybin with IV midazolam, and finally 4) psilocybin (1 mg) with IV saline (n = 22). Midazolam is a benzodiazepine that acts at cortical $GABA_A$ receptors to block memory by blocking neural plasticity [45, 46]. This study marks the first use of midazolam as an intervention to modify psychedelic-induced neuroplasticity. It is used as an amnestic agent in clinical and research settings [61, 101]. The amnesia is thought to be the behavioral consequence of the inhibition of long-term potentiation (LTP) between synapses at the cellular level [102]. Thus, we can say that the memory for the psychedelic experience itself is a biomarker for neuroplasticity acutely induced by psilocybin.

Recruitment

To recruit a diverse and multi-generational sample of subjects, a variety of recruitment methods will be used including both direct and indirect methods. The PIs and co-investigators are connected to numerous departments throughout campus (e.g., Psychiatry, Family and Community Medicine, Anesthesiology, School of Pharmacy, Asian Languages and Cultures, etc.) so will be able to easily promote their research throughout the campus. They will use multiple formats (e.g., emails, direct conversation, presentations at department meetings, etc.) to engage colleagues and students in being potential participants. Additional outreach efforts may include the following: the University's mass email of faculty, staff, and students, local radio and TV advertising, posting of flyers, newspaper advertisements, and web-based postings (e.g., Craigslist, social media campaigns, UW-Madison's Transdisciplinary Center for Research in Psychoactive Substances

website). UW-Madison's Institute for Clinical and Translational Research (ICTR) also provides an on-line recruitment tool called StudyFinder which will also be used to enhance enrollment.

Informed Consent

During the pre-screening process, an overview of the study will be provided by a qualified study team member. For this trial, written informed consent will occur at the in-person screening visit. Study participants will be considered enrolled in the study when they provide written informed consent. Written informed consent will be obtained in person from all participants before undergoing any study procedures. Prisoners, pregnant women and mentally impaired persons will not be included. Competent persons meeting other eligibility criteria will be welcomed, regardless of race, gender, ethnicity, religion, or socioeconomic status. Additionally, students, employees, patients or family members affiliated with the PI(s) will not be enrolled, and study team family members will be prohibited from participating in this study.

Eligibility Criteria

To ensure safety of the participants and maintain experimental integrity, various inclusionary and exclusionary criteria will be employed and adhered to. Please refer to Table 1 for the complete list of these criteria.

Inclusion Criteria
Individuals aged 18 – 65 years.
Willing and able to provide informed consent.

Willing to comply with all study procedures and be available for the duration of the study.

Able to read, speak and understand English.

Ability to take oral medication.

WEMWBS score less than or equal to 53.

Women of childbearing potential must use an acceptable method of contraception from 30 days prior to dosing and agree to use an acceptable method of contraception until study completion. Males must agree to avoid impregnation of women throughout study participation through use of an acceptable method of contraception. *Note: Includes, but is not limited to, barrier with additional spermicidal foam or jelly, intrauterine device, hormonal contraception (started at least 30 days prior to study enrollment), intercourse with men who underwent vasectomy.*

Eastern Cooperative Oncology Group (ECOG) performance status of 0 based on physical exam, which indicates that the participant is fully functioning.

Agree to refrain from using legal psychoactive substance for the following defined time periods (the exception is caffeine):

Tobacco and Nicotine: from Screening until Study Termination
Alcohol: 72 hour window prior to the Dosing Visit

. Non-smokers.

. No psychedelic substance use within 3 months prior to their investigational product dosing visit by self-report.

Exclusion Criteria

Calculated Body Mass Index (BMI) >35 at Screening

Clinically significant abnormal chemistry or hematologic laboratory results (from a screen of Complete Blood Count with Differential and Comprehensive Metabolic Panel and urinalysis), using the UWHC lab reference intervals.

Urine drug test containing non-prescribed drugs of abuse (i.e., non-prescribed opioids, benzodiazepines, amphetamines, cocaine) at Baseline.

Evidence of ischemic disease, ventricular arrhythmias, or cardiac conduction defects on ECG at Screening.

History of Prolonged QT syndrome as determined by self-report or QTc interval > 450 milliseconds as calculated by the Screening ECG.

Clinically significant abnormalities on physical examination.

Women of childbearing potential with positive urine pregnancy test at Screening or Dosing or woman who are breastfeeding.

At Screening and Baseline, pre-hypertension defined as systolic blood pressure ≥130/80 and tachycardia/bradycardia defined as heart rate ≥ 100 beats per minute or < 45 beats per minute. On dosing day, acute hypertension/tachycardia/bradycardia (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, heart rate > 100 beats per minute or < 45 beats per minute)..

Currently meets diagnostic criteria for any DSM-5 psychiatric condition as assessed by the MINI International Neuropsychiatric Interview (MINI). Prior history of primary psychotic disorder (unless substance-induced or due to a medical condition), bipolar disorder Type I or Type II, or schizophrenia, as determined by the MINI will be exclusionary.
Concurrent or recent (within 1 year) history of major depressive disorder, obsessive-compulsive disorder, generalized anxiety disorder, panic disorder, anorexia nervosa, or bulimia nervosa, posttraumatic stress disorder, as determined by the MINI and psychiatric history.
Active suicidal ideation or attempt in prior 12 months as assessed by the MINI and/or C-SSRS
First degree family history of primary psychotic disorder, bipolar disorder Type I, bipolar disorder Type II, or schizophrenia, as determined via self-report.
MRI-incompatible implants or devices such as certain cardiac pacemakers or defibrillators, insulin pumps, cochlear implants, metal in orbit, implanted neural stimulators, CNS aneurysm clips and other medical implants that have not been certified for MRI.
Not able to fit in the scanner coil (e.g., weight greater than 300 pounds, very large shoulders, etc.)
Use of psychotropic medications, including antidepressants, mood stabilizers (lithium, carbamazepine, valproic acid, lamotrigine), antipsychotics, benzodiazepines, psychostimulants, or supplement-type antidepressants/anxiolytics (St. John's Wort, SAMe, L-methylfolate, valerian) within 3 months of Screening.
Any regular use of medication, with the exception of females taking birth control or individuals taking medications approved for use by the study PI (or designee).
As determined by self report, history of cardiovascular disease, stroke, seizure disorder, acute narrow angle glaucoma, clinically-significant autoimmune conditions, claustrophobia, neoplasm (other than resected basal or squamous cell skin cancer), Type I or insulin-dependent Type II diabetes.
Chronic viral infection, including hepatitis C, hepatitis B and human immunodeficiency virus
Inability to perform CVLT, assessed by screening CVLT score of less than or equal to 5th percentile.
Lack of a local support person who is available during the participant's 24-hour treatment and observation period, as determined by self-report.
No IV access by self-report based on prior attempts.
Any physical or psychological symptom, based on the clinical judgment of the study physician and/or psychologist that would make a participant unsuitable for the study.

Table 1. Inclusion and exclusion criterion that will be adhered to for the entirety of the study.

Dosing Day Assessments

Dosing sessions will occur in the UW Hospital Clinical Research unit and will be supervised by facilitators who will also conduct both the preparatory and integration sessions with the participant. Various assessments will be conducted on the dosing days (Figure 2). Memory is an essential behavioral readout of psilocybin-induced neural plasticity. The California Verbal Learning Test (CVLT) will be used to assess memory during the dosing session and to adjust the dose of midazolam for Aims 2 and 3. Brief questionnaires and narrative reports will be used to assess the subjective quality of the psychedelic experience. Questions drawn from the Altered State of Consciousness questionnaire (ASC) [105] along with an additional question from the Persisting Effects Questionnaire capturing emotional salience will be administered. Participants will also provide a 60-second free-form narrative report of their subjective experience that will be audio recorded. Along with this, visual art displays will be presented in a digital frame at each of these times and participants will be directed to draw their attention to this item for a 4-second trial [424]. Participants will subsequently be asked to attend to a musical soundtrack for a 4-second period. All assessments above will be grouped together with vital sign assessments to limit

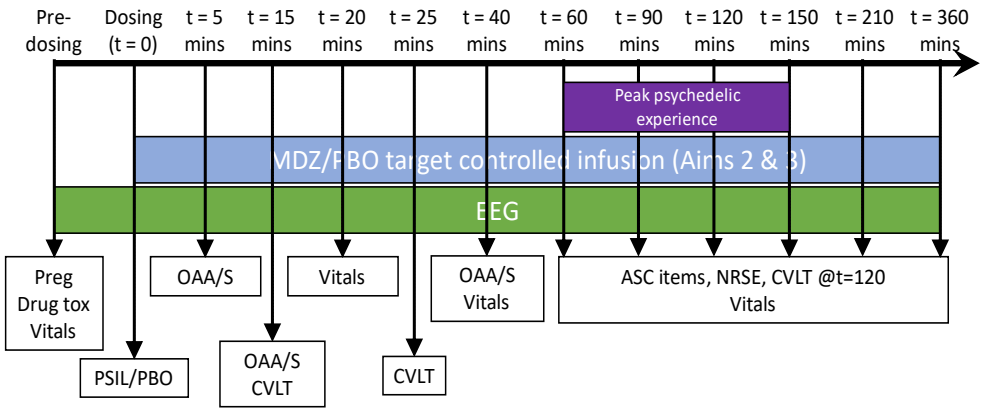


Figure 2. Dosing day experimental schedule. ASC = Altered States of Consciousness Questionnaire; CVLT = California Verbal Learning Test; MDZ = midazolam; NRSE = narrative report of subjective experience; OAA/S: Observer's Assessment of Arousal and Sedation; PBO = placebo; Preg = pregnancy test; PSIL: psilocybin; Tox = urine drug screen; Vitals = blood pressure, heart rate, pO_2.

the number of interruptions to the psychedelic experience, and they will be timed to capture the peak of the subjective experience as it correlated to peak plasma concentrations of psilocin [109].

In addition to behavioral assessments, brain activity will be assessed through monitoring resting state brain activity using hd-EEG continuously throughout the dosing session. From this we will derive two independent and objective measures of the subjective quality of the psychedelic experience: 1) Lempel-Ziv complexity [110] and 2) eyes closed alpha power (8-13 Hz) [111]. Previous studies have evidenced psilocybin and other serotonergic psychedelic induced increases in the complexity of scalp EEG, which likely reflects an increase in network repertoire or information capacity [112, 113]. These drugs have also been evidenced to attenuate the decrease in alpha band power typically observed under control conditions when participants close their eyes [425]. This is linked to the visual cortex entering an 'idling mode' during periods of decreased visual processing and so the psychedelic induced blockade of this decrease may reflect the presence of visual hallucinations and increased activity in the visual cortex [113, 115, 116, 117]. hd-EEG data will be filtered at 0.1-100 Hz, sampled at 250 Hz and stored for offline analysis.

Finally, along with behavioral and electrophysiological measurements, saliva will be collected at pre-dosing and at 90 minutes post-dosing session and assessed for 1) circulating cortisol concentrations as a biomarker of drug-induced anxiety and stress responses, and 2) progesterone levels for female participants to help standardize measurements of plasticity relative to the menstrual cycle.

Post-Dosing Day Assessments

Behavioral Assessments. On the day immediately following dosing (Day 1) hit rates, false alarms, memory accuracy, recollection accuracy, familiarity accuracy, and bias will be calculated from each assessment (CVLT, ASC, NRSE, audio/visual) using Yes-No Recognition tasks. Confidence measures will be used to assess memory, familiarity, recognition accuracy, as well as metacognitive accuracy and efficiency for each task. These outcomes will be compared between treatment groups as a bias-corrected, difficulty-insensitive approach for identifying differences arising in objective memory performance or assessment of performance across different tasks [118]. These measurements will be repeated on Day 7 to assess recovery of baseline memory and metacognitive function following elimination of the drugs from the system. Tasks on this day will include items from Day 1 as well as new distractors from thematically similar scales not used prior to Day 7.

Long-term changes in behavior relating to wellbeing will be assessed using the WEMWBS at baseline and on Days 1, 7, 14, and 28. The subjective quality of the psychedelic experience will be assessed on Day 1 using the full ASC, Mystical Experiences Questionnaire, Emotional Breakthrough Inventory, Challenging Experiences Questionnaire, and the Psychological insight Questionnaire. Long-term reflections of the subjective experience will be assessed on Day 28 using the PEQ and the Psychological Insight survey. Further, microphenomenological interviews will be conducted on Day 14. This data will be used to assess the emotional salience of the experience and its noetic quality, a feature that contributes strongly to the MEQ [122,123].

Behavioral plasticity will be measured using various self-report and performance-based measures administered at baseline and at Days 7 and 28. These measures are focused on psychological flexibility, shown previously to increase in response to psilocybin [266, 426]. Performance-based measures will include the Penn Conditional Exclusion Test (PCET) [427] a measure of task switching, and the Alternative Use Task (AUT) [428], a measure of divergent thinking [429]. Self-perceived changes across different domains of psychological and cognitive flexibility will be assessed using the brief experiential avoidance questionnaire [430] (BEAQ; degree of engaging challenging emotions, thoughts, and situations), cognitive control and flexibility questionnaire [431] (CCF; self-regulation in the context of internal and external stress), and the personalized psychological flexibility index[432] (PPFI; ability to pursue goals amidst situational challenges). The Visual Contrast Detection Threshold task (VCDT), shown to rely on neural activity in primary visual cortex [433], will serve as control.

Neuroplasticity assessments.

Participants will be assessed using TMS-EEG at baseline and on Days 7 and 28. Each participant will be fitted with 64 TMS-compatible EEG electrodes (EASYCAP GmbH) using a Nexstim head tracker. EEG data will be recorded at 5 kHz and streamed from the BrainVision recorder software (Brain Products) to a separate computer displaying the TMS-evoked potentials in real time [434]. After target optimization, we will administer a sequence of at least 200 TMS pulses @0.5 Hz. Repetitive TMS stimulation (1500 pulses) will then be applied as described [435], with pulses organized according to safety guidelines [436]. Specifically, pulses will be organized into bursts (50 pulses @ 5 Hz), bursts organized into trains of 6 bursts (separated by 5

sec), with 5 trains delivered (separated by 1 min). A second TEP will then be obtained as above to evaluate the magnitude of LTP.

Facilitation

In this study, the primary objective of facilitation is to review study procedures, monitor safety and adverse events, and administer assessments at preparation, dosing, and integration. Facilitation will not involve targeted psychosocial strategies to improve wellbeing outside of psychosocial history gathering, providing acute emotional support during dosing and integration, and establishing rapport and a therapeutic alliance which are critical for safety, minimizing participant burden, and study completion [437]. Facilitation checklists and a validated measure of the therapeutic alliance (Working Alliance Inventory [438]) completed by the facilitator and participant will be administered to ensure fidelity to study procedures and account for non-specific therapeutic factors [439, 440].

Outcome Measures

This study will consist of various primary, secondary, and exploratory outcomes. See table 2.

Type (Primary, Secondary, Other)	Name	Time Frame	Brief Description
Primary	WEMWBS	Baseline, 28-days post-treatment.	WEMWBS is a standard measure of wellbeing and is the primary behavioral outcome for all three aims.

Primary	Prefrontal TEP amplitude	Baseline, 7-days post-treatment	TEP (transcranial magnetic stimulation evoked potential) amplitude measures neural excitability in the stimulated region of the brain.
Primary	PCET	Baseline, 7-days post-treatment	The PCET (Penn Conditional Exclusion Test) is a performance-based measure of psychological flexibility (task-switching).
Secondary	TEP amplitude	28-days post-treatment	TEP (transcranial magnetic stimulation evoked potential) amplitude measures neural excitability in the stimulated region of the brain. Hypothesis testing will be performed if effect on TEP amplitude at 7-days is found to be significant.
Secondary	PCET	28-days post-treatment	The PCET (Penn Conditional Exclusion Test) is a performance-based measure of psychological flexibility (task-switching). Hypothesis testing will be performed if effect on PCET at 7-days is found to be significant.
Secondary	AUT	Baseline, 7- and 28-days post-treatment.	The Alternative Use Task (AUT) is a performance-based measure of psychological flexibility (task-switching). Hypothesis testing will be performed if effect on

			PCET at 7-days is found to be significant.
Secondary	BEAQ	Baseline, 7- and 28-days post-treatment.	The brief experiential avoidance questionnaire (BEAQ) is a self-report measure of psychological flexibility, specifically the degree of engaging challenging emotions, thoughts, and situations. Hypothesis testing will be performed if effect on PCET at 7-days is found to be significant.
Secondary	CCF	Baseline, 7- and 28-days post-treatment.	The cognitive control and flexibility questionnaire (CCF) is a self-report measure of psychological flexibility, specifically self-regulation in the context of internal and external stress. Hypothesis testing will be performed if effect on PCET at 7-days is found to be significant.
Secondary	PPFI	Baseline, 7- and 28-days post-treatment.	The personalized psychological flexibility index (PPFI) is a self-report measure of psychological flexibility, specifically the ability to pursue goals amidst situational challenges. Hypothesis testing will be performed if effect on PCET at 7-days is found to be significant.
Secondary	ASC	Dosing day, Day 1 post-dosing	The Altered States of Consciousness questionnaire will be used as a manipulation check, i.e., to confirm

			that midazolam is working as expected (blocking memory without affecting subjective quality of psychedelic experience.
Other	SETS	Day 1 post-dosing	SETS will measure expectation for treatment outcomes
Other	Blinding	Day 1 post-dosing	Blinding will be assessed with confidence measures using AUROCs for Treatment Group and Dose ID
Other	WEMWBS	7- and 14-days post-treatment	WEMWBS is a standard measure of wellbeing. Changes at these additional time points will be analyzed on an exploratory basis.
Other	TEP_{LTP}	7-, 28-days post-dosing	Log of ratio of TEP amplitude after vs. before high frequency stimulation (TEP_{LTP}) will be analyzed on an exploratory basis.
Other	VCDT	Baseline, 7- and 28-days post-treatment	The Visual Contrast Detection Task (VCDT) is a performance-based measure of visual acuity. Performance on the VCDT will be analyzed on an exploratory basis.
Other	Occipital TEP amplitude	Baseline, 7-days post-treatment	TEP (transcranial magnetic stimulation evoked potential) amplitude measures neural excitability in the stimulated region of the brain. Occipital TEP amplitude changes will be analyzed on an exploratory basis.

Other	CVLT, ASC, NRSE, AUDVIS	1 and 7 Days post-treatment	Changes in yes-no signal discrimination with confidence will be assessed using AUROCs as an exploratory measure of memory
Other	Salivary Cortisol	Baseline and 90 min	Salivary cortisol will be used as a biological marker of stress and HPA axis activation

Statistical Analysis

Sample size and power analysis. Sample size for this study is based on the hypothesis that midazolam interferes with psilocybin's effect on wellbeing (WEMWBS). We conservatively use this hypothesis for power analysis because we anticipate the difference between *psilocybin+placebo* and *psilocybin+midazolam* will be smaller than differences between *psilocybin+placebo* and *placebo+placebo* used in Aim 1. A sample size of 20 per group will be sufficient to detect a significant interaction between psilocybin and midazolam, assuming a 7.5-point difference in WEMWBS for *psilocybin+placebo* compared to *placebo+placebo*, 67% reduction of this effect in the *psilocybin+midazolam* group, 80% power, and two-sided $\alpha = 0.05$. Power analysis simulations assumed a latent individual WEMWBS score = 51, SD = 6.34, and within-subject SD = 2.89, corresponding to retest reliability of 0.83 and total SD = 7 [441]. We mimicked recruitment criteria by excluding cases with baseline WEMWBS>=51.

Analysis of resting state hd-EEG data and memory assessments. hd-EEG data will be analyzed using EEGLAB, a custom MATLAB-based toolbox [127]. Visual inspection will discard bad epochs and channels. The hd-EEG data will be epoched and average-referenced, then independent

component analysis will be applied to remove eye movements and spontaneous muscle activity. Lempel-Ziv complexity and alpha-band power will be computed as previously described [128,129]. CVLT, ASC, and NRSE data will be analyzed using the standard definitions for hit rates, false alarm rates, bias, and memory accuracy [130]. Recollection and familiarity accuracy will be assessed using an independence remember/know model and dual process signal detection analysis validated in memory studies with MDMA [442].

Confidence ratings will be measured on a six-point receiver operating characteristic (ROC) curve, plotting cumulative (H) against (F) at each confidence level, and calculating area under the ROC curve (AUROC) for each condition to assess objective state discrimination. AUROC's for metacognitive accuracy will use analogous definitions for hits, false alarms, bias, and rates as above; metacognitive efficiency will be defined by meta-d' / d' [443]. Two-way ANOVAs (group x time) will be used to compare performance across groups for each accuracy and efficiency measure for CVLT, ASC, NRSE, and audio/visual (AUDVIS) items on Day 1 versus 7. A large (~135,000 trial) dataset [424] will be used as a normative benchmark for decay in visual item recognition over time.

Expectancy. Pre-treatment expectancy has been shown to mediate outcomes in psychotherapy and pharmacotherapy clinical trials [444-446] and is associated with the quality of the subjective experience and outcomes in psychedelic trials [447, 448]. Expectancy effects for psilocybin will be examined in all groups using the Stanford Expectations of Treatment Scale (SETS) [449], a validated measure of treatment expectancy adapted to account for pre-dosing beliefs about psilocybin and facilitation on outcome measures. Measurements of midazolam interactions with

expectancy effects and the psychosocial facilitation elements will also be performed, including factors associated with facilitation, such as perceived therapeutic (working) alliance [439].

Blinding efficacy. Given the importance of blinding in psychedelic clinical trials[447], participants, facilitators, and anesthesiologists will be asked about identity and dosage of compound(s) received (psilocybin and/or midazolam) and the confidence in their responses at post-dose follow-up[450]. The hypothesis that midazolam's amnestic effects will increase blinding effectiveness (for participants) will be tested by comparing the AUROC's generated for treatment group and dose ID accuracy between +/-midazolam groups.

Relevant biological variables. Sex-differences in the effects of psychedelics in human participants have not been reported previously. However, the effects of psychedelics in pre-clinical models are reported to be sex-dependent [451-453]. Furthermore, plasticity in response to direct cortical stimulation in human participants has been shown to be sex-dependent [454, 455], an effect that may be hormonally-mediated [456, 457]. Data will be stratified according to sex, and female participants' menstrual phase will be included as a covariate in exploratory analyses to investigate whether plasticity-inducing effects of psychedelics exhibit similar sex- and hormone-dependence.

Data protection

Loss of confidentiality is a risk of research participation. Every effort will be made to maintain subject confidentiality throughout the study. Data entry forms will be developed using the REDCap® database system developed at the Vanderbilt Institute for Clinical and Translational Research (CTSA). REDCap® (Research Electronic Data Capture) is an Oracle-based, secure, password-protected, HIPAA-compliant, web-based application designed to support data capture

for research studies. All electronic and hard entry forms will include unique subject identifiers to maintain subject confidentiality and will not relate to the subject in any way. Any protected personal health information will be encrypted and stored separately. The only link between identifying information and project data will be in a key stored on a password-protected computer accessible only to the PIs and to the project coordinators, who will be making appointments and assigning research personnel to meet with the participant.

Timeline

We estimate study start-up will take approximately 6-8 months. During this time, appropriate personnel will be established and trained, procedures for protocol implementation will be developed and practiced, study materials will be curated, and the REDCap® database will be developed and undergo user acceptance testing. Additionally, we will obtain IRB approval, submit the study application to the FDA, and register the study at clincialtrials.gov. Recruitment may occur through various means including flyers, web-based postings, mass emails, etc. Based on previous experience, we expect a 2:1 screen fail rate in this population (i.e., for every 2 subjects screened, 1 will screen fail and 1 will be eligible). We feel a 4-year enrollment period is necessary because of the time and effort that goes into each screening and dosing visit. Based on the Pilot RECAP study leading up to this study, we estimate that we will enroll up to ~3 *evaluable* subjects/month.

At this rate, we are well positioned to enroll 100 *evaluable* subjects by the 4th year. Data collection will be continuous from year 1 to the second quarter of year 5. UW-Madison's Office of Clinical Trials (OCT) will conduct study monitoring throughout the duration of the study. Monitoring will occur approximately every 6 months throughout the enrollment and data

collection period. Study monitors will conduct data quality assessments, ensure regulatory compliance and compliance with the study protocol. A study initiation visit will occur prior to enrollment and a study close-out visit will occur at the end of data collection. The PIs will be responsible for reporting issues (e.g., adverse events, noncompliance) to the IRB and FDA throughout the data collection periods. Additionally, the PIs are responsible for submitting annual and final reports to the FDA, continuing reviews and study closeout reports to the IRB, and NIH progress reports. Finally, cleaning of imaging data will occur continuously throughout the study. After enrollment is complete, six months will be needed for data analysis, and results write-up.

6.4. Discussion

Currently, psychedelic clinical trials are increasing in number and rigor, and as such, it is likely that psychedelic assisted psychotherapy will receive FDA approval in the next few years for major depressive disorder. It is vital that we clarify the role of neural plasticity and the psychedelic experience to contribute to optimization of clinical approaches. This work represents one of the attempts to disentangle the contributions of the acute psychedelic experience from the biological changes to neuroplasticity. This study will provide vital data towards understanding the mechanistic basis for therapeutic benefit after psychedelic administration.

Further, there are still many details regarding psychedelic effects that remain unclear. Regional specificity of psychedelic-induced behavioral and neural plasticity remains untested. Deficits in glutamatergic signaling in PFC are associated with multiple psychiatric disorders [179, 458, 459], occur in animal models of these disorders [40, 460-462], and are ameliorated in these models by serotoninergic psychedelics[283, 286]. Surprisingly, analyses of gene expression

patterns suggest that the density of serotonin type 2A receptors, which underlie the phenomenology of the 'psychedelic experience'[97, 99, 100], is higher in visual cortex compared to PFC [99], consistent with strong visual hallucinations during the dosing session[98]. Thus, it is unclear whether psychedelic-induced neural plasticity is specific to the PFC or is more widespread. Two elements of our study will directly address this question. First, we plan to record responses to TMS stimulation in two locations, one prefrontal and one occipital, and hypothesize that we will observe plasticity in the former and not the latter. Second, behavioral plasticity will be evaluated in two domains: psychological flexibility, previously shown to depend on activity in PFC [266, 463-465], and visual contrast detection, previously shown to depend on activity in primary visual cortex [433].

Additionally, fundamental questions about the mechanisms underlying the development and expression of psychiatric disorders such as major depressive disorder and anxiety remain unanswered. The monoamine theory of depression, based largely on the success of antidepressants that acutely elevate brain levels of serotonin, norepinephrine or dopamine [466], has been largely supplanted by a model based on impaired neural plasticity in key brain regions including PFC [422]. A mechanism of action for serotonergic psychedelics based on neural plasticity will be strong supporting evidence for the plasticity model of psychiatric disorders.

References

1. Banks, M.I., et al., *Catalysts for change: the cellular neurobiology of psychedelics*. Mol Biol Cell, 2021. **32**(12): p. 1135-1144.

2. Gulyaeva, N.V., *Molecular Mechanisms of Neuroplasticity: An Expanding Universe*. Biochemistry (Mosc), 2017. **82**(3): p. 237-242.

3. Preston, A.R. and H. Eichenbaum, *Interplay of hippocampus and prefrontal cortex in memory*. Curr Biol, 2013. **23**(17): p. R764-73.

4. Phillips, M.L., H.A. Robinson, and L. Pozzo-Miller, *Ventral hippocampal projections to the medial prefrontal cortex regulate social memory*. Elife, 2019. **8**.

5. Tripathi, A., et al., *The hippocampal to prefrontal cortex circuit in mice: a promising electrophysiological signature in models for psychiatric disorders*. Brain Struct Funct, 2016. **221**(4): p. 2385-91.

6. Pallavicini, C., et al., *Spectral signatures of serotonergic psychedelics and glutamatergic dissociatives*. Neuroimage, 2019. **200**: p. 281-291.

7. Jones, N.T., et al., *Transient Elevation of Plasma Glucocorticoids Supports Psilocybin-Induced Anxiolysis in Mice*. ACS Pharmacol Transl Sci, 2023. **6**(8): p. 1221-1231.

8. Halberstadt, A.L. and M.A. Geyer, *Characterization of the head-twitch response induced by hallucinogens in mice: detection of the behavior based on the dynamics of head movement*. Psychopharmacology (Berl), 2013. **227**(4): p. 727-39.

9. Association, A.P. *Stress in American 2023 A Nation Recovering from Collective Trauma*. 2023 [cited 2024 June 15]; Available from: https://www.apa.org/news/press/releases/stress/2023/collective-trauma-recovery.

10. Maddy Reinert, T.N., and Danielle Fritze. *The State of Mental Health in America*. 2022; Available from: https://mhanational.org/sites/default/files/2023-State-of-Mental-Health-in-America-Report.pdf.

11. Illness, N.A.o.M. *Mental Health By the Numbers*. 2023; Available from: https://www.nami.org/mhstats.

12. Control, C.f.D. *Suicide Data and Statistics*. 2024 April 2024; Available from: https://www.cdc.gov/suicide/facts/data.html?CDC_AAref_Val=https://www.cdc.gov/suicide/suicide-data-statistics.html.

13. Broerman, R., *Diathesis-Stress Model*, in *Encyclopedia of Personality and Individual Differences*, V. Zeigler-Hill and T.K. Shackelford, Editors. 2017, Springer International Publishing: Cham. p. 1-3.

14. Richter-Levin, G. and L. Xu, *How could stress lead to major depressive disorder?* IBRO Rep, 2018. **4**: p. 38-43.

15. Daviu, N., et al., *Neurobiological links between stress and anxiety.* Neurobiology of Stress, 2019. **11**: p. 100191.

16. Maeng, L.Y. and M.R. Milad, *Post-Traumatic Stress Disorder: The Relationship Between the Fear Response and Chronic Stress.* Chronic Stress (Thousand Oaks), 2017. **1**: p. 2470547017713297.

17. Peavy, G.M., et al., *The influence of chronic stress on dementia-related diagnostic change in older adults.* Alzheimer Dis Assoc Disord, 2012. **26**(3): p. 260-6.

18. Sinha, R., *Chronic stress, drug use, and vulnerability to addiction.* Ann N Y Acad Sci, 2008. **1141**: p. 105-30.

19. O'Connor, D.B., N. Gartland, and R.C. O'Connor, *Chapter Five - Stress, cortisol and suicide risk*, in *International Review of Neurobiology*, A. Clow and N. Smyth, Editors. 2020, Academic Press. p. 101-130.

20. Liu, R.T., S.M. Cheek, and B.A. Nestor, *Non-suicidal self-injury and life stress: A systematic meta-analysis and theoretical elaboration.* Clin Psychol Rev, 2016. **47**: p. 1-14.

21. Cay, M., et al., *Effect of increase in cortisol level due to stress in healthy young individuals on dynamic and static balance scores*. North Clin Istanb, 2018. **5**(4): p. 295-301.

22. Sudheimer, K.D., et al., *Cortisol, cytokines, and hippocampal volume interactions in the elderly*. Frontiers in Aging Neuroscience, 2014. **6**.

23. Dhabhar, F.S., *The short-term stress response - Mother nature's mechanism for enhancing protection and performance under conditions of threat, challenge, and opportunity*. Front Neuroendocrinol, 2018. **49**: p. 175-192.

24. Radley, J., et al., *Chronic stress and brain plasticity: Mechanisms underlying adaptive and maladaptive changes and implications for stress-related CNS disorders*. Neurosci Biobehav Rev, 2015. **58**: p. 79-91.

25. Kumar, A., et al., *Acute-stress induces the structural plasticity in hippocampal neurons of 15 and 30-day-old chick, Gallus gallus domesticus*. Annals of Anatomy - Anatomischer Anzeiger, 2023. **245**: p. 151996.

26. McEwen, B.S. and J.H. Morrison, *The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course*. Neuron, 2013. **79**(1): p. 16-29.

27. Kumar, A., et al., *Acute stress-induced neuronal plasticity in the corticoid complex of 15-day-old chick, Gallus domesticus*. Journal of Anatomy, 2021. **239**(4): p. 869-891.

28. Mizumori, S.J. and Y.S. Jo, *Homeostatic regulation of memory systems and adaptive decisions*. Hippocampus, 2013. **23**(11): p. 1103-24.

29. Thierry, A.M., et al., *Hippocampo-prefrontal cortex pathway: anatomical and electrophysiological characteristics*. Hippocampus, 2000. **10**(4): p. 411-9.

30. Funahashi, S. and J.M. Andreau, *Prefrontal cortex and neural mechanisms of executive function*. Journal of Physiology-Paris, 2013. **107**(6): p. 471-482.

31. Šimić, G., et al., *Understanding Emotions: Origins and Roles of the Amygdala*. Biomolecules, 2021. **11**(6).

32. Anand, K.S. and V. Dhikav, *Hippocampus in health and disease: An overview.* Ann Indian Acad Neurol, 2012. **15**(4): p. 239-46.

33. McEwen, B.S., C. Nasca, and J.D. Gray, *Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex.* Neuropsychopharmacology, 2016. **41**(1): p. 3-23.

34. Yavas, E., S. Gonzalez, and M.S. Fanselow, *Interactions between the hippocampus, prefrontal cortex, and amygdala support complex learning and memory.* F1000Res, 2019. **8**.

35. Zelikowsky, M., et al., *Neuronal Ensembles in Amygdala, Hippocampus, and Prefrontal Cortex Track Differential Components of Contextual Fear.* The Journal of Neuroscience, 2014. **34**(25): p. 8462-8466.

36. Dixon, M.L. and C.S. Dweck, *The amygdala and the prefrontal cortex: The co-construction of intelligent decision-making.* Psychological Review, 2022. **129**(6): p. 1414-1441.

37. Marin, M.F., et al., *Chronic stress, cognitive functioning and mental health.* Neurobiol Learn Mem, 2011. **96**(4): p. 583-95.

38. Scott, S.B., et al., *The Effects of Stress on Cognitive Aging, Physiology and Emotion (ESCAPE) Project.* BMC Psychiatry, 2015. **15**: p. 146.

39. Holsboer, F., *The corticosteroid receptor hypothesis of depression.* Neuropsychopharmacology, 2000. **23**(5): p. 477-501.

40. Yuen, E.Y., et al., *Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex.* Neuron, 2012. **73**(5): p. 962-77.

41. Elnazer, H.Y. and D.S. Baldwin, *Investigation of Cortisol Levels in Patients with Anxiety Disorders: A Structured Review*, in *Behavioral Neurobiology of Stress-related Disorders*, C.M. Pariante and M.D. Lapiz-Bluhm, Editors. 2014, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 191-216.

42. Kim, E.J., B. Pellman, and J.J. Kim, *Stress effects on the hippocampus: a critical review.* Learn Mem, 2015. **22**(9): p. 411-6.

43. Vyas, A., et al., *Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons.* J Neurosci, 2002. **22**(15): p. 6810-8.

44. Arnsten, A.F.T., et al., *The effects of stress exposure on prefrontal cortex: Translating basic research into successful treatments for post-traumatic stress disorder.* Neurobiology of Stress, 2015. **1**: p. 89-99.

45. Schilling, O.K., et al., *Emotional reactivity to daily stressors: Does stressor pile-up within a day matter for young-old and very old adults?* Psychol Aging, 2022. **37**(2): p. 149-162.

46. Patriquin, M.A. and S.J. Mathew, *The Neurobiological Mechanisms of Generalized Anxiety Disorder and Chronic Stress.* Chronic Stress, 2017. **1**: p. 2470547017703993.

47. Kessler, R.C., et al., *Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication.* Arch Gen Psychiatry, 2005. **62**(6): p. 617-27.

48. Papola, D., et al., *Psychotherapies for Generalized Anxiety Disorder in Adults: A Systematic Review and Network Meta-Analysis of Randomized Clinical Trials.* JAMA Psychiatry, 2024. **81**(3): p. 250-259.

49. Garakani, A., et al., *Pharmacotherapy of Anxiety Disorders: Current and Emerging Treatment Options.* Front Psychiatry, 2020. **11**: p. 595584.

50. Bruce, S.E., et al., *Influence of psychiatric comorbidity on recovery and recurrence in generalized anxiety disorder, social phobia, and panic disorder: a 12-year prospective study.* Am J Psychiatry, 2005. **162**(6): p. 1179-87.

51. Ned H. Kalin, M.D., *The Critical Relationship Between Anxiety and Depression.* American Journal of Psychiatry, 2020. **177**(5): p. 365-367.

52. Raison, C.L., et al., *Effects of Naturalistic Psychedelic Use on Depression, Anxiety, and Well-Being: Associations With Patterns of Use, Reported Harms, and Transformative Mental States*. Front Psychiatry, 2022. **13**: p. 831092.

53. Petersen, M., U. Garg, and H. Ketha, *Chapter 16 - Hallucinogens—psychedelics and dissociative drugs*, in *Toxicology Cases for the Clinical and Forensic Laboratory*, H. Ketha and U. Garg, Editors. 2020, Academic Press. p. 295-303.

54. Carhart-Harris, R.L., et al., *Psilocybin for treatment-resistant depression: fMRI-measured brain mechanisms*. Sci Rep, 2017. **7**(1): p. 13187.

55. Mateos-Aparicio, P. and A. Rodríguez-Moreno, *The Impact of Studying Brain Plasticity*. Frontiers in Cellular Neuroscience, 2019. **13**.

56. Abraham, W.C. and M.F. Bear, *Metaplasticity: the plasticity of synaptic plasticity*. Trends Neurosci, 1996. **19**(4): p. 126-30.

57. Froese, T., G. Guzmán, and L. Guzmán-Dávalos, *On the Origin of the Genus Psilocybe and Its Potential Ritual Use in Ancient Africa and Europe1*. Economic Botany, 2016. **70**(2): p. 103-114.

58. Guerra-Doce, E., *Psychoactive Substances in Prehistoric Times: Examining the Archaeological Evidence*. Time and Mind, 2015. **8**(1): p. 91-112.

59. Sayin, U., *The Consumption of Psychoactive Plants in Ancient Global and Anatolian Cultures During Religious Rituals: The Roots of the Eruption of Mythological Figures and Common Symbols in Religions and Myths*. NeuroQuantology, 2014. **12**.

60. Nichols, D.E. and H. Walter, *The History of Psychedelics in Psychiatry*. Pharmacopsychiatry, (EFirst).

61. Carbonaro, T.M., et al., *Survey study of challenging experiences after ingesting psilocybin mushrooms: Acute and enduring positive and negative consequences*. J Psychopharmacol, 2016. **30**(12): p. 1268-1278.

62. Roseman, L., D.J. Nutt, and R.L. Carhart-Harris, *Quality of Acute Psychedelic Experience Predicts Therapeutic Efficacy of Psilocybin for Treatment-Resistant Depression*. Front Pharmacol, 2017. **8**: p. 974.

63. Hasler, F., et al., *Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose-effect study.* Psychopharmacology (Berl), 2004. **172**(2): p. 145-56.

64. Strajhar, P., et al., *Acute Effects of Lysergic Acid Diethylamide on Circulating Steroid Levels in Healthy Subjects.* J Neuroendocrinol, 2016. **28**(3): p. 12374.

65. Johansen, P. and T.S. Krebs, *Psychedelics not linked to mental health problems or suicidal behavior: a population study.* J Psychopharmacol, 2015. **29**(3): p. 270-9.

66. Hendricks, P.S., et al., *Classic psychedelic use is associated with reduced psychological distress and suicidality in the United States adult population.* J Psychopharmacol, 2015. **29**(3): p. 280-8.

67. Garcia-Romeu, A., B. Kersgaard, and P.H. Addy, *Clinical applications of hallucinogens: A review.* Exp Clin Psychopharmacol, 2016. **24**(4): p. 229-68.

68. Reiff, C.M., et al., *Psychedelics and Psychedelic-Assisted Psychotherapy.* Am J Psychiatry, 2020. **177**(5): p. 391-410.

69. Muttoni, S., M. Ardissino, and C. John, *Classical psychedelics for the treatment of depression and anxiety: A systematic review.* J Affect Disord, 2019. **258**: p. 11-24.

70. Goldberg, S.B., et al., *The experimental effects of psilocybin on symptoms of anxiety and depression: A meta-analysis.* Psychiatry Res, 2020. **284**: p. 112749.

71. Johnson, M.W., et al., *Pilot study of the 5-HT2AR agonist psilocybin in the treatment of tobacco addiction.* J Psychopharmacol, 2014. **28**(11): p. 983-92.

72. Bogenschutz, M.P., et al., *Psilocybin-assisted treatment for alcohol dependence: a proof-of-concept study.* J Psychopharmacol, 2015. **29**(3): p. 289-99.

73. Moreno, F.A., et al., *Safety, tolerability, and efficacy of psilocybin in 9 patients with obsessive-compulsive disorder.* J Clin Psychiatry, 2006. **67**(11): p. 1735-40.

74. Carhart-Harris, R.L., et al., *Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study.* Lancet Psychiatry, 2016. **3**(7): p. 619-27.

75. Ross, S., et al., *Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial.* J Psychopharmacol, 2016. **30**(12): p. 1165-1180.

76. Griffiths, R.R., et al., *Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial.* J Psychopharmacol, 2016. **30**(12): p. 1181-1197.

77. Johnson, M.W., A. Garcia-Romeu, and R.R. Griffiths, *Long-term follow-up of psilocybin-facilitated smoking cessation.* Am J Drug Alcohol Abuse, 2017. **43**(1): p. 55-60.

78. Griffiths, R.R., et al., *Psilocybin occasioned mystical-type experiences: immediate and persisting dose-related effects.* Psychopharmacology (Berl), 2011. **218**(4): p. 649-65.

79. Nicholas, C.R., et al., *High dose psilocybin is associated with positive subjective effects in healthy volunteers.* J Psychopharmacol, 2018. **32**(7): p. 770-778.

80. Canal, C.E., *Serotonergic Psychedelics: Experimental Approaches for Assessing Mechanisms of Action.* Handb Exp Pharmacol, 2018. **252**: p. 227-260.

81. Canal, C.E. and D. Morgan, *Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: a comprehensive history, a re-evaluation of mechanisms, and its utility as a model.* Drug Test Anal, 2012. **4**(7-8): p. 556-76.

82. Halberstadt, A.L. and M.A. Geyer, *Effect of Hallucinogens on Unconditioned Behavior.* Curr Top Behav Neurosci, 2018. **36**: p. 159-199.

83. Planchez, B., A. Surget, and C. Belzung, *Animal models of major depression: drawbacks and challenges.* J Neural Transm (Vienna), 2019. **126**(11): p. 1383-1408.

84. Campos, A.C., et al., *Animal models of anxiety disorders and stress.* Braz J Psychiatry, 2013. **35 Suppl 2**: p. S101-11.

85. Hibicke, M., et al., *Psychedelics, but Not Ketamine, Produce Persistent Antidepressant-like Effects in a Rodent Experimental System for the Study of Depression*. ACS Chem Neurosci, 2020. **11**(6): p. 864-871.

86. Jefsen, O., et al., *Psilocybin lacks antidepressant-like effect in the Flinders Sensitive Line rat*. Acta Neuropsychiatr, 2019. **31**(4): p. 213-219.

87. Harvey, J.A., *Role of the serotonin 5-HT(2A) receptor in learning*. Learn Mem, 2003. **10**(5): p. 355-62.

88. Williams, G.V., S.G. Rao, and P.S. Goldman-Rakic, *The physiological role of 5-HT2A receptors in working memory*. J Neurosci, 2002. **22**(7): p. 2843-54.

89. Zhang, G., et al., *Stimulation of serotonin 2A receptors facilitates consolidation and extinction of fear memory in C57BL/6J mice*. Neuropharmacology, 2013. **64**: p. 403-13.

90. Catlow, B.J., et al., *Effects of psilocybin on hippocampal neurogenesis and extinction of trace fear conditioning*. Exp Brain Res, 2013. **228**(4): p. 481-91.

91. Halberstadt, A.L. and M.A. Geyer, *Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens*. Neuropharmacology, 2011. **61**(3): p. 364-81.

92. Rickli, A., et al., *Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens*. Eur Neuropsychopharmacol, 2016. **26**(8): p. 1327-37.

93. Dinis-Oliveira, R.J., *Metabolism of psilocybin and psilocin: clinical and forensic toxicological relevance*. Drug Metab Rev, 2017. **49**(1): p. 84-91.

94. Tylš, F., T. Páleníček, and J. Horáček, *Psilocybin--summary of knowledge and new perspectives*. Eur Neuropsychopharmacol, 2014. **24**(3): p. 342-56.

95. Zhuk, O., et al., *Research on acute toxicity and the behavioral effects of methanolic extract from psilocybin mushrooms and psilocin in mice*. Toxins (Basel), 2015. **7**(4): p. 1018-29.

96. Brown, R.T., et al., *Pharmacokinetics of Escalating Doses of Oral Psilocybin in Healthy Adults*. Clin Pharmacokinet, 2017. **56**(12): p. 1543-1554.

97. Vollenweider, F.X., et al., *Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action*. Neuroreport, 1998. **9**(17): p. 3897-902.

98. Valle, M., et al., *Inhibition of alpha oscillations through serotonin-2A receptor activation underlies the visual effects of ayahuasca in humans*. Eur Neuropsychopharmacol, 2016. **26**(7): p. 1161-75.

99. Preller, K.H., et al., *Psilocybin Induces Time-Dependent Changes in Global Functional Connectivity*. Biol Psychiatry, 2020. **88**(2): p. 197-207.

100. Madsen, M.K., et al., *Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels*. Neuropsychopharmacology, 2019.

101. Pokorny, T., et al., *Modulatory effect of the 5-HT1A agonist buspirone and the mixed non-hallucinogenic 5-HT1A/2A agonist ergotamine on psilocybin-induced psychedelic experience*. Eur Neuropsychopharmacol, 2016. **26**(4): p. 756-66.

102. Weber, E.T. and R. Andrade, *Htr2a Gene and 5-HT(2A) Receptor Expression in the Cerebral Cortex Studied Using Genetically Modified Mice*. Front Neurosci, 2010. **4**.

103. Jakab, R.L. and P.S. Goldman-Rakic, *5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites*. Proc Natl Acad Sci U S A, 1998. **95**(2): p. 735-40.

104. Bhagwagar, Z., et al., *Increased 5-HT(2A) receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [(11)C]MDL 100,907*. Am J Psychiatry, 2006. **163**(9): p. 1580-7.

105. Meyer, J.H., et al., *Dysfunctional attitudes and 5-HT2 receptors during depression and self-harm*. Am J Psychiatry, 2003. **160**(1): p. 90-9.

106. Meyer, J.H., et al., *The effect of paroxetine on 5-HT(2A) receptors in depression: an [(18)F]setoperone PET imaging study*. Am J Psychiatry, 2001. **158**(1): p. 78-85.

107. Yatham, L.N., et al., *Decrease in brain serotonin 2 receptor binding in patients with major depression following desipramine treatment: a positron emission tomography study with fluorine-18-labeled setoperone.* Arch Gen Psychiatry, 1999. **56**(8): p. 705-11.

108. Buckholtz, N.S., D.F. Zhou, and D.X. Freedman, *Serotonin2 agonist administration down-regulates rat brain serotonin2 receptors.* Life Sci, 1988. **42**(24): p. 2439-45.

109. Raote, I.B., A.; Panicker,M.M., *Serotonin 2A (5-HT2A) Receptor Function: Ligand-Dependent Mechanisms and Pathways*, in *Serotonin Receptors in Neurobiology*, A. Chattopadhyay, Editor. 2007, CRC Press/Taylor & Francis: Boca Raton, FL.

110. Xia, Z., et al., *A direct interaction of PSD-95 with 5-HT2A serotonin receptors regulates receptor trafficking and signal transduction.* J Biol Chem, 2003. **278**(24): p. 21901-8.

111. Abbas, A.I., et al., *PSD-95 is essential for hallucinogen and atypical antipsychotic drug actions at serotonin receptors.* J Neurosci, 2009. **29**(22): p. 7124-36.

112. Gresch, P.J., et al., *Behavioral tolerance to lysergic acid diethylamide is associated with reduced serotonin-2A receptor signaling in rat cortex.* Neuropsychopharmacology, 2005. **30**(9): p. 1693-702.

113. Isbell, H., et al., *Cross tolerance between LSD and psilocybin.* Psychopharmacologia, 1961. **2**: p. 147-59.

114. Lopez-Gimenez, J.F. and J. Gonzalez-Maeso, *Hallucinogens and Serotonin 5-HT2A Receptor-Mediated Signaling Pathways.* Curr Top Behav Neurosci, 2018. **36**: p. 45-73.

115. Sleight, A.J., et al., *Radiolabelling of the human 5-HT2A receptor with an agonist, a partial agonist and an antagonist: effects on apparent agonist affinities.* Biochem Pharmacol, 1996. **51**(1): p. 71-6.

116. Day, M., et al., *Stimulation of 5-HT(2) receptors in prefrontal pyramidal neurons inhibits Ca(v)1.2 L type Ca(2+) currents via a PLCbeta/IP3/calcineurin signaling cascade.* J Neurophysiol, 2002. **87**(5): p. 2490-504.

117. Hoyer, D., et al., *International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin).* Pharmacol Rev, 1994. **46**(2): p. 157-203.

118. Garnovskaya, M.N., et al., *5-Hydroxytryptamine2A receptors expressed in rat renal mesangial cells inhibit cyclic AMP accumulation.* Mol Pharmacol, 1995. **48**(2): p. 230-7.

119. Betke, K.M., C.A. Wells, and H.E. Hamm, *GPCR mediated regulation of synaptic transmission.* Prog Neurobiol, 2012. **96**(3): p. 304-21.

120. Tedford, H.W. and G.W. Zamponi, *Direct G protein modulation of Cav2 calcium channels.* Pharmacol Rev, 2006. **58**(4): p. 837-62.

121. Kurrasch-Orbaugh, D.M., et al., *Serotonin 5-hydroxytryptamine 2A receptor-coupled phospholipase C and phospholipase A2 signaling pathways have different receptor reserves.* J Pharmacol Exp Ther, 2003. **304**(1): p. 229-37.

122. Kenakin, T.P., *Biased signalling and allosteric machines: new vistas and challenges for drug discovery.* Br J Pharmacol, 2012. **165**(6): p. 1659-1669.

123. Cussac, D., et al., *Agonist-directed trafficking of signalling at serotonin 5-HT2A, 5-HT2B and 5-HT2C-VSV receptors mediated Gq/11 activation and calcium mobilisation in CHO cells.* Eur J Pharmacol, 2008. **594**(1-3): p. 32-8.

124. Egan, C., et al., *Agonist high and low affinity state ratios predict drug intrinsic activity and a revised ternary complex mechanism at serotonin 5-HT(2A) and 5-HT(2C) receptors.* Synapse, 2000. **35**(2): p. 144-50.

125. Fitzgerald, L.W., et al., *High-affinity agonist binding correlates with efficacy (intrinsic activity) at the human serotonin 5-HT2A and 5-HT2C receptors: evidence favoring the ternary complex and two-state models of agonist action.* J Neurochem, 1999. **72**(5): p. 2127-34.

126. Berg, K.A., et al., *Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus.* Mol Pharmacol, 1998. **54**(1): p. 94-104.

127. Roth, B.L., et al., *High-affinity agonist binding is not sufficient for agonist efficacy at 5-hydroxytryptamine2A receptors: evidence in favor of a modified ternary complex model*. J Pharmacol Exp Ther, 1997. **280**(2): p. 576-83.

128. Wang, C.D., T.K. Gallaher, and J.C. Shih, *Site-directed mutagenesis of the serotonin 5-hydroxytrypamine2 receptor: identification of amino acids necessary for ligand binding and receptor activation*. Mol Pharmacol, 1993. **43**(6): p. 931-40.

129. Kanagarajadurai, K., et al., *Molecular modeling and docking studies of human 5-hydroxytryptamine 2A (5-HT2A) receptor for the identification of hotspots for ligand binding*. Mol Biosyst, 2009. **5**(12): p. 1877-88.

130. Shapiro, D.A., et al., *Differential modes of agonist binding to 5-hydroxytryptamine(2A) serotonin receptors revealed by mutation and molecular modeling of conserved residues in transmembrane region 5*. Mol Pharmacol, 2000. **58**(5): p. 877-86.

131. Wacker, D., et al., *Crystal Structure of an LSD-Bound Human Serotonin Receptor*. Cell, 2017. **168**(3): p. 377-389.e12.

132. McCorvy, J.D. and B.L. Roth, *Structure and function of serotonin G protein-coupled receptors*. Pharmacol Ther, 2015. **150**: p. 129-42.

133. Kim, K., et al., *Structure of a Hallucinogen-Activated Gq-Coupled 5-HT(2A) Serotonin Receptor*. Cell, 2020. **182**(6): p. 1574-1588.e19.

134. Banerjee, A.A. and V.A. Vaidya, *Differential signaling signatures evoked by DOI versus lisuride stimulation of the 5-HT(2A) receptor*. Biochem Biophys Res Commun, 2020. **531**(4): p. 609-614.

135. Garcia, E.E., R.L. Smith, and E. Sanders-Bush, *Role of G(q) protein in behavioral effects of the hallucinogenic drug 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane*. Neuropharmacology, 2007. **52**(8): p. 1671-7.

136. Karaki, S., et al., *Quantitative phosphoproteomics unravels biased phosphorylation of serotonin 2A receptor at Ser280 by hallucinogenic versus nonhallucinogenic agonists*. Mol Cell Proteomics, 2014. **13**(5): p. 1273-85.

137. González-Maeso, J., et al., *Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior.* Neuron, 2007. **53**(3): p. 439-52.

138. Pottie, E., P. Dedecker, and C.P. Stove, *Identification of psychedelic new psychoactive substances (NPS) showing biased agonism at the 5-HT(2A)R through simultaneous use of β-arrestin 2 and miniGα(q) bioassays*. Biochem Pharmacol, 2020. **182**: p. 114251.

139. Schmid, C.L. and L.M. Bohn, *Serotonin, but not N-methyltryptamines, activates the serotonin 2A receptor via a ß-arrestin2/Src/Akt signaling complex in vivo.* J Neurosci, 2010. **30**(40): p. 13513-24.

140. Schmid, C.L., K.M. Raehal, and L.M. Bohn, *Agonist-directed signaling of the serotonin 2A receptor depends on beta-arrestin-2 interactions in vivo.* Proc Natl Acad Sci U S A, 2008. **105**(3): p. 1079-84.

141. Pottie, E., A. Cannaert, and C.P. Stove, *In vitro structure-activity relationship determination of 30 psychedelic new psychoactive substances by means of β-arrestin 2 recruitment to the serotonin 2A receptor.* Arch Toxicol, 2020. **94**(10): p. 3449-3460.

142. Iwata, Y., et al., *Effects of glutamate positive modulators on cognitive deficits in schizophrenia: a systematic review and meta-analysis of double-blind randomized controlled trials*. Mol Psychiatry, 2015. **20**(10): p. 1151-60.

143. Wise, T., et al., *Glutamatergic hypofunction in medication-free major depression: Secondary effects of affective diagnosis and relationship to peripheral glutaminase*. J Affect Disord, 2018. **234**: p. 214-219.

144. Gonzalez-Burgos, G. and D.A. Lewis, *NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia*. Schizophr Bull, 2012. **38**(5): p. 950-7.

145. Sanacora, G., G. Treccani, and M. Popoli, *Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders*. Neuropharmacology, 2012. **62**(1): p. 63-77.

146. Muschamp, J.W., et al., *Lysergic acid diethylamide and [-]-2,5-dimethoxy-4-methylamphetamine increase extracellular glutamate in rat prefrontal cortex.* Brain Res, 2004. **1023**(1): p. 134-40.

147. Scruggs, J.L., D. Schmidt, and A.Y. Deutch, *The hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) increases cortical extracellular glutamate levels in rats.* Neurosci Lett, 2003. **346**(3): p. 137-40.

148. Holze, F., et al., *Acute dose-dependent effects of lysergic acid diethylamide in a double-blind placebo-controlled study in healthy subjects.* Neuropsychopharmacology, 2020.

149. de Almeida, R.N., et al., *Modulation of Serum Brain-Derived Neurotrophic Factor by a Single Dose of Ayahuasca: Observation From a Randomized Controlled Trial.* Front Psychol, 2019. **10**: p. 1234.

150. Hutten, N.R.P.W., et al., *Low Doses of LSD Acutely Increase BDNF Blood Plasma Levels in Healthy Volunteers.* ACS Pharmacology & Translational Science, 2020.

151. Lisman, J., R. Yasuda, and S. Raghavachari, *Mechanisms of CaMKII action in long-term potentiation.* Nat Rev Neurosci, 2012. **13**(3): p. 169-82.

152. Mukherjee, S. and D. Manahan-Vaughan, *Role of metabotropic glutamate receptors in persistent forms of hippocampal plasticity and learning.* Neuropharmacology, 2013. **66**: p. 65-81.

153. Zhai, Y., et al., *Group II metabotropic glutamate receptor modulation of DOI-induced c-fos mRNA and excitatory responses in the cerebral cortex.* Neuropsychopharmacology, 2003. **28**(1): p. 45-52.

154. Gonzalez-Maeso, J., et al., *Identification of a serotonin/glutamate receptor complex implicated in psychosis.* Nature, 2008. **452**(7183): p. 93-7.

155. Benvenga, M.J., et al., *Metabotropic Glutamate(2) Receptors Play a Key Role in Modulating Head Twitches Induced by a Serotonergic Hallucinogen in Mice.* Front Pharmacol, 2018. **9**: p. 208.

156. Moreno, J.L., et al., *Metabotropic glutamate mGlu2 receptor is necessary for the pharmacological and behavioral effects induced by hallucinogenic 5-HT2A receptor agonists*. Neurosci Lett, 2011. **493**(3): p. 76-9.

157. Moreno, J.L., et al., *Chronic treatment with LY341495 decreases 5-HT(2A) receptor binding and hallucinogenic effects of LSD in mice*. Neurosci Lett, 2013. **536**: p. 69-73.

158. Benneyworth, M.A., R.L. Smith, and E. Sanders-Bush, *Chronic phenethylamine hallucinogen treatment alters behavioral sensitivity to a metabotropic glutamate 2/3 receptor agonist*. Neuropsychopharmacology, 2008. **33**(9): p. 2206-16.

159. Hanks, J.B. and J. González-Maeso, *Animal models of serotonergic psychedelics*. ACS Chem Neurosci, 2013. **4**(1): p. 33-42.

160. Marek, G.J., et al., *Physiological antagonism between 5-hydroxytryptamine(2A) and group II metabotropic glutamate receptors in prefrontal cortex*. J Pharmacol Exp Ther, 2000. **292**(1): p. 76-87.

161. Moreno, J.L., et al., *Allosteric signaling through an mGlu2 and 5-HT2A heteromeric receptor complex and its potential contribution to schizophrenia*. Sci Signal, 2016. **9**(410): p. ra5.

162. Walker, A.G., et al., *Metabotropic glutamate receptor 3 activation is required for long-term depression in medial prefrontal cortex and fear extinction*. Proc Natl Acad Sci U S A, 2015. **112**(4): p. 1196-201.

163. Kometer, M., et al., *Psilocybin-induced spiritual experiences and insightfulness are associated with synchronization of neuronal oscillations*. Psychopharmacology (Berl), 2015. **232**(19): p. 3663-76.

164. Muthukumaraswamy, S.D., et al., *Broadband cortical desynchronization underlies the human psychedelic state*. J Neurosci, 2013. **33**(38): p. 15171-83.

165. Timmermann, C., et al., *Neural correlates of the DMT experience assessed with multivariate EEG*. Sci Rep, 2019. **9**(1): p. 16324.

166. Klimesch, W., *Alpha-band oscillations, attention, and controlled access to stored information*. Trends Cogn Sci, 2012. **16**(12): p. 606-17.

167. de Araujo, D.B., et al., *Seeing with the eyes shut: neural basis of enhanced imagery following Ayahuasca ingestion*. Hum Brain Mapp, 2012. **33**(11): p. 2550-60.

168. Timmermann, C., et al., *LSD modulates effective connectivity and neural adaptation mechanisms in an auditory oddball paradigm*. Neuropharmacology, 2018. **142**: p. 251-262.

169. Bravermanova, A., et al., *Psilocybin disrupts sensory and higher order cognitive processing but not pre-attentive cognitive processing-study on P300 and mismatch negativity in healthy volunteers*. Psychopharmacology (Berl), 2018. **235**(2): p. 491-503.

170. Michaiel, A.M., P.R.L. Parker, and C.M. Niell, *A Hallucinogenic Serotonin-2A Receptor Agonist Reduces Visual Response Gain and Alters Temporal Dynamics in Mouse V1*. Cell Rep, 2019. **26**(13): p. 3475-3483 e4.

171. Schartner, M.M., et al., *Global and local complexity of intracranial EEG decreases during NREM sleep*. Neurosci Conscious, 2017. **2017**(1): p. niw022.

172. Studerus, E., A. Gamma, and F.X. Vollenweider, *Psychometric evaluation of the altered states of consciousness rating scale (OAV)*. PLoS One, 2010. **5**(8): p. e12412.

173. Schmid, Y., et al., *Acute Effects of Lysergic Acid Diethylamide in Healthy Subjects*. Biol Psychiatry, 2015. **78**(8): p. 544-53.

174. Maurer, D., et al., *Reduced perceptual narrowing in synesthesia*. Proceedings of the National Academy of Sciences, 2020. **117**(18): p. 10089-10096.

175. Tagliazucchi, E., et al., *Increased Global Functional Connectivity Correlates with LSD-Induced Ego Dissolution*. Curr Biol, 2016. **26**(8): p. 1043-50.

176. Preller, K.H., et al., *Changes in global and thalamic brain connectivity in LSD-induced altered states of consciousness are attributable to the 5-HT2A receptor*. Elife, 2018. **7**.

177. Preller, K.H., et al., *Effective connectivity changes in LSD-induced altered states of consciousness in humans*. Proc Natl Acad Sci U S A, 2019. **116**(7): p. 2743-2748.

178. Quirk, G.J. and D.R. Gehlert, *Inhibition of the amygdala: key to pathological states?* Ann N Y Acad Sci, 2003. **985**: p. 263-72.

179. Negron-Oyarzo, I., F. Aboitiz, and P. Fuentealba, *Impaired Functional Connectivity in the Prefrontal Cortex: A Mechanism for Chronic Stress-Induced Neuropsychiatric Disorders*. Neural Plast, 2016. **2016**: p. 7539065.

180. Rocha, J.M., et al., *Serotonergic hallucinogens and recognition of facial emotion expressions: a systematic review of the literature.* Ther Adv Psychopharmacol, 2019. **9**: p. 2045125319845774.

181. Barrett, F.S., et al., *Emotions and brain function are altered up to one month after a single high dose of psilocybin*. Sci Rep, 2020. **10**(1): p. 2214.

182. Carhart-Harris, R.L., et al., *Neural correlates of the psychedelic state as determined by fMRI studies with psilocybin*. Proc Natl Acad Sci U S A, 2012. **109**(6): p. 2138-43.

183. Palhano-Fontes, F., et al., *The psychedelic state induced by ayahuasca modulates the activity and connectivity of the default mode network*. PLoS One, 2015. **10**(2): p. e0118143.

184. Davey, C.G., J. Pujol, and B.J. Harrison, *Mapping the self in the brain's default mode network*. Neuroimage, 2016. **132**: p. 390-397.

185. Berman, M.G., et al., *Depression, rumination and the default network*. Soc Cogn Affect Neurosci, 2011. **6**(5): p. 548-55.

186. Sheline, Y.I., et al., *The default mode network and self-referential processes in depression*. Proc Natl Acad Sci U S A, 2009. **106**(6): p. 1942-7.

187. Smigielski, L., et al., *Psilocybin-assisted mindfulness training modulates self-consciousness and brain default mode network connectivity with lasting effects*. Neuroimage, 2019. **196**: p. 207-215.

188. Sampedro, F., et al., *Assessing the Psychedelic "After-Glow" in Ayahuasca Users: Post-Acute Neurometabolic and Functional Connectivity Changes Are Associated with Enhanced Mindfulness Capacities*. Int J Neuropsychopharmacol, 2017. **20**(9): p. 698-711.

189. Barrett, F.S., et al., *Psilocybin acutely alters the functional connectivity of the claustrum with brain networks that support perception, memory, and attention*. Neuroimage, 2020. **218**: p. 116980.

190. Roseman, L., et al., *The effects of psilocybin and MDMA on between-network resting state functional connectivity in healthy volunteers*. Front Hum Neurosci, 2014. **8**: p. 204.

191. Carhart-Harris, R.L., et al., *Neural correlates of the LSD experience revealed by multimodal neuroimaging*. Proc Natl Acad Sci U S A, 2016. **113**(17): p. 4853-8.

192. Geiger, H.A., M.G. Wurst, and R.N. Daniels, *DARK Classics in Chemical Neuroscience: Psilocybin*. ACS Chem Neurosci, 2018. **9**(10): p. 2438-2447.

193. Carhart-Harris, R.L. and K.J. Friston, *REBUS and the Anarchic Brain: Toward a Unified Model of the Brain Action of Psychedelics*. Pharmacol Rev, 2019. **71**(3): p. 316-344.

194. Aghajanian, G.K. and G.J. Marek, *Serotonin, via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release*. Brain Res, 1999. **825**(1-2): p. 161-71.

195. Lambe, E.K. and G.K. Aghajanian, *Hallucinogen-induced UP states in the brain slice of rat prefrontal cortex: role of glutamate spillover and NR2B-NMDA receptors*. Neuropsychopharmacology, 2006. **31**(8): p. 1682-9.

196. Scruggs, J.L., et al., *DOI-Induced activation of the cortex: dependence on 5-HT2A heteroceptors on thalamocortical glutamatergic neurons*. J Neurosci, 2000. **20**(23): p. 8846-52.

197. Aghajanian, G.K. and H.J. Hailgler, *Hallucinogenic indoleamines: Preferential action upon presynaptic serotonin receptors*. Psychopharmacol Commun, 1975. **1**(6): p. 619-29.

198. Puig, M.V., et al., *In vivo modulation of the activity of pyramidal neurons in the rat medial prefrontal cortex by 5-HT2A receptors: relationship to thalamocortical afferents*. Cereb Cortex, 2003. **13**(8): p. 870-82.

199. Riga, M.S., et al., *The natural hallucinogen 5-MeO-DMT, component of Ayahuasca, disrupts cortical function in rats: reversal by antipsychotic drugs*. Int J Neuropsychopharmacol, 2014. **17**(8): p. 1269-82.

200. Celada, P., M.V. Puig, and F. Artigas, *Serotonin modulation of cortical neurons and networks*. Front Integr Neurosci, 2013. **7**: p. 25.

201. Wood, J., Y. Kim, and B. Moghaddam, *Disruption of prefrontal cortex large scale neuronal activity by different classes of psychotomimetic drugs*. J Neurosci, 2012. **32**(9): p. 3022-31.

202. Riga, M.S., et al., *The serotonin hallucinogen 5-MeO-DMT alters cortico-thalamic activity in freely moving mice: Regionally-selective involvement of 5-HT1A and 5-HT2A receptors*. Neuropharmacology, 2018. **142**: p. 219-230.

203. Puig, M.V., et al., *Serotonin modulates fast-spiking interneuron and synchronous activity in the rat prefrontal cortex through 5-HT1A and 5-HT2A receptors*. J Neurosci, 2010. **30**(6): p. 2211-22.

204. Amargos-Bosch, M., et al., *Co-expression and in vivo interaction of serotonin1A and serotonin2A receptors in pyramidal neurons of prefrontal cortex*. Cereb Cortex, 2004. **14**(3): p. 281-99.

205. Araneda, R. and R. Andrade, *5-Hydroxytryptamine2 and 5-hydroxytryptamine 1A receptors mediate opposing responses on membrane excitability in rat association cortex*. Neuroscience, 1991. **40**(2): p. 399-412.

206. Benko, J. and S. Vrankova, *Natural Psychoplastogens As Antidepressant Agents*. Molecules, 2020. **25**(5).

207. Liu, W., et al., *The Role of Neural Plasticity in Depression: From Hippocampus to Prefrontal Cortex*. Neural Plast, 2017. **2017**: p. 6871089.

208. Moda-Sava, R.N., et al., *Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation*. Science, 2019. **364**(6436).

209. Barre, A., et al., *Presynaptic serotonin 2A receptors modulate thalamocortical plasticity and associative learning*. Proc Natl Acad Sci U S A, 2016. **113**(10): p. E1382-91.

210. Berthoux, C., et al., *Sustained Activation of Postsynaptic 5-HT2A Receptors Gates Plasticity at Prefrontal Cortex Synapses*. Cereb Cortex, 2019. **29**(4): p. 1659-1669.

211. Jones, K.A., et al., *Rapid modulation of spine morphology by the 5-HT2A serotonin receptor through kalirin-7 signaling*. Proc Natl Acad Sci U S A, 2009. **106**(46): p. 19575-80.

212. Ly, C., et al., *Psychedelics Promote Structural and Functional Neural Plasticity*. Cell Rep, 2018. **23**(11): p. 3170-3182.

213. Meunier, C.N., P. Chameau, and P.M. Fossier, *Modulation of Synaptic Plasticity in the Cortex Needs to Understand All the Players*. Front Synaptic Neurosci, 2017. **9**: p. 2.

214. Lima da Cruz, R.V., et al., *A Single Dose of 5-MeO-DMT Stimulates Cell Proliferation, Neuronal Survivability, Morphological and Functional Changes in Adult Mice Ventral Dentate Gyrus*. Front Mol Neurosci, 2018. **11**: p. 312.

215. Morales-Garcia, J.A., et al., *The alkaloids of Banisteriopsis caapi, the plant source of the Amazonian hallucinogen Ayahuasca, stimulate adult neurogenesis in vitro*. Sci Rep, 2017. **7**(1): p. 5309.

216. Martin, D.A. and C.D. Nichols, *The Effects of Hallucinogens on Gene Expression*. Curr Top Behav Neurosci, 2018. **36**: p. 137-158.

217. Jefsen, O.H., et al., *Transcriptional regulation in the rat prefrontal cortex and hippocampus after a single administration of psilocybin*. Journal of Psychopharmacology, 2020: p. 026988112095961 4.

218. Dakic, V., et al., *Short term changes in the proteome of human cerebral organoids induced by 5-MeO-DMT*. Sci Rep, 2017. **7**(1): p. 12863.

219. Martin, D.A. and C.D. Nichols, *Psychedelics Recruit Multiple Cellular Types and Produce Complex Transcriptional Responses Within the Brain*. EBioMedicine, 2016. **11**: p. 262-277.

220. Vaidya, V.A., et al., *5-HT2A receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex*. J Neurosci, 1997. **17**(8): p. 2785-95.

221. Musazzi, L., et al., *Pharmacological characterization of BDNF promoters I, II and IV reveals that serotonin and norepinephrine input is sufficient for transcription activation*. Int J Neuropsychopharmacol, 2014. **17**(5): p. 779-91.

222. Jaggar, M. and V. Vaidya, *5-HT2A Receptors and BDNF Regulation: Implications for Psychopathology*. 2018. p. 395-438.

223. Pei, Q., et al., *Glutamate receptor activation is involved in 5-HT2 agonist-induced Arc gene expression in the rat cortex*. Neuropharmacology, 2004. **46**(3): p. 331-9.

224. Pei, Q., et al., *Serotonergic regulation of mRNA expression of Arc, an immediate early gene selectively localized at neuronal dendrites*. Neuropharmacology, 2000. **39**(3): p. 463-70.

225. Benekareddy, M., et al., *Induction of the plasticity-associated immediate early gene Arc by stress and hallucinogens: role of brain-derived neurotrophic factor*. Int J Neuropsychopharmacol, 2013. **16**(2): p. 405-15.

226. Murnane, K.S., *Serotonin 2A receptors are a stress response system: implications for post-traumatic stress disorder*. Behav Pharmacol, 2019. **30**(2 and 3-Spec Issue): p. 151-162.

227. Brouwer, A. and R.L. Carhart-Harris, *Pivotal mental states*. J Psychopharmacol, 2020: p. 269881120959637.

228. McEwen, B.S., *Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders*. Ann N Y Acad Sci, 2004. **1032**: p. 1-7.

229. Huang, C.C., C.H. Yang, and K.S. Hsu, *Do stress and long-term potentiation share the same molecular mechanisms?* Mol Neurobiol, 2005. **32**(3): p. 223-35.

230. Cadle, C.E. and P.R. Zoladz, *Stress time-dependently influences the acquisition and retrieval of unrelated information by producing a memory of its own.* Front Psychol, 2015. **6**: p. 910.

231. Hemrick-Luecke, S.K. and D.C. Evans, *Comparison of the potency of MDL 100,907 and SB 242084 in blocking the serotonin (5-HT)(2) receptor agonist-induced increases in rat serum corticosterone concentrations: evidence for 5-HT(2A) receptor mediation of the HPA axis.* Neuropharmacology, 2002. **42**(2): p. 162-9.

232. Galvão, A.C.M., et al., *Cortisol Modulation by Ayahuasca in Patients With Treatment Resistant Depression and Healthy Controls.* Front Psychiatry, 2018. **9**: p. 185.

233. Van de Kar, L.D., et al., *5-HT2A receptors stimulate ACTH, corticosterone, oxytocin, renin, and prolactin release and activate hypothalamic CRF and oxytocin-expressing cells.* J Neurosci, 2001. **21**(10): p. 3572-9.

234. Jorgensen, H., et al., *Serotonergic stimulation of corticotropin-releasing hormone and pro-opiomelanocortin gene expression.* J Neuroendocrinol, 2002. **14**(10): p. 788-95.

235. Contesse, V., et al., *Role of 5-HT in the regulation of the brain-pituitary-adrenal axis: effects of 5-HT on adrenocortical cells.* Can J Physiol Pharmacol, 2000. **78**(12): p. 967-83.

236. Wolff, M., et al., *Learning to Let Go: A Cognitive-Behavioral Model of How Psychedelic Therapy Promotes Acceptance.* Front Psychiatry, 2020. **11**: p. 5.

237. Inserra, A., D. De Gregorio, and G. Gobbi, *Psychedelics in Psychiatry: Neuroplastic, Immunomodulatory, and Neurotransmitter Mechanisms.* Pharmacol Rev, 2021. **73**(1): p. 202-277.

238. Beurel, E., M. Toups, and C.B. Nemeroff, *The Bidirectional Relationship of Depression and Inflammation: Double Trouble.* Neuron, 2020. **107**(2): p. 234-256.

239. Thompson, C. and A. Szabo, *Psychedelics as a novel approach to treating autoimmune conditions*. Immunol Lett, 2020. **228**: p. 45-54.

240. Wang, D.S., et al., *Memory deficits induced by inflammation are regulated by α5-subunit-containing GABAA receptors*. Cell Rep, 2012. **2**(3): p. 488-96.

241. Vezzani, A. and B. Viviani, *Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability.* Neuropharmacology, 2015. **96**(Pt A): p. 70-82.

242. Herr, N., C. Bode, and D. Duerschmied, *The Effects of Serotonin in Immune Cells*. Front Cardiovasc Med, 2017. **4**: p. 48.

243. Galvão-Coelho, N.L., et al., *Changes in inflammatory biomarkers are related to the antidepressant effects of Ayahuasca*. J Psychopharmacol, 2020. **34**(10): p. 1125-1133.

244. Uthaug, M.V., et al., *Prospective examination of synthetic 5-methoxy-N,N-dimethyltryptamine inhalation: effects on salivary IL-6, cortisol levels, affect, and non-judgment*. Psychopharmacology (Berl), 2020. **237**(3): p. 773-785.

245. Nau, F., Jr., et al., *Serotonin 5-HT2A receptor activation blocks TNF-α mediated inflammation in vivo.* PLoS One, 2013. **8**(10): p. e75426.

246. Yu, B., et al., *Serotonin 5-hydroxytryptamine(2A) receptor activation suppresses tumor necrosis factor-alpha-induced inflammation with extraordinary potency.* J Pharmacol Exp Ther, 2008. **327**(2): p. 316-23.

247. Glebov, K., et al., *Serotonin stimulates secretion of exosomes from microglia cells*. Glia, 2015. **63**(4): p. 626-34.

248. Krabbe, G., et al., *Activation of serotonin receptors promotes microglial injury-induced motility but attenuates phagocytic activity.* Brain Behav Immun, 2012. **26**(3): p. 419-28.

249. Haroon, E., A.H. Miller, and G. Sanacora, *Inflammation, Glutamate, and Glia: A Trio of Trouble in Mood Disorders*. Neuropsychopharmacology, 2017. **42**(1): p. 193-215.

250. Perry, V.H. and C. Holmes, *Microglial priming in neurodegenerative disease*. Nat Rev Neurol, 2014. **10**(4): p. 217-24.

251. Family, N., et al., *Safety, tolerability, pharmacokinetics, and pharmacodynamics of low dose lysergic acid diethylamide (LSD) in healthy older volunteers*. Psychopharmacology (Berl), 2020. **237**(3): p. 841-853.

252. Pascual-Leone, A., et al., *Characterizing brain cortical plasticity and network dynamics across the age-span in health and disease with TMS-EEG and TMS-fMRI*. Brain Topogr, 2011. **24**(3-4): p. 302-15.

253. Yoo, K.-S., et al., *Postsynaptic density protein 95 (PSD-95) is transported by KIF5 to dendritic regions*. Molecular Brain, 2019. **12**(1): p. 97.

254. Zhao, X., et al., *Psilocybin promotes neuroplasticity and induces rapid and sustained antidepressant-like effects in mice*. Journal of Psychopharmacology, 2024. **38**(5): p. 489-499.

255. Du, Y., et al., *Psilocybin facilitates fear extinction in mice by promoting hippocampal neuroplasticity*. Chin Med J (Engl), 2023. **136**(24): p. 2983-2992.

256. Shao, L.-X., et al., *Psilocybin induces rapid and persistent growth of dendritic spines in frontal cortex in vivo*. Neuron, 2021. **109**(16): p. 2535-2544.e4.

257. Jefferson, S.J., et al., *5-MeO-DMT modifies innate behaviors and promotes structural neural plasticity in mice*. Neuropsychopharmacology, 2023. **48**(9): p. 1257-1266.

258. de la Fuente Revenga, M., et al., *Prolonged epigenomic and synaptic plasticity alterations following single exposure to a psychedelic in mice*. Cell Rep, 2021. **37**(3): p. 109836.

259. De Gregorio, D., et al., *Lysergic acid diethylamide (LSD) promotes social behavior through mTORC1 in the excitatory neurotransmission*. Proc Natl Acad Sci U S A, 2021. **118**(5).

260. Nardou, R., et al., *Psychedelics reopen the social reward learning critical period*. Nature, 2023. **618**(7966): p. 790-798.

261. Wegman-Points, L., et al., *Corticosterone as a potential confounding factor in delineating mechanisms underlying ketamine's rapid antidepressant actions*. bioRxiv, 2019: p. 2019.12.30.891333.

262. Walsh, Z., et al., *Ketamine for the treatment of mental health and substance use disorders: comprehensive systematic review*. BJPsych Open, 2021. **8**(1): p. e19.

263. Fitzgerald, P.J., J.Y. Yen, and B.O. Watson, *Stress-sensitive antidepressant-like effects of ketamine in the mouse forced swim test*. PLoS One, 2019. **14**(4): p. e0215554.

264. Fadahunsi, N., et al., *Acute and long-term effects of psilocybin on energy balance and feeding behavior in mice*. Transl Psychiatry, 2022. **12**(1): p. 330.

265. Bouso, J.C., et al., *Serotonergic psychedelics and personality: A systematic review of contemporary research*. Neurosci Biobehav Rev, 2018. **87**: p. 118-132.

266. Doss, M.K., et al., *Psilocybin therapy increases cognitive and neural flexibility in patients with major depressive disorder*. Transl Psychiatry, 2021. **11**(1): p. 574.

267. Yaden, D.B. and R.R. Griffiths, *The Subjective Effects of Psychedelics Are Necessary for Their Enduring Therapeutic Effects*. ACS Pharmacol Transl Sci, 2021. **4**(2): p. 568-572.

268. Davis, A.K., F.S. Barrett, and R.R. Griffiths, *Psychological flexibility mediates the relations between acute psychedelic effects and subjective decreases in depression and anxiety*. J Contextual Behav Sci, 2020. **15**: p. 39-45.

269. Murphy-Beiner, A. and K. Soar, *Ayahuasca's 'afterglow': improved mindfulness and cognitive flexibility in ayahuasca drinkers*. Psychopharmacology (Berl), 2020. **237**(4): p. 1161-1169.

270. Uthaug, M.V., et al., *Sub-acute and long-term effects of ayahuasca on affect and cognitive thinking style and their association with ego dissolution*. Psychopharmacology (Berl), 2018. **235**(10): p. 2979-2989.

271. Gukasyan, N., et al., *Efficacy and safety of psilocybin-assisted treatment for major depressive disorder: Prospective 12-month follow-up*. J Psychopharmacol, 2022. **36**(2): p. 151-158.

272. Agin-Liebes, G.I., et al., *Long-term follow-up of psilocybin-assisted psychotherapy for psychiatric and existential distress in patients with life-threatening cancer.* J Psychopharmacol, 2020. **34**(2): p. 155-166.

273. Mion, G. and T. Villevieille, *Ketamine pharmacology: an update (pharmacodynamics and molecular aspects, recent findings).* CNS Neurosci Ther, 2013. **19**(6): p. 370-80.

274. Zhang, M.W. and R.C. Ho, *Controversies of the Effect of Ketamine on Cognition.* Front Psychiatry, 2016. **7**: p. 47.

275. Bălăeţ, M., *Psychedelic Cognition-The Unreached Frontier of Psychedelic Science.* Front Neurosci, 2022. **16**: p. 832375.

276. Mans, K., et al., *Sustained, Multifaceted Improvements in Mental Well-Being Following Psychedelic Experiences in a Prospective Opportunity Sample.* Front Psychiatry, 2021. **12**: p. 647909.

277. McCulloch, D.E., et al., *Psilocybin-Induced Mystical-Type Experiences are Related to Persisting Positive Effects: A Quantitative and Qualitative Report.* Front Pharmacol, 2022. **13**: p. 841648.

278. Aleksandrova, L.R. and A.G. Phillips, *Neuroplasticity as a convergent mechanism of ketamine and classical psychedelics.* Trends Pharmacol Sci, 2021. **42**(11): p. 929-942.

279. Kadriu, B., et al., *Ketamine and Serotonergic Psychedelics: Common Mechanisms Underlying the Effects of Rapid-Acting Antidepressants.* Int J Neuropsychopharmacol, 2020.

280. Moliner, R., et al., *Psychedelics promote plasticity by directly binding to BDNF receptor TrkB.* Nature Neuroscience, 2023. **26**(6): p. 1032-1041.

281. Vargas, M.V., et al., *Psychedelics and Other Psychoplastogens for Treating Mental Illness.* Front Psychiatry, 2021. **12**: p. 727117.

282. Jett, J.D., et al., *Antidepressant-like cognitive and behavioral effects of acute ketamine administration associated with plasticity in the ventral hippocampus to*

medial prefrontal cortex pathway. Psychopharmacology (Berl), 2015. **232**(17): p. 3123-33.

283. Hesselgrave, N., et al., *Harnessing psilocybin: antidepressant-like behavioral and synaptic actions of psilocybin are independent of 5-HT2R activation in mice.* Proc Natl Acad Sci U S A, 2021. **118**(17).

284. Zhang, J., et al., *(R)-Ketamine Rapidly Ameliorates the Decreased Spine Density in the Medial Prefrontal Cortex and Hippocampus of Susceptible Mice After Chronic Social Defeat Stress.* Int J Neuropsychopharmacol, 2019. **22**(10): p. 675-679.

285. Li, N., et al., *Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure.* Biol Psychiatry, 2011. **69**(8): p. 754-61.

286. Shao, L.X., et al., *Psilocybin induces rapid and persistent growth of dendritic spines in frontal cortex in vivo.* Neuron, 2021. **109**(16): p. 2535-2544 e4.

287. Wu, M., et al., *Ketamine Rapidly Enhances Glutamate-Evoked Dendritic Spinogenesis in Medial Prefrontal Cortex Through Dopaminergic Mechanisms.* Biol Psychiatry, 2021. **89**(11): p. 1096-1105.

288. Cavalleri, L., et al., *Ketamine enhances structural plasticity in mouse mesencephalic and human iPSC-derived dopaminergic neurons via AMPAR-driven BDNF and mTOR signaling.* Molecular Psychiatry, 2018. **23**(4): p. 812-823.

289. Rawat, R., et al., *Ketamine activates adult-born immature granule neurons to rapidly alleviate depression-like behaviors in mice.* Nat Commun, 2022. **13**(1): p. 2650.

290. Yamada, J. and S. Jinno, *Potential link between antidepressant-like effects of ketamine and promotion of adult neurogenesis in the ventral hippocampus of mice.* Neuropharmacology, 2019. **158**: p. 107710.

291. Clarke, M., et al., *Ketamine modulates hippocampal neurogenesis and pro-inflammatory cytokines but not stressor induced neurochemical changes.* Neuropharmacology, 2017. **112**(Pt A): p. 210-220.

292. González-Maeso, J., et al., *Transcriptome fingerprints distinguish hallucinogenic and nonhallucinogenic 5-hydroxytryptamine 2A receptor agonist effects in mouse somatosensory cortex.* J Neurosci, 2003. **23**(26): p. 8836-43.

293. Nichols, C.D. and E. Sanders-Bush, *A single dose of lysergic acid diethylamide influences gene expression patterns within the mammalian brain.* Neuropsychopharmacology, 2002. **26**(5): p. 634-42.

294. Nichols, C.D., E.E. Garcia, and E. Sanders-Bush, *Dynamic changes in prefrontal cortex gene expression following lysergic acid diethylamide administration.* Brain Res Mol Brain Res, 2003. **111**(1-2): p. 182-8.

295. Nichols, C.D. and E. Sanders-Bush, *Molecular genetic responses to lysergic acid diethylamide include transcriptional activation of MAP kinase phosphatase-1, C/EBP-beta and ILAD-1, a novel gene with homology to arrestins.* J Neurochem, 2004. **90**(3): p. 576-84.

296. Viana, G.S.B., et al., *Rapid and long-lasting antidepressant-like effects of ketamine and their relationship with the expression of brain enzymes, BDNF, and astrocytes.* Braz J Med Biol Res, 2020. **54**(2): p. e10107.

297. Davoudian, P.A., L.-X. Shao, and A.C. Kwan, *Shared and Distinct Brain Regions Targeted for Immediate Early Gene Expression by Ketamine and Psilocybin.* ACS Chemical Neuroscience, 2023. **14**(3): p. 468-480.

298. Nardou, R., et al., *Psychedelics reopen the social reward learning critical period.* Nature, 2023. **618**(7966): p. 790-798.

299. de la Fuente Revenga, M., et al., *Prolonged epigenomic and synaptic plasticity alterations following single exposure to a psychedelic in mice.* Cell Reports, 2021. **37**(3).

300. Bathje, G.J., E. Majeski, and M. Kudowor, *Psychedelic integration: An analysis of the concept and its practice.* Front Psychol, 2022. **13**: p. 824077.

301. Carhart-Harris, R.L. and D.J. Nutt, *Serotonin and brain function: a tale of two receptors.* J Psychopharmacol, 2017. **31**(9): p. 1091-1120.

302. Lepow, L., H. Morishita, and R. Yehuda, *Critical Period Plasticity as a Framework for Psychedelic-Assisted Psychotherapy.* Frontiers in Neuroscience, 2021. **15**.

303. Abraham, W.C., *Metaplasticity: tuning synapses and networks for plasticity.* Nature Reviews Neuroscience, 2008. **9**(5): p. 387-387.

304. Ewald, A.H., et al., *Designer drugs 2,5-dimethoxy-4-bromo-amphetamine (DOB) and 2,5-dimethoxy-4-bromo-methamphetamine (MDOB): studies on their metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques.* J Mass Spectrom, 2006. **41**(4): p. 487-98.

305. Glatfelter, G.C., et al., *Structure–Activity Relationships for Psilocybin, Baeocystin, Aeruginascin, and Related Analogues to Produce Pharmacological Effects in Mice.* ACS Pharmacology & Translational Science, 2022. **5**(11): p. 1181-1196.

306. Bittar, T.P. and B. Labonte, *Functional Contribution of the Medial Prefrontal Circuitry in Major Depressive Disorder and Stress-Induced Depressive-Like Behaviors.* Front Behav Neurosci, 2021. **15**: p. 699592.

307. Shin, L.M. and I. Liberzon, *The neurocircuitry of fear, stress, and anxiety disorders.* Neuropsychopharmacology, 2010. **35**(1): p. 169-91.

308. Kessler, R., et al., *Long-Term Neuroanatomical Consequences of Childhood Maltreatment: Reduced Amygdala Inhibition by Medial Prefrontal Cortex.* Front Syst Neurosci, 2020. **14**: p. 28.

309. Amada, N., et al., *Psychedelic experience and the narrative self: An exploratory qualitative study.* Journal of Consciousness Studies, 2020. **27**: p. 6-33.

310. Logue, J., et al., *Sex-specific effects of social isolation stress and ketamine on hippocampal plasticity.* Neurosci Lett, 2022. **766**: p. 136301.

311. Torrado Pacheco, A., et al., *Acute psilocybin enhances cognitive flexibility in rats.* Neuropsychopharmacology, 2023. **48**(7): p. 1011-1020.

312. Darmani, N.A., et al., *Do functional relationships exist between 5-HT1A and 5-HT2 receptors?* Pharmacol Biochem Behav, 1990. **36**(4): p. 901-6.

313. Jiang, K., X. Liu, and R. Su, *Contrasting effects of DOI and lisuride on impulsive decision-making in delay discounting task*. Psychopharmacology, 2022. **239**(11): p. 3551-3565.

314. Berquist, M.D. and W.E. Fantegrossi, *Effects of 5-HT2A receptor agonist 2,5-dimethoxy-4-iodoamphetamine on alcohol consumption in Long-Evans rats*. Behav Pharmacol, 2021. **32**(5): p. 382-391.

315. Choi, K.H., et al., *Effects of Ketamine on Rodent Fear Memory.* Int J Mol Sci, 2020. **21**(19).

316. Morena, M., et al., *Ketamine anesthesia enhances fear memory consolidation via noradrenergic activation in the basolateral amygdala*. Neurobiology of Learning and Memory, 2021. **178**: p. 107362.

317. Zanos, P., et al., *Ketamine and Ketamine Metabolite Pharmacology: Insights into Therapeutic Mechanisms*. Pharmacol Rev, 2018. **70**(3): p. 621-660.

318. Figueroa, A.G., et al., *Hippocampal β2-GABA(A) receptors mediate LTP suppression by etomidate and contribute to long-lasting feedback but not feedforward inhibition of pyramidal neurons*. J Neurophysiol, 2021. **126**(4): p. 1090-1100.

319. Zucker, R.S. and W.G. Regehr, *Short-term synaptic plasticity.* Annu Rev Physiol, 2002. **64**: p. 355-405.

320. Singh, N., et al., *Presynaptic endoplasmic reticulum regulates short-term plasticity in hippocampal synapses*. Commun Biol, 2021. **4**(1): p. 241.

321. Majić, T., T.T. Schmidt, and J. Gallinat, *Peak experiences and the afterglow phenomenon: when and how do therapeutic effects of hallucinogens depend on psychedelic experiences?* J Psychopharmacol, 2015. **29**(3): p. 241-53.

322. Sumner, R.L., et al., *A qualitative and quantitative account of patient's experiences of ketamine and its antidepressant properties*. J Psychopharmacol, 2021. **35**(8): p. 946-961.

323. Bear, M.F., *A synaptic basis for memory storage in the cerebral cortex.* Proc Natl Acad Sci U S A, 1996. **93**(24): p. 13453-9.

324. de la Fuente Revenga, M., et al., *Fully automated head-twitch detection system for the study of 5-HT2A receptor pharmacology in vivo.* Scientific Reports, 2019. **9**(1): p. 14247.

325. Ly, C., et al., *Transient Stimulation with Psychoplastogens Is Sufficient to Initiate Neuronal Growth.* ACS Pharmacol Transl Sci, 2021. **4**(2): p. 452-460.

326. Kim, J.W., et al., *Sustained effects of rapidly acting antidepressants require BDNF-dependent MeCP2 phosphorylation.* Nat Neurosci, 2021. **24**(8): p. 1100-1109.

327. Jang, G. and M.B. MacIver, *Ketamine Produces a Long-Lasting Enhancement of CA1 Neuron Excitability.* Int J Mol Sci, 2021. **22**(15).

328. Gass, N., et al., *Differences between ketamine's short-term and long-term effects on brain circuitry in depression.* Translational Psychiatry, 2019. **9**(1): p. 172.

329. Park, P., et al., *NMDA receptor-dependent long-term potentiation comprises a family of temporally overlapping forms of synaptic plasticity that are induced by different patterns of stimulation.* Philos Trans R Soc Lond B Biol Sci, 2014. **369**(1633): p. 20130131.

330. Volianskis, A., G.L. Collingridge, and M.S. Jensen, *The roles of STP and LTP in synaptic encoding.* PeerJ, 2013. **1**: p. e3.

331. Schulz, P.E., E.P. Cook, and D. Johnston, *Using paired-pulse facilitation to probe the mechanisms for long-term potentiation (LTP).* J Physiol Paris, 1995. **89**(1): p. 3-9.

332. Fuhrmann, G., et al., *Coding of Temporal Information by Activity-Dependent Synapses.* Journal of Neurophysiology, 2002. **87**(1): p. 140-148.

333. Fung, C.C.A., et al., *Dynamical Synapses Enhance Neural Information Processing: Gracefulness, Accuracy, and Mobility.* Neural Computation, 2012. **24**(5): p. 1147-1185.

334. Tsodyks, M. and S. Wu, *Short-term synaptic plasticity.* Scholarpedia, 2013. **8**.

335. Barak, O. and M. Tsodyks, *Persistent Activity in Neural Networks with Dynamic Synapses.* PLOS Computational Biology, 2007. **3**(2): p. e35.

336. Melamed, O., et al., *Slow oscillations in neural networks with facilitating synapses*. Journal of Computational Neuroscience, 2008. **25**(2): p. 308-316.

337. France, G., et al., *Multiple roles of GluN2B-containing NMDA receptors in synaptic plasticity in juvenile hippocampus*. Neuropharmacology, 2017. **112**: p. 76-83.

338. Barre, A., et al., *Presynaptic serotonin 2A receptors modulate thalamocortical plasticity and associative learning*. Proceedings of the National Academy of Sciences, 2016. **113**(10): p. E1382-E1391.

339. Arvanov, V.L., et al., *A pre- and postsynaptic modulatory action of 5-HT and the 5-HT2A, 2C receptor agonist DOB on NMDA-evoked responses in the rat medial prefrontal cortex*. Eur J Neurosci, 1999. **11**(8): p. 2917-34.

340. Scruggs, J.L., D. Schmidt, and A.Y. Deutch, *The hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) increases cortical extracellular glutamate levels in rats*. Neuroscience Letters, 2003. **346**(3): p. 137-140.

341. Wojtas, A., et al., *Effect of Psilocybin and Ketamine on Brain Neurotransmitters, Glutamate Receptors, DNA and Rat Behavior*. Int J Mol Sci, 2022. **23**(12).

342. Chen, A., C.J. Hough, and H. Li, *Serotonin type II receptor activation facilitates synaptic plasticity via N-methyl-D-aspartate-mediated mechanism in the rat basolateral amygdala*. Neuroscience, 2003. **119**(1): p. 53-63.

343. Fanselow, M.S. and H.W. Dong, *Are the dorsal and ventral hippocampus functionally distinct structures?* Neuron, 2010. **65**(1): p. 7-19.

344. Marek, G.J. and G.K. Aghajanian, *LSD and the phenethylamine hallucinogen DOI are potent partial agonists at 5-HT2A receptors on interneurons in rat piriform cortex*. J Pharmacol Exp Ther, 1996. **278**(3): p. 1373-82.

345. Porter, R.H., et al., *Functional characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C receptors in CHO-K1 cells*. Br J Pharmacol, 1999. **128**(1): p. 13-20.

346. Canal, C.E., R.G. Booth, and D. Morgan, *Support for 5-HT2C receptor functional selectivity in vivo utilizing structurally diverse, selective 5-HT2C receptor ligands and*

the 2,5-dimethoxy-4-iodoamphetamine elicited head-twitch response model. Neuropharmacology, 2013. **70**: p. 112-21.

347. Halberstadt, A.L., et al., *5-HT(2A) and 5-HT(2C) receptors exert opposing effects on locomotor activity in mice.* Neuropsychopharmacology, 2009. **34**(8): p. 1958-67.

348. Yaden, D.B., et al., *Psychedelics and Consciousness: Distinctions, Demarcations, and Opportunities.* Int J Neuropsychopharmacol, 2021. **24**(8): p. 615-623.

349. Winkelman, M.J., *The Mechanisms of Psychedelic Visionary Experiences: Hypotheses from Evolutionary Psychology.* Front Neurosci, 2017. **11**: p. 539.

350. Zafar, R., et al., *Psychedelic therapy in the treatment of addiction: the past, present and future.* Front Psychiatry, 2023. **14**: p. 1183740.

351. Wallach, J., et al., *Identification of 5-HT2A receptor signaling pathways associated with psychedelic potential.* Nature Communications, 2023. **14**(1): p. 8221.

352. Wiepking, L., E. de Bruin, and A. Ghiţă, *The potential of psilocybin use to enhance well-being in healthy individuals – A scoping review.* Journal of Psychedelic Studies, 2023. **7**(3): p. 184-199.

353. Nayak, S.M., et al., *Naturalistic psilocybin use is associated with persisting improvements in mental health and wellbeing: results from a prospective, longitudinal survey.* Front Psychiatry, 2023. **14**: p. 1199642.

354. Appelbaum, L.G., et al., *Synaptic plasticity and mental health: methods, challenges and opportunities.* Neuropsychopharmacology, 2023. **48**(1): p. 113-120.

355. Nichols, C.D. and E. Sanders-Bush, *A Single Dose of Lysergic Acid Diethylamide Influences Gene Expression Patterns within the Mammalian Brain.* Neuropsychopharmacology, 2002. **26**(5): p. 634-642.

356. Desouza, L.A., et al., *The Hallucinogenic Serotonin(2A) Receptor Agonist, 2,5-Dimethoxy-4-Iodoamphetamine, Promotes cAMP Response Element Binding Protein-Dependent Gene Expression of Specific Plasticity-Associated Genes in the Rodent Neocortex.* Front Mol Neurosci, 2021. **14**: p. 790213.

357. de Vos, C.M.H., N.L. Mason, and K.P.C. Kuypers, *Psychedelics and Neuroplasticity: A Systematic Review Unraveling the Biological Underpinnings of Psychedelics*. Frontiers in Psychiatry, 2021. **12**: p. 1575.

358. Raval, N.R., et al., *A Single Dose of Psilocybin Increases Synaptic Density and Decreases 5-HT(2A) Receptor Density in the Pig Brain*. Int J Mol Sci, 2021. **22**(2).

359. Zahid, Z., Sultan, Z., Krause, B., Wenthur, C., Pearce, R., Banks, M., *Divergent Effects of Ketamine and the Serotoninergic Psychedelic 2,5-Dimethoxy-4-Iodoamphetamine on Hippocampal Plasticity and Metaplasticity*. Psychedelic Medicine. **0**(0): p. null.

360. Li, M., C. Long, and L. Yang, *Hippocampal-prefrontal circuit and disrupted functional connectivity in psychiatric and neurodegenerative disorders*. Biomed Res Int, 2015. **2015**: p. 810548.

361. Place, R., et al., *Bidirectional prefrontal-hippocampal interactions support context-guided memory*. Nat Neurosci, 2016. **19**(8): p. 992-4.

362. Fernandez-Leon, J.A., et al., *Neural correlates and determinants of approach–avoidance conflict in the prelimbic prefrontal cortex*. eLife, 2021. **10**: p. e74950.

363. Sánchez-Bellot, C., et al., *Two opposing hippocampus to prefrontal cortex pathways for the control of approach and avoidance behaviour*. Nat Commun, 2022. **13**(1): p. 339.

364. Padilla-Coreano, N., et al., *Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior*. Neuron, 2016. **89**(4): p. 857-66.

365. Kirlic, N., J. Young, and R.L. Aupperle, *Animal to human translational paradigms relevant for approach avoidance conflict decision making*. Behav Res Ther, 2017. **96**: p. 14-29.

366. Bryant, K.G. and J.M. Barker, *Arbitration of Approach-Avoidance Conflict by Ventral Hippocampus*. Front Neurosci, 2020. **14**: p. 615337.

367. La-Vu, M., et al., *To Approach or Avoid: An Introductory Overview of the Study of Anxiety Using Rodent Assays*. Front Behav Neurosci, 2020. **14**: p. 145.

368. Jimenez, J.C., et al., *Anxiety Cells in a Hippocampal-Hypothalamic Circuit.* Neuron, 2018. **97**(3): p. 670-683.e6.

369. Kim, W.B. and J.-H. Cho, *Synaptic Targeting of Double-Projecting Ventral CA1 Hippocampal Neurons to the Medial Prefrontal Cortex and Basal Amygdala*. The Journal of Neuroscience, 2017. **37**(19): p. 4868-4882.

370. Jones, N.T., et al., *In vivo validation of psilacetin as a prodrug yielding modestly lower peripheral psilocin exposure than psilocybin*. Front Psychiatry, 2023. **14**: p. 1303365.

371. Spellman, T., et al., *Hippocampal-prefrontal input supports spatial encoding in working memory.* Nature, 2015. **522**(7556): p. 309-14.

372. Liu, X. and A.G. Carter, *Ventral Hippocampal Inputs Preferentially Drive Corticocortical Neurons in the Infralimbic Prefrontal Cortex.* J Neurosci, 2018. **38**(33): p. 7351-7363.

373. Sotres-Bayon, F., et al., *Gating of fear in prelimbic cortex by hippocampal and amygdala inputs*. Neuron, 2012. **76**(4): p. 804-12.

374. Siapas, A.G., E.V. Lubenov, and M.A. Wilson, *Prefrontal phase locking to hippocampal theta oscillations*. Neuron, 2005. **46**(1): p. 141-51.

375. Kozell, L.B., et al., *Pharmacologic Activity of Substituted Tryptamines at 5-Hydroxytryptamine (5-HT)(2A) Receptor (5-HT(2A)R), 5-HT(2C)R, 5-HT(1A)R, and Serotonin Transporter.* J Pharmacol Exp Ther, 2023. **385**(1): p. 62-75.

376. Klein, A.K., et al., *Investigation of the Structure-Activity Relationships of Psilocybin Analogues*. ACS Pharmacol Transl Sci, 2021. **4**(2): p. 533-542.

377. Takaba, R., et al., *Ethopharmacological evaluation of antidepressant-like effect of serotonergic psychedelics in C57BL/6J male mice.* Res Sq, 2023.

378. Dieterich, A., et al., *Chronic corticosterone administration induces negative valence and impairs positive valence behaviors in mice*. Transl Psychiatry, 2019. **9**(1): p. 337.

379. Gukasyan, N. and S.K. Narayan, *Menstrual Changes and Reversal of Amenorrhea Induced by Classic Psychedelics: A Case Series*. J Psychoactive Drugs, 2024. **56**(1): p. 50-55.

380. Peck, S.K., et al., *Psilocybin therapy for females with anorexia nervosa: a phase 1, open-label feasibility study*. Nature Medicine, 2023. **29**(8): p. 1947-1953.

381. Bornemann, J., et al., *Self-Medication for Chronic Pain Using Classic Psychedelics: A Qualitative Investigation to Inform Future Research*. Frontiers in Psychiatry, 2021. **12**.

382. Gao, R., *Interpreting the electrophysiological power spectrum*. J Neurophysiol, 2016. **115**(2): p. 628-30.

383. Buzsáki, G. and A. Draguhn, *Neuronal Oscillations in Cortical Networks*. Science, 2004. **304**(5679): p. 1926-1929.

384. Vejmola, Č., et al., *Psilocin, LSD, mescaline, and DOB all induce broadband desynchronization of EEG and disconnection in rats with robust translational validity*. Translational Psychiatry, 2021. **11**(1): p. 506.

385. Alamia, A., et al., *DMT alters cortical travelling waves*. eLife, 2020. **9**: p. e59784.

386. Le, G.H., et al., *Spectral signatures of psilocybin, lysergic acid diethylamide (LSD) and ketamine in healthy volunteers and persons with major depressive disorder and treatment-resistant depression: A systematic review*. Journal of Affective Disorders, 2024. **355**: p. 342-354.

387. Souza, A.C., et al., *5-MeO-DMT induces sleep-like LFP spectral signatures in the hippocampus and prefrontal cortex of awake rats*. Scientific Reports, 2024. **14**(1): p. 11281.

388. Hohaia, W., et al., *Occipital alpha-band brain waves when the eyes are closed are shaped by ongoing visual processes*. Scientific Reports, 2022. **12**(1): p. 1194.

389. Sheng, J.A., et al., *The Hypothalamic-Pituitary-Adrenal Axis: Development, Programming Actions of Hormones, and Maternal-Fetal Interactions*. Front Behav Neurosci, 2020. **14**: p. 601939.

390. Hazell, G.G., et al., *G protein-coupled receptors in the hypothalamic paraventricular and supraoptic nuclei--serpentine gateways to neuroendocrine homeostasis*. Front Neuroendocrinol, 2012. **33**(1): p. 45-66.

391. Zhang, Y., et al., *Desensitization of 5-HT1A receptors by 5-HT2A receptors in neuroendocrine neurons in vivo*. J Pharmacol Exp Ther, 2004. **310**(1): p. 59-66.

392. Dos Santos, T.S., et al., *Distribution of serotonin 5-HT1A-binding sites in the brainstem and the hypothalamus, and their roles in 5-HT-induced sleep and ingestive behaviors in rock pigeons (Columba livia)*. Behav Brain Res, 2015. **295**: p. 45-63.

393. Shi, J., et al., *Sustained treatment with a 5-HT(2A) receptor agonist causes functional desensitization and reductions in agonist-labeled 5-HT(2A) receptors despite increases in receptor protein levels in rats*. Neuropharmacology, 2008. **55**(5): p. 687-92.

394. Mitchell, J.P., M.R. Banaji, and C.N. Macrae, *The link between social cognition and self-referential thought in the medial prefrontal cortex*. J Cogn Neurosci, 2005. **17**(8): p. 1306-15.

395. Venkatachalam, K.L., J.E. Herbrandson, and S.J. Asirvatham, *Signals and Signal Processing for the Electrophysiologist*. Circulation: Arrhythmia and Electrophysiology, 2011. **4**(6): p. 974-981.

396. Carhart-Harris, R., et al., *Trial of Psilocybin versus Escitalopram for Depression*. N Engl J Med, 2021. **384**(15): p. 1402-1411.

397. Raison, C.L., et al., *Single-Dose Psilocybin Treatment for Major Depressive Disorder: A Randomized Clinical Trial*. Jama, 2023. **330**(9): p. 843-853.

398. Varker, T., et al., *Efficacy of Psychoactive Drugs for the Treatment of Posttraumatic Stress Disorder: A Systematic Review of MDMA, Ketamine, LSD and Psilocybin*. J Psychoactive Drugs, 2021. **53**(1): p. 85-95.

399. Frohlich, J., et al., *Paradoxical pharmacological dissociations result from drugs that enhance delta oscillations but preserve consciousness*. Communications Biology, 2023. **6**(1): p. 654.

400. Timmermann, C., et al., *Human brain effects of DMT assessed via EEG-fMRI.* Proc Natl Acad Sci U S A, 2023. **120**(13): p. e2218949120.

401. Thomas, C.W., et al., *Psilocin acutely alters sleep-wake architecture and cortical brain activity in laboratory mice.* Translational Psychiatry, 2022. **12**(1): p. 77.

402. Harmony, T., *The functional significance of delta oscillations in cognitive processing.* Front Integr Neurosci, 2013. **7**: p. 83.

403. O'Neill, P.K., J.A. Gordon, and T. Sigurdsson, *Theta oscillations in the medial prefrontal cortex are modulated by spatial working memory and synchronize with the hippocampus through its ventral subregion.* J Neurosci, 2013. **33**(35): p. 14211-24.

404. Attar, E.T., *Review of electroencephalography signals approaches for mental stress assessment.* Neurosciences (Riyadh), 2022. **27**(4): p. 209-215.

405. Jensen, O. and A. Mazaheri, *Shaping functional architecture by oscillatory alpha activity: gating by inhibition.* Front Hum Neurosci, 2010. **4**: p. 186.

406. Ippolito, G., et al., *The Role of Alpha Oscillations among the Main Neuropsychiatric Disorders in the Adult and Developing Human Brain: Evidence from the Last 10 Years of Research.* Biomedicines, 2022. **10**(12).

407. Schmidt, R., et al., *Beta Oscillations in Working Memory, Executive Control of Movement and Thought, and Sensorimotor Function.* J Neurosci, 2019. **39**(42): p. 8231-8238.

408. Lundqvist, M., et al., *Gamma and beta bursts during working memory readout suggest roles in its volitional control.* Nat Commun, 2018. **9**(1): p. 394.

409. Yao, Z., et al., *Acute or Chronic Exposure to Corticosterone Promotes Wakefulness in Mice.* Brain Sci, 2023. **13**(10).

410. Preller, K.H., et al., *Role of the 5-HT(2A) Receptor in Self- and Other-Initiated Social Interaction in Lysergic Acid Diethylamide-Induced States: A Pharmacological fMRI Study.* J Neurosci, 2018. **38**(14): p. 3603-3611.

411. Krediet, E., et al., *Reviewing the Potential of Psychedelics for the Treatment of PTSD*. Int J Neuropsychopharmacol, 2020. **23**(6): p. 385-400.

412. Weston, N.M., et al., *Historic psychedelic drug trials and the treatment of anxiety disorders*. Depress Anxiety, 2020.

413. Davis, A.K., et al., *Effects of Psilocybin-Assisted Therapy on Major Depressive Disorder: A Randomized Clinical Trial*. JAMA Psychiatry, 2020.

414. Andersen, K.A.A., et al., *Therapeutic effects of classic serotonergic psychedelics: A systematic review of modern-era clinical studies*. Acta Psychiatr Scand, 2020.

415. DiVito, A.J. and R.F. Leger, *Psychedelics as an emerging novel intervention in the treatment of substance use disorder: a review*. Mol Biol Rep, 2020.

416. Krediet, E., et al., *Reviewing the Potential of Psychedelics for the Treatment of PTSD*. International Journal of Neuropsychopharmacology, 2020. **23**(6): p. 385-400.

417. Doss, M.K., et al., *Psilocybin therapy increases cognitive and neural flexibility in patients with major depressive disorder*. Translational Psychiatry, 2021. **11**(1): p. 574.

418. Yaden, D.B., et al., *Psychedelics and Consciousness: Distinctions, Demarcations, and Opportunities*. International Journal of Neuropsychopharmacology, 2021. **24**(8): p. 615-623.

419. Yaden, D.B. and R.R. Griffiths, *The Subjective Effects of Psychedelics Are Necessary for Their Enduring Therapeutic Effects*. ACS Pharmacology & Translational Science, 2021. **4**(2): p. 568-572.

420. Brunoni, A.R., M. Lopes, and F. Fregni, *A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression*. Int J Neuropsychopharmacol, 2008. **11**(8): p. 1169-80.

421. Price, R.B. and R. Duman, *Neuroplasticity in cognitive and psychological mechanisms of depression: an integrative model*. Mol Psychiatry, 2020. **25**(3): p. 530-543.

422. Boku, S., et al., *Neural basis of major depressive disorder: Beyond monoamine hypothesis*. Psychiatry Clin Neurosci, 2018. **72**(1): p. 3-12.

423. Liu, B., et al., *From Serotonin to Neuroplasticity: Evolvement of Theories for Major Depressive Disorder.* Front Cell Neurosci, 2017. **11**: p. 305.

424. Vanasse, T.J., et al., *Multiple traces and altered signal-to-noise in systems consolidation: Evidence from the 7T fMRI Natural Scenes Dataset.* Proc Natl Acad Sci U S A, 2022. **119**(44): p. e2123426119.

425. Tyls, F., et al., *PM506. Psilocybin Clinical Trial: Acute Effects and its relationship to the brain activity as measured by quantitative EEG.* Int J Neuropsychopharmacol, 2016. **19**(Suppl 1): p. 84.

426. Mason, N.L., et al., *Spontaneous and deliberate creative cognition during and after psilocybin exposure.* Translational Psychiatry, 2021. **11**(1): p. 209.

427. Kurtz, M.M., et al., *The Penn Conditional Exclusion Test: a new measure of executive-function with alternate forms of repeat administration.* Arch Clin Neuropsychol, 2004. **19**(2): p. 191-201.

428. Kaufman, J.C., J.A. Lucker, and J. Baer, *Essentials of Creativity Assessment*. 2008, New York: Wiley.

429. Kuypers, K.P., et al., *Ayahuasca enhances creative divergent thinking while decreasing conventional convergent thinking.* Psychopharmacology (Berl), 2016. **233**(18): p. 3395-403.

430. Gamez, W., et al., *The brief experiential avoidance questionnaire: development and initial validation.* Psychol Assess, 2014. **26**(1): p. 35-45.

431. Gabrys, R.L., et al., *Cognitive Control and Flexibility in the Context of Stress and Depressive Symptoms: The Cognitive Control and Flexibility Questionnaire.* Front Psychol, 2018. **9**: p. 2219.

432. Kashdan, T.B., et al., *Understanding psychological flexibility: A multimethod exploration of pursuing valued goals despite the presence of distress.* Psychol Assess, 2020. **32**(9): p. 829-850.

433. Bao, M., et al., *Perceptual learning increases the strength of the earliest signals in visual cortex.* J Neurosci, 2010. **30**(45): p. 15080-4.

434. Casarotto, S., et al., *The rt-TEP tool: real-time visualization of TMS-Evoked Potentials to maximize cortical activation and minimize artifacts.* J Neurosci Methods, 2022. **370**: p. 109486.

435. Esser, S.K., et al., *A direct demonstration of cortical LTP in humans: a combined TMS/EEG study.* Brain Res Bull, 2006. **69**(1): p. 86-94.

436. Wassermann, E.M., *Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996.* Electroencephalogr Clin Neurophysiol, 1998. **108**(1): p. 1-16.

437. Johnson, M., W. Richards, and R. Griffiths, *Human hallucinogen research: guidelines for safety.* J Psychopharmacol, 2008. **22**(6): p. 603-20.

438. Horvath, A.O. and L.S. Greenberg, *Development and validation of the Working Alliance Inventory.* Journal of Counseling Psychology, 1989. **36**(2): p. 223–233.

439. Baier, A.L., A.C. Kline, and N.C. Feeny, *Therapeutic alliance as a mediator of change: A systematic review and evaluation of research.* Clin Psychol Rev, 2020. **82**: p. 101921.

440. Murphy, R., et al., *Therapeutic Alliance and Rapport Modulate Responses to Psilocybin Assisted Therapy for Depression.* Front Pharmacol, 2021. **12**: p. 788155.

441. Tennant, R., et al., *The Warwick-Edinburgh Mental Well-being Scale (WEMWBS): development and UK validation.* Health Qual Life Outcomes, 2007. **5**: p. 63.

442. Doss, M.K., et al., *MDMA Impairs Both the Encoding and Retrieval of Emotional Recollections.* Neuropsychopharmacology, 2018. **43**(4): p. 791-800.

443. Fleming, S.M. and H.C. Lau, *How to measure metacognition.* Frontiers in Human Neuroscience, 2014. **8**.

444. Horvath, A.O., et al., *Alliance in individual psychotherapy.* Psychotherapy (Chic), 2011. **48**(1): p. 9-16.

445. Rutherford, B.R., et al., *Patient Expectancy as a Mediator of Placebo Effects in Antidepressant Clinical Trials.* Am J Psychiatry, 2017. **174**(2): p. 135-142.

446. Rutherford, B.R. and S.P. Roose, *A model of placebo response in antidepressant clinical trials.* Am J Psychiatry, 2013. **170**(7): p. 723-33.

447. Muthukumaraswamy, S.D., A. Forsyth, and T. Lumley, *Blinding and expectancy confounds in psychedelic randomized controlled trials.* Expert Rev Clin Pharmacol, 2021. **14**(9): p. 1133-1152.

448. Kaertner, L.S., et al., *Positive expectations predict improved mental-health outcomes linked to psychedelic microdosing.* Sci Rep, 2021. **11**(1): p. 1941.

449. Younger, J., et al., *Development of the Stanford Expectations of Treatment Scale (SETS): a tool for measuring patient outcome expectancy in clinical trials.* Clin Trials, 2012. **9**(6): p. 767-76.

450. Bogenschutz, M.P., et al., *Percentage of Heavy Drinking Days Following Psilocybin-Assisted Psychotherapy vs Placebo in the Treatment of Adult Patients With Alcohol Use Disorder: A Randomized Clinical Trial.* JAMA Psychiatry, 2022. **79**(10): p. 953-962.

451. de la Fuente Revenga, M., et al., *Tolerance and Cross-Tolerance among Psychedelic and Nonpsychedelic 5-HT(2A) Receptor Agonists in Mice.* ACS Chem Neurosci, 2022. **13**(16): p. 2436-2448.

452. Jaster, A.M., et al., *Differences across sexes on head-twitch behavior and 5-HT(2A) receptor signaling in C57BL/6J mice.* Neurosci Lett, 2022. **788**: p. 136836.

453. Alper, K., et al., *Psilocybin sex-dependently reduces alcohol consumption in C57BL/6J mice.* Front Pharmacol, 2022. **13**: p. 1074633.

454. Kuo, M.F., W. Paulus, and M.A. Nitsche, *Sex differences in cortical neuroplasticity in humans.* Neuroreport, 2006. **17**(16): p. 1703-7.

455. Swissa, Y., et al., *Sensorimotor performance after high-definition transcranial direct current stimulation over the primary somatosensory or motor cortices in men versus women*. Sci Rep, 2022. **12**(1): p. 11117.

456. Fernández, G., et al., *Menstrual Cycle-Dependent Neural Plasticity in the Adult Human Brain Is Hormone, Task, and Region Specific*. The Journal of Neuroscience, 2003. **23**(9): p. 3790-3795.

457. Sumner, R.L., et al., *Neural plasticity is modified over the human menstrual cycle: Combined insight from sensory evoked potential LTP and repetition suppression*. Neurobiology of Learning and Memory, 2018. **155**: p. 422-434.

458. Arnsten, A.F., *Stress weakens prefrontal networks: molecular insults to higher cognition*. Nat Neurosci, 2015. **18**(10): p. 1376-85.

459. Godsil, B.P., et al., *The hippocampal-prefrontal pathway: the weak link in psychiatric disorders?* Eur Neuropsychopharmacol, 2013. **23**(10): p. 1165-81.

460. Cook, S.C. and C.L. Wellman, *Chronic stress alters dendritic morphology in rat medial prefrontal cortex*. J Neurobiol, 2004. **60**(2): p. 236-48.

461. Radley, J.J., et al., *Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex*. Cereb Cortex, 2006. **16**(3): p. 313-20.

462. Liston, C., et al., *Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting*. J Neurosci, 2006. **26**(30): p. 7870-4.

463. Cohen, Y., E. Schneidman, and R. Paz, *The geometry of neuronal representations during rule learning reveals complementary roles of cingulate cortex and putamen*. Neuron, 2021. **109**(5): p. 839-851 e9.

464. Dajani, D.R., et al., *Measuring Cognitive Flexibility with the Flexible Item Selection Task: From fMRI Adaptation to Individual Connectome Mapping*. J Cogn Neurosci, 2020. **32**(6): p. 1026-1045.

465. Ghahremani, D.G., et al., *Neural components underlying behavioral flexibility in human reversal learning*. Cereb Cortex, 2010. **20**(8): p. 1843-52.

466. Delgado, P.L., *Depression: the case for a monoamine deficiency.* J Clin Psychiatry, 2000. **61 Suppl 6**: p. 7-11.