Pharmacodynamic and Pharmacokinetic Profiling of Psilocybin and Other Serotonergic Psychedelics

By:

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A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Molecular and Cellular Pharmacology)

at the

UNIVERSITY OF WISCONSIN-MADISON

2024

Date of the final oral examination: 06/17/2024

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ACKNOWLEDGEMENTS

Starting a new journey in life is like standing on the edge of an uncharted path, where every step holds the promise of discovery and transformation. It is an invitation to break free from the familiar and embrace the unknown with open arms. This moment, filled with both trepidation and excitement, symbolizes a rebirth of sorts—a chance to redefine oneself and one's aspirations. The beauty of embarking on a new journey lies not only in the destination but in the experiences and lessons gathered along the way. It is through these journeys that we encounter the true essence of life, where every challenge becomes a lesson, every setback a moment of growth, and every success a testament to our resilience. Embracing a new path means trusting in the process, finding joy in uncertainty, and ultimately, allowing the journey to shape us into more profound versions of ourselves.

In this spirit, the journey of completing my PhD in Molecular and Cellular Pharmacology at the University of Wisconsin-Madison embodies this transformative journey. Pursuing a PhD challenges you in countless ways, pushing you to your limits while fostering immense personal growth and self-discovery. This academic endeavor was not just a pursuit of scientific knowledge, but a profound voyage that embarked on a journey of self-discovery. Each experiment and late-night study session tests your resilience, determination, and intellect. My journey through the complexities of pharmacology and neuroscience mirrored my own self-discovery, revealing the depths of my character and abilities. This journey shaped me into a more insightful, innovative, and confident scientist and person. Through the trials and triumphs of this rigorous path, I discovered not only the mysteries of science but also my true potential and identity.

This journey would not be possible without the unwavering support and guidance of an incredible network of mentors, colleagues, friends, and family. Dr. Cody Wenthur's mentorship was instrumental in guiding me to become the scientist I am today. At the beginning of my journey,

I spent countless hours in the vivarium, trying to handle the unruly mice. I often found myself frustrated, avoiding their bites and struggling with the delicate task of coloring their tails before administering injections. When I was not yet licensed to inject psilocybin, Cody would come down to help, effortlessly picking up the mice to inject them. His ease and skill inspired me to spend even more time in the vivarium, determined to achieve that same level of proficiency. Cody's exceptional abilities extended beyond working with mice. He had a remarkable talent for making complex concepts in pharmacology, pharmacokinetics, organic chemistry, and other subjects clear and understandable. He would walk over to the whiteboard and explain these topics so effectively that other lab members and I would wonder how these concepts ever seemed complicated in the first place.

Cody's mentorship extended beyond just explaining complex concepts and greatly influenced my approach to experimental design. Initially, he provided detailed structure and guidance on experimental parameters and design. Gradually, he transitioned to allowing me to conceptualize and design the experiments myself, fostering my independence and confidence as a researcher. In addition, we often spent hours in his office discussing my "quick questions" about the papers I had read. These sessions, which usually started with a single query, would lead us down fascinating rabbit holes, leaving me with new ideas and directions for my research. Cody's willingness to engage in these deep, exploratory discussions shaped my scientific thinking and approach. Moreover, Cody provided invaluable mentorship and support throughout my PhD.

In addition, Jill Kyzer was an exceptional mentor and a good friend throughout my PhD journey. During the perils of the COVID pandemic, Jill and I were assigned the morning shift in the lab to do our research. Even though I spent most of my time in the vivarium, it was knowing that I would get to talk with her every day that helped motivate me, and as time went on, greatly looked forward to talking with her every day. It never ceased to amaze me the number of stories she had, from going to "smart school" as a kid to canoeing/kayaking into class at the College of

Charleston or wearing her "lucky" rock concert clown jacket during each organic chemistry exam, that terrified the student sitting behind her. During COVID, Jill's life and stories took a delightful turn with the addition of the sweetest, most innocent dog, Poppy. Every day brought new adventures of Poppy often accompanied by photos, adding joy to my day and our lab. In addition to the stories told, Jill also contributed to many stories of my own, from helping me plan dates, and trips with my friends, being an absolute boss at bar trivia, providing new podcasts, always listening, and providing support, even at times when I wouldn't ask for help, but moreover for being a good friend. She always provided insightful and informative critiques of my presentations, posters, and dissertation. Although she often thought she was being too harsh, her feedback was invaluable in improving my ability to present effectively and create impactful posters. Her caring nature and ability to tell me what I needed to hear, rather than what I wanted to hear truly influenced who I am today as a person and a scientist.

Adam Worob, as the senior graduate student, played a significant role in my development as a researcher. He mentored me in various essential skills, including handling mice, mixing drugs, critically analyzing research papers, and formulating precise research questions. Our spontaneous conversations, which often lasted for hours, were a source of immense learning and friendship. We would pick up these conversations every few weeks, each time diving deep into topics that expanded my understanding and perspective. Adam was more than just a mentor; he was a good friend. During a particularly difficult period when I was in a deep state of depression, Adam understood what I was going through better than most. He recognized my unspoken pain and encouraged me to start talking about it. His willingness to listen and share insights from his life made a significant difference in my journey toward healing. Beyond his supportive nature, Adam was a brilliant and talented graduate student. His guidance and friendship were invaluable, both in my academic pursuits and in navigating life's challenges.

Joining the lab at the same time as Zarmeen Zahid was an absolute honor. As the first two students focused on psychedelics and neuroscience, we spent countless hours reading papers, presenting weekly at psychedelic group meetings, and establishing new protocols for behavioral analysis and electrophysiology. Together, we established the foundation for our joint labs to produce innovative research findings that challenged the current literature. Zarmeen took on the tedious and challenging task of electrophysiology recording, measuring changes in brain waves and neuroplasticity. Her resilience in the face of obstacles was truly inspiring, as she continually sought new methods and optimized data measurements. All the while, she never gave up, even when faced with setbacks and challenges that would have discouraged many. Zarmeen's unwavering dedication to perfecting the electrophysiology recordings, despite the tedious and often frustrating nature of the work, demonstrated her exceptional commitment to our research. She consistently showed a remarkable ability to think creatively and adapt to new situations, finding innovative solutions to complex problems. This relentless pursuit of excellence not only advanced our joint research but also set a high standard for everyone in the lab. Zarmeen's perseverance and determination were a constant source of motivation for me and others, pushing us to strive for the same level of persistence and innovation in our own work. Her ability to maintain such high spirits and enthusiasm, even during the most challenging times, is a testament to her passion for science and her belief in the potential of our research to make a meaningful impact in developing psychedelic treatments for suicidal ideations and depression.

John Razidlo was one of the first neuroscience and biology-focused graduate students to join the lab amidst a group of chemists. It was wonderful to have another student to talk with about biology, pharmacology, and behavioral neuroscience. His critical thinking skills were invaluable as we navigated the complexities of stress and psychedelics research papers together. John's resilience and determination are truly admirable and something to behold as he progresses through graduate school. Despite the numerous challenges and intense workload of graduate

studies, he has consistently demonstrated an unwavering commitment to his studies and research. His ability to maintain focus and drive, even in the face of setbacks, serves as a powerful inspiration to those around him. John's work ethic is reflected in the quality and depth of his research. He approaches every project with a level of diligence and thoroughness that sets him apart. Whether he's delving into complex scientific literature, meticulously designing experiments, or analyzing data, John does so with a level of precision and dedication that is truly remarkable. His resilience is not just about persistence; it's about his willingness to go the extra mile, to explore new methodologies, and to push the boundaries of our understanding in the field of neuroscience. Moreover, John's determination extends beyond his academic pursuits. He is always ready to lend a helping hand to his peers, offering support and sharing his knowledge generously. His collaborative spirit and positive attitude create an encouraging environment for everyone in the lab. This combination of personal resilience, academic excellence, and supportive nature makes John not only a standout graduate student but also a role model and a source of motivation for his colleagues.

John also became a good friend and travel buddy for all our conference trips, including those to San Diego and Washington, D.C., for the Society for Neuroscience conference. These trips were filled with unforgettable memories, from crashing a famous beach and visiting as many Smithsonian museums as possible in two days to exploring the San Diego Zoo. A cherished tradition emerged from these trips: getting Pad Thai together. Additionally, John introduced me to Warhammer's fantastic (and expensive) world, further enriching our friendship.

Matthew Enriquez brought a new and vibrant energy to the lab, significantly enriching our research environment. Since joining the lab, he has grown immensely as both a scientist and a person. Witnessing his progress has been a true joy as he forges into unexplored territories of synthetic chemistry, pushing the boundaries of our collective knowledge. Beyond the lab, it was an honor to introduce Matt to fishing, even though he initially thought it mainly involved drinking

beer on the shoreline. Additionally, we bonded over our shared passion for music, frequently attending concerts together, and our mutual love for anime, which provided countless hours of enjoyment and discussion. Matt's enthusiasm and growth have been a source of inspiration, and his presence has made a lasting impact on both our research and our personal lives. His journey in the lab exemplifies the fusion of scientific exploration and personal camaraderie, making our collective experience richer and more fulfilling.

It was a pleasure to mentor and work with Laura Wagner. I could not have asked for a more passionate, intelligent, and outstanding undergraduate student to guide. Her enthusiasm for learning and dedication to her work made the mentorship experience incredibly rewarding. Laura's commitment to our research projects and her keen intellect contributed to our publication's success. Beyond the lab, I enjoyed introducing Laura to the Renaissance fair and taking her thrift shopping to find the perfect outfit for the event. These shared experiences outside of our academic pursuits helped to strengthen our mentor-mentee relationship and allowed us to connect on a more personal level. Laura's curiosity and adventurous spirit made these activities even more enjoyable, and they are memories I will always cherish.

Dylan Sebo, a graduate student in another lab, has been a great friend whose support and insights have been invaluable. From the beginning, Dylan's friendship and encouragement have provided a steady source of motivation. Despite being in different labs, our shared passion for science and mutual interests fostered a strong bond. His perspectives, often from a different angle, enriched my understanding and approach to research. Dylan's support went beyond academic discussions. He was always there to lend a listening ear during challenging times, offering thoughtful advice and empathy. His ability to balance rigorous research demands with a genuine concern for my well-being made a significant difference in my graduate school experience. Whether we were discussing complex scientific theories, sharing the latest updates in our respective projects, or simply hanging out, Dylan's friendship was a cornerstone of my

support network. His contributions, both as a friend and a fellow scientist, have been instrumental in my journey through graduate school.

Rhiann Sato, a pharmacy student who helped in the lab, played a role in my PhD journey. Her unique perspective and dedication significantly contributed to our research efforts. Your constant encouragement and unwavering support helped me push through the most challenging times. Your insights and advice were invaluable, often providing the clarity and perspective I needed to tackle complex problems. Beyond academics, your friendship has been a source of immense strength and comfort. Our shared laughs, tears, discussions about our aspirations, and the venting sessions provided a much-needed reprieve from the stresses of graduate school. It's meant far more to me than you know and has greatly helped me. Knowing that I could rely on you, whether for academic advice or personal support, made a world of difference. Your resilience and positivity were infectious, often lifting my spirits when I needed it most. Thank you for being an incredible friend and for making this journey not only bearable but truly memorable.

Dr. Cameron Scarlett and Molly Pellitteri Hahn have been exceptional mentors, providing invaluable assistance with LC-MS/MS and pharmacokinetic (PK) studies. Their expertise has been instrumental in the success of my research, guiding me through complex methodologies and ensuring reliable results. Dr. Scarlett's deep knowledge of mass spectrometry and ability to explain intricate concepts clearly have greatly enhanced my understanding and improved my experimental design. His feedback consistently pushed me to think critically and refine my work. Molly Pellitteri Hahn's hands-on mentorship and practical insights into PK studies have provided a solid foundation for my research. Her attention to detail and skill in optimizing protocols ensured rigorous and reproducible studies. Her constructive feedback and extensive experience were invaluable, especially during challenging phases. Together, Dr. Scarlett and Molly have created a supportive learning environment that advanced my technical skills and deepened my

appreciation for meticulous research. Their contributions have been pivotal in shaping my PhD journey, and I am deeply grateful for their unwavering support.

In addition to my academic and professional network, my family's love, encouragement, and belief in me provided the support needed to get through this challenging journey. My father, Keith, and my mother, Becky, have always believed in me, offering love and encouragement. Although they didn't fully understand my goals and research aspirations at first, over time, they came to appreciate and support them wholeheartedly. Their support has been a foundation throughout my academic journey, despite them not always understanding why I had to make the decisions I did. Whether celebrating my successes or providing comfort during challenging times, their presence has been a source of strength and motivation. My younger sister, Jamie, has been a source of joy and inspiration. Her lively spirit and interest in my work have reminded me to stay grounded and focused. My grandparents, Grandma Marj, and Grandpa Jim, have been steadfast in their support. Their wisdom and warmth have guided me through the most challenging times, offering both practical advice and emotional comfort. Their unwavering belief in my potential has been a beacon of hope and resilience.

Dr. Cody Wenthur's mentorship, wisdom, and guidance, has been a cornerstone of my development. Jill Kyzer's unwavering support and friendship provided much-needed motivation during the most challenging times. Adam Worob, with his insight and empathy, John Razidlo's resilience and friendship, Rhiann Sato's steadfast friendship, Dylan Sebo's invaluable insights, and Matthew Enriquez's vibrant energy have all contributed uniquely to this journey, making each step memorable and enriching.

Dr. Cameron Scarlett and Molly Pellitteri Hahn's assistance with LC-MS/MS and PK studies has been instrumental, offering the technical expertise and support crucial to my research. Laura Wagner's enthusiasm and dedication as an undergraduate mentee added a layer of fulfillment to my experience. Beyond the lab, the support of my family—my father Keith, my mother

Becky, my sister Jamie, and my grandparents, Marj and Jim—provided the love and support necessary to navigate the rigors of graduate school.

This journey would not have been possible without the collective support of these remarkable individuals. Their belief in me, their guidance, and their friendship have made this path not only possible but profoundly meaningful. Each challenge faced, and each milestone reached has been a testament to their unwavering support and the collaborative spirit that has defined my PhD experience. As I look back on this journey, I am deeply grateful for the resilience, wisdom, and love that have surrounded me, shaping my path and propelling me forward into the next journey that awaits.

ABSTRACT

While correlations between drug-induced cortisol elevation, self-reported anxiety, and treatment outcomes have been reported for human studies during psilocybin-assisted psychotherapy, the mechanistic relationship between psychedelic-associated alterations in plasma glucocorticoid responses and the time course of anxious responsiveness remains unclear. Using rodents, both time-bound manipulation of glucocorticoid concentrations and assessment of anxiety-like behaviors can be achieved. Here, 3 mg/kg IP psilocybin was found to have anxiolytic-like effects in C57BL/6 male mice at 4 h after treatment. These effects were not altered by pretreatment with a 5-HT_{2A} A antagonist but were blunted by pretreatment with a glucocorticoid receptor antagonist or suppression of psilocybin-induced corticosterone elevations. Anxiolytic-like effects were also observed at 4 h following treatment with the nonpsychedelic 5-HT_{2A} agonist lisuride at a dose causing a similar increase in plasma glucocorticoids as that seen with psilocybin, as well as following stress-induced (via repeated injection) glucocorticoid release alone. Psilocybin's anxiolytic-like effects persisted at 7 days following administration. The long-term anxiolytic effects of psilocybin were lost when psilocybin was administered to animals with ongoing chronic elevations in plasma corticosterone concentrations. Overall, these experiments indicate that acute, resolvable psilocybin-induced glucocorticoid release drives the post-acute anxiolytic-like effects of psilocybin in mice and that its long-term anxiolytic-like effects can be abolished in the presence of chronically elevated plasma glucocorticoid elevations.

In light of these findings, it is crucial to consider the pharmacokinetic properties of psilocybin and its derivatives. Psilocybin is a Schedule I substance that is dephosphorylated *in vivo* to form an active metabolite, psilocin. Psilacetin, also known as O-acetyl psilocin or 4-acetoxy-N, N-dimethyltryptamine (4-AcO-DMT), is an unscheduled compound that has long been suggested as an alternative psilocin prodrug. However, direct *in vivo* support for this hypothesis

has thus far been lacking. This study employed liquid chromatography-tandem mass spectrometry (LC-MS/MS) to assess the time-course and plasma concentrations of psilocin following the intraperitoneal (IP) administration of psilacetin fumarate or psilocybin to male and female C57Bl6/J mice. Direct comparisons of the time courses for psilocin exposure arising from psilocybin and psilacetin found that psilocybin led to 10-25% higher psilocin concentrations than psilacetin at 15-min post-injection. The half-life of psilocin remained approximately 30 min, irrespective of whether it came from psilocybin or psilacetin. Overall, the relative amount of psilocin exposure from psilacetin fumarate was found to be approximately 70% of that from psilocybin. These findings provide the first direct support for the long-standing assumption in the field that psilacetin functions as a prodrug for psilocin *in vivo*. In addition, these results indicate that psilacetin fumarate results in lower peripheral psilocin exposure than psilocybin when dosed on an equimolar basis. Thoughtful substitution of psilocybin with psilacetin fumarate appears to be a viable approach for conducting mechanistic psychedelic research in C57Bl6/J mice.

Beyond these specific findings, there is considerable evidence from the literature that psychedelics, such as N, and N-dimethyltryptamine (DMT), are safe and effective treatments for depression. However, clinical administration to induce psychedelic effects and expensive psychotherapy-assisted treatments likely limit accessibility to the average patient. There is emerging evidence that DMT promotes positive behavioral changes *in vivo* at sub-hallucinogenic dosages, and depending on the target indication, subjecting patients to high, bolus dosages may not be necessary. Due to rapid metabolic degradation, achieving target levels of DMT in subjects is difficult, requiring IV administration, which poses risks to patients during the intense hallucinogenic and subjective drug effects. The chemical and physical properties of DMT make it an excellent candidate for non-invasive, transdermal delivery platforms. This paper outlines the formulation development, *in vitro*, and *in vivo* testing of transdermal drug-in-adhesive DMT patches using various adhesives and permeation enhancers. *In vivo* behavioral and

pharmacokinetic studies were performed with lead patch formulation (F5) in male and female Swiss Webster mice. The resulting DMT levels in plasma and brain samples were quantified using LC/MS/MS. Notable differences were seen in female versus male mice during IV administration; however, transdermal administration provided consistent, extended drug release at a non-hallucinogenic dose. The IV half-life of DMT was extended by 20-fold with administration of the transdermal delivery system at sub-hallucinogenic plasma concentrations not exceeding 60 ng/mL. Results of a translational head twitch assay (a surrogate for hallucinogenic effects in non-human organisms) were consistent with the absence of hallucinations at low plasma levels achieved with our TDDS. Despite the reported low bioavailability of DMT, the non-invasive transdermal DMT patch F5 afforded an impressive 77 % bioavailability compared to IV at two dosages. This unique transdermal delivery option has the potential to provide an out-patient treatment option for ailments not requiring higher bolus doses. It is especially intriguing for therapeutic indications requiring non-hallucinogenic alternatives.

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CHAPTER 1: INTRODUCTION

1.1 Psychiatric Disorders and Antidepressant Use in the United States

Psychiatric disorders have become an increasingly prevalent concern, afflicting 47.6 million people in the United States (1,2). Among these, major depressive disorder (MDD) affects 17.3 million American adults, accounting for 7.1% of the total US adult population each year (3,4). **Figure 1** illustrates the prevalence of major depressive episodes among U.S. adults aged 18 or older in 2021. In 2021, an estimated 21.0 million adults in the United States experienced at least one major depressive episode, representing 8.3% of the total U.S. adult population (3,4). The prevalence data presented here for major depressive episodes are from the 2021 National Survey on Drug Use and Health (NSDUH). To understand the scale and impact of depression, it's essential to look at how major depressive episodes are defined. The NSDUH study definition of a major depressive episode is based mainly on the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5): “a period of at least two weeks when a person experienced a depressed mood or loss of interest or pleasure in daily activities, and had a majority of specified symptoms, such as problems with sleep, eating, energy, concentration, or self-worth” (3,4). Given the high prevalence of depression, it is not surprising that more than 32 million individuals aged 12 and older take antidepressants daily, and one in ten Americans over 18 are on antidepressants (5). Consequently, finding alternative and more effective treatment options for MDD have become a significant concern, as first-line therapeutics fail to treat two-thirds of patients effectively (5–8). These alarming statistics underscore the limitations of current antidepressant medications and highlights the need for improved treatment strategies and the evaluation of current therapeutic approaches.

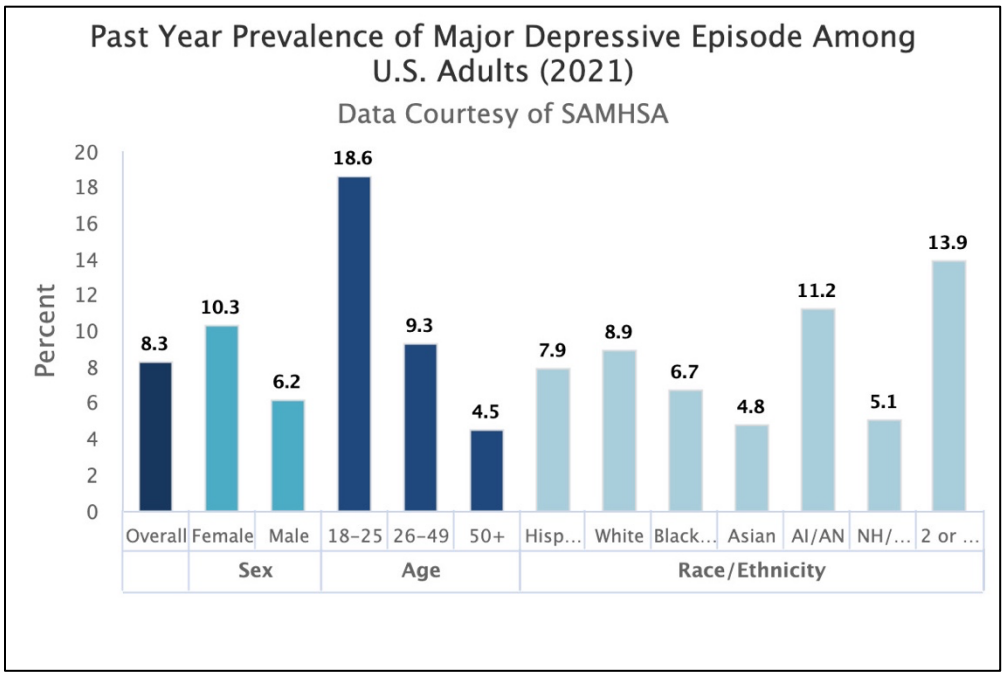

Figure 1: Past Year Prevalence of Major Depressive Episodes Among U.S. Adults (2021). This figure illustrates the prevalence of major depressive episodes among U.S. adults aged 18 or older in 2021, categorized by sex, age, and race/ethnicity. The data courtesy of the Substance Abuse and Mental Health Services Administration (SAMHSA) and the figure taken from the NMIH website on the prevalence of depression.

To better understand and address these treatment challenges, *The Sequenced Treatment Alternatives to Relieve Depression (STAR*D)* study, a large-scale clinical trial funded by the National Institutes of Health, aimed to address the effectiveness of antidepressant treatments (6). This study is one of the most comprehensive investigations into MDD treatments, enrolling 4,041 participants across various treatment settings. Remarkably, the study revealed that only 25% of patients demonstrated a clinical response when taking antidepressants in conjunction with therapy over a one-year period. Only about 30% of participants achieved remission after the first treatment step. With subsequent steps, the cumulative remission rate increased to approximately 67%, indicating that multiple treatment attempts are often necessary (6,7). Furthermore, these findings emphasize the necessity for continued research and development of more effective treatment approaches. They also illustrate the importance of personalized and adaptive treatment

plans, acknowledging that multiple treatment attempts may be necessary to achieve remission. The STAR*D study's multi-step process, involving various medication options and therapeutic interventions, provides a valuable framework for understanding and improving the management of MDD. The study underscores the complexity and challenges of treating MDD. In conclusion, despite being one of the most comprehensive investigations into MDD treatment, it revealed that a significant portion of patients do not achieve remission with initial antidepressant therapy.

Building on these findings, recent studies and meta-analyses have provided updated insights into the efficacy of selective serotonin reuptake inhibitors (SSRIs) and other antidepressant medications. Recent data suggest that the response rate to antidepressants is around 50-60%, with remission rates varying between 30-50% depending on the population and specific medication as seen in **Table 1** (8–10). SSRIs, such as fluoxetine and sertraline, remain among the most commonly prescribed antidepressants. However, not all patients respond adequately to these traditional medications, highlighting the need for alternative treatment options. In response to this challenge, the introduction of novel antidepressants like Vortioxetine© and Esketamine© has provided alternative options and additional avenues for patients who do not benefit from SSRIs (10,11). These medications have shown promise in clinical trials, with response rates comparable to or slightly higher than older medications.

Efficacy of Various Antidepressant Medications			
Medication	**Response Rate**	**Remission Rate**	**Notes**
SSRIs (e.g., fluoxetine, sertraline)	50-60%	30-40%	Widely used, generally well-tolerated
SNRIs (e.g., venlafaxine, duloxetine)	55-65%	35-45%	Effective for both depression and anxiety
Bupropion	50-60%	30-40%	Favorable side effect profile
Vortioxetine	55-65%	35-45%	Cognitive benefits in addition to mood
Esketamine	50-70%	40-50%	Rapid-acting, used for treatment-resistant depression

Table 1: Efficacy of Antidepressant Medications. Presents the efficacy of various antidepressant medications, comparing response and remission rates based on recent studies and meta-analyses. Recent Efficacy of SSRIs and Antidepressants**:** Cipriani *et al.,* (2018) in The Lancet. Newer Antidepressant Medications: Thase *et al.,* (2016) from ScienceDirect and Daly *et al.,* (2019), from JAMA Psychiatry.

The STAR*D study and recent statistics underscore the complexity of treating MDD and the inherent variability in patient response to antidepressant medications. While SSRIs and newer medications offer valuable treatment options, the high rates of treatment resistance and partial responses highlight the need for continued research and the development of more effective therapeutic strategies. Given that traditional antidepressants fail to achieve effective outcomes for approximately two-thirds of patients, there has been a growing interest in exploring new therapeutic options. In this context, there has been a growing interest in exploring alternative treatments. This search has led to the resurgence of psychedelic substances, which, despite long-standing stigmatization and legal restrictions, have re-emerged as promising candidates for psychiatric therapy.

1.2 Psychedelic-Assisted Therapy and It's Promising Therapeutic Potential

Psychedelic substances, long stigmatized and restricted, have re-emerged as promising candidates for psychiatric therapy. Compounds, such as psilocybin from '*Psilocybe'* containing mushrooms and N, N-Dimethyltryptamine (DMT) brewed from the Banisteriopsis caapi vine, have a rich history of use spanning centuries in various traditional cultures (12–15). Indigenous peoples

in regions like the Amazon, Mexico, and North America have long harnessed the profound effects these substances can have on consciousness, emotional well-being, and communal bonding. Historically, shamans or healers in these cultural contexts employed psychedelics to facilitate visionary experiences, promote psychological healing, and strengthen connections with the spiritual world (16–18). These compounds were often integral to ceremonies and rituals, serving as personal and communal transformation tools. In the Amazon, ayahuasca—a potent brew made from the *Banisteriopsis caapi* vine and other plants—containing DMT has been used by indigenous tribes for centuries. Shamans would lead ayahuasca ceremonies, guiding participants through intense, often transformative journeys aimed at healing emotional wounds, gaining spiritual insights, and connecting with the natural and spiritual realms (14,15,17). Similarly, in Mexico, the Mazatec people have long incorporated psilocybin mushrooms into their religious ceremonies, believing that these fungi opened channels to the divine and provided profound personal revelations (18–20). These cultural practices underscore the significance of psilocybin, which is predominantly found in mushrooms belonging to the genus *Psilocybe*, such as *Psilocybe cubensis* and *Psilocybe semilanceata* (12,21).

In recent decades, there has been a renewed interest in these ancient medicinal plants, driven by promising research into their therapeutic potential (22–28). This reemergence in modern medicine, known as psychedelic-assisted therapy, involves the careful administration of these substances under controlled, supportive conditions guided by trained professionals. The therapy typically involves preparation sessions to set intentions and build trust, the psychedelic sessions themselves, where the patient is supported in navigating their experience, and integration sessions to help make sense of the insights gained and apply them to everyday life (22,29–33). Clinical studies have demonstrated that psychedelic-assisted therapy can produce significant and lasting improvements in mental health conditions such as depression (34–37), anxiety(35,38–40), post-traumatic stress disorder (PTSD) (41–43), and substance use disorders (44–46). For

example, clinical trials with psilocybin have demonstrated remarkable success in reducing symptoms of treatment-resistant depression. The benefits have been observed to last from one month to a year after just a few therapeutic sessions (34,47). Similarly, MDMA-assisted therapy has been highly effective in treating PTSD, with many participants experiencing profound relief from their symptoms after just a few sessions (41–43). This innovative therapeutic approach combines the therapeutic potential of psychedelics with contemporary clinical practices, offering new options for treating psychiatric conditions.

Psychedelic-assisted therapy bridges the gap between traditional healing practices and modern medicine, offering a new and promising approach to mental health treatment. This therapy leverages the potential of psychedelic substances to alleviate symptoms and promote profound personal growth and transformation. As research progresses, the potential of psychedelic-assisted therapy to revolutionize psychiatric treatment options and mental health care is becoming increasingly evident. This innovative approach provides hope for individuals suffering from MDD and other psychological conditions, underscoring its promise in transforming treatment options. The potential for this transformation has roots in the mid-20th century, a period marked by a significant shift in the perception and understanding of psychedelic substances. This shift brought psychedelics from obscurity to the forefront of cultural and scientific exploration, laying the groundwork for their current resurgence in therapeutic contexts.

1.3 The Birth of Psychedelic Science: Hofmann, Huxley, and Osmond's Legacy

The mid-20th century underwent a significant shift in the perception and understanding of psychedelic substances from obscurity to the forefront of cultural and scientific exploration. Albert Hofmann's serendipitous discovery of the effects of *‘lysergic acid diethylamide’* (LSD) can be considered the catalyst for this paradigm shift. Hofmann, a Swiss chemist, first synthesized LSD in 1938 while researching lysergic acid derivatives at Sandoz Laboratories. Hofmann’s synthesis

was initially focused on developing a stimulant for the respiratory and circulatory systems (analeptic) without affecting the uterus by incorporating this functional group into lysergic acid (48,49). However, it was not until five years later, on April 19, 1943—a day now celebrated as "Bicycle Day"—that Hofmann ingested a relatively small amount of LSD and experienced its profound psychoactive effects (48). This accidental ingestion of LSD led to the serendipitous finding of this compound's profound mind-altering effects. As a result, this led to the scientific inquiry into the psychological and potential therapeutic effects of LSD and other psychedelic compounds. In addition, Hofmann's contributions to psychedelic research extended beyond LSD. Fifteen years later, in 1958, Hofmann was the first to synthesize psilocybin, the active compound in *Psilocybe* mushrooms, after isolating it from its natural form (50,51). This synthesis marked a significant milestone in psychedelic research, as it allowed for more controlled and systematic studies of psilocybin's effects.

Building on the foundational work of Hofmann, Aldous Huxley, and Humphry Osmond brought psychedelics to the attention of the public. Huxley documented his experiences with mescaline in the highly influential and controversial book *"The Doors of Perception"* (52). When the book was initially released, psychoactive substances like mescaline were primarily associated with delinquency and unconventional behavior. However, Huxley's positive portrayal of mescaline as a means to achieve higher states of consciousness challenged these societal norms and conventions. Huxley's suggestions that psychedelics could offer profound insights and enhance human understanding directly opposed the dominant view that such substances were merely recreational drugs that caused harm, with no medicinal purpose (52–54). His vivid and compelling descriptions painted a picture of psychedelics as tools for expanding the mind and exploring the depths of human consciousness.

Inspired by such perspectives, Humphry Osmond, a British psychiatrist, played a crucial role in the development of psychedelic research (55). Moreover, his clinical investigations into the

therapeutic potential of psychedelics were groundbreaking. It was Osmond who coined the term *"psychedelic,"* derived from the Greek words for *"mind-revealing,"* to describe the mind-expanding properties of these substances he observed in his clinical work. He believed that psychedelics, such as LSD and mescaline, could be used to treat various mental health conditions, including both alcoholism and schizophrenia (56,57). Osmond's research began in the 1950s when he conducted experiments with LSD and mescaline to explore their effects on the human psyche. He hypothesized that these substances could induce a state similar to hallucinations, altered stated of perception, and psychosis found with schizophrenia, allowing psychiatrists to understand these positive symptoms better and treat the condition (56). In addition, Osmond's studies showed promising results, particularly in the treatment of alcoholism. In one notable study, Osmond and his colleagues administered LSD to chronic alcoholics, resulting in a significant number of patients achieving sobriety after their psychedelic experience (55,57).

Furthermore, Osmond's collaboration with Aldous Huxley was instrumental in bringing psychedelics to public attention. It was Osmond who first introduced Huxley to mescaline in 1953, leading to Huxley's profound experiences documented in *"The Doors of Perception."* (55)This collaboration between a psychiatrist and a literary figure helped bridge the gap between clinical research and public understanding. Osmond's clinical investigations and Huxley's literary and ethnographic descriptions were major catalysts in initiating scientific and therapeutic discourse around psychedelics (54,55). Their work also influenced the emerging counterculture movement of the 1960s, which embraced these compounds as a means of personal and societal transformation (32,58). However, the association of these compounds with radical and non-conformist movements added to the controversy surrounding Huxley's and Osmond's research. Despite this, their contributions remain pivotal points in the history of psychedelic research and public perception, continuing to provoke discussion and debate to this day.

1.4 The Harvard Psilocybin Project and its Influence on Psychedelic Science

The *'Harvard Psilocybin Project,'* spearheaded by Timothy Leary and Richard Alpert (later known as Ram Dass), began in the early 1960s. The project's aim and focus was in exploring the potential therapeutic benefits of psilocybin, particularly its ability to alter perception, enhance creativity, and provide profound spiritual insights (58–61). Based on early reports and data collected from their research, participants reported that psilocybin induced intense subjective, mystical experiences, which increased overall mood and well-being. These findings led them to the overall hypothesis that psilocybin serves as a key to unlocking a new therapeutic avenue and might be a powerful tool for psychological healing and personal growth (59,60,62). However, unlike Osmond, they did not follow a strict code of research ethics and rigor in their efforts.

One of the most famous experiments conducted by the Harvard Psilocybin Project was the Concord Prison Experiment. This study, led by Timothy Leary and his team, occurred between 1961 and 1963 at the Concord State Prison in Massachusetts (60). The primary objective was to investigate whether psilocybin could reduce recidivism, relapsing back into crime rates among inmates, by providing introspective and transformative life-changing experiences. Leary and his colleagues hypothesized that the profound psychological and introspective insights induced by psilocybin would lead to significant behavioral changes, fostering personal growth and rehabilitation. They believed that by addressing the deeper psychological issues underlying criminal behavior, psilocybin could help inmates reintegrate into society more successfully. The experiment involved 32 prisoners, all of whom volunteered to participate. These participants were divided into two groups: one that received psilocybin and another that served as a control group. Once split into groups, the psilocybin sessions were conducted in a controlled and supportive environment, often described as a therapeutic setting. During these sessions, inmates were encouraged to enter deep states of introspection and explore their thoughts and emotions (60,63).

The initial results of the Concord Prison Experiment were promising. Many participants reported profound insights and a renewed sense of purpose, experiencing intense emotional and spiritual revelations that they felt had a lasting impact on their attitudes and behaviors. Some inmates gained clarity about their past actions and committed to positive changes in their lives. Moreover, follow-up studies suggested that inmates who underwent psilocybin sessions had lower recidivism rates compared to the control group. Leary and his team were optimistic about psilocybin's potential as a rehabilitation tool, believing these early findings supported their hypothesis that psychedelics could reduce criminal behavior and aid in reintegration (60,63). Nevertheless, the experiment faced challenges and controversies. One of the most controversial aspects was the unconventional use of psychedelics in a prison setting. This practice drew significant criticism, raising ethical concerns about the consent and well-being of the participants. Additionally, critics questioned the scientific rigor of the study, pointing to potential biases and methodological flaws (63–65).

Drawing from the prison study and transitioning to the broader implications of their work, the ethical concerns surrounding the psilocybin studies led by Leary and Alpert were multifaceted. These concerns reflected the era's less stringent informed consent standards and the pioneering nature of psychedelic research. Participants, which partially consisted of students of Leary and Alpert, may not have fully comprehended the studies' experimental nature or associated risks, partly due to skewed power dynamics between them and the professors. This issue was exacerbated by uncertainties about the long-term effects of psilocybin, with potential risks not yet fully understood. The most prominent is that psilocybin, along with other psychedelics, can trigger latent predispositions to psychiatric illnesses such as psychosis or schizophrenia (66–68). These fears of potential long-term negative impacts and Leary and Alpert’s narrative-focused approach, emphasizing subjective experiences over empirical data, distanced their work from mainstream scientific methods. In addition, this approach challenged the reproducibility and scientific value of

their findings.

Compounding these issues, their decision to partake in some of the psilocybin studies alongside the patients blurred the lines between observer and subject, introducing bias and undermining objectivity. The lack of control groups and clear protocols for drug administration further highlighted methodological shortcomings, complicating efforts to attribute observed effects directly to psilocybin (64,65). The individual nature of psychedelic experiences also complicated the reproducibility and verification of findings by other researchers, hindering broader scientific acceptance. Adding to these complexities, Leary and Alpert's experimental design, methods, and personal use of psychedelics were closely aligned with the countercultural movement of the 1960s. This alignment contrasted sharply with mainstream societal norms and scientific practices, further contributing to the controversy and skepticism surrounding their work.

Their emphasis on personal experience and spiritual growth influenced the counterculture, distancing them from the scientific community. The ethical and methodological issues ultimately led to Leary and Alpert's dismissal from Harvard University in 1963 (63,69,70). The controversies surrounding the Harvard Psilocybin Project finally prompted tighter regulations regarding human subjects' research. Shortly after these influential studies, in July 1974, Institutional Review Boards (IRBs) became crucial in safeguarding human subjects, mandating informed consent, voluntary participation, and minimizing harm (71). The public outcry and reaction, combined with the overall association of psychedelic compounds with the counter-cultural movement, significantly influenced the legal stance on psilocybin. This ultimately led to its classification as a controlled substance. Psilocybin was listed as a Schedule I drug under the United Nations 1971 Convention on Psychotropic Substances, specifying they are considered to have a high potential for abuse and no recognized medical potential (72). This cultural stigmatization and scheduling of psilocybin halted psychedelic research in the Unites States for almost thirty years, further conflating these substances with recreational drug use and undermining their potential therapeutic applications

(25,26,73). Although Leary and Alpert's research provided valuable insights into the potential benefits of psychedelics, the project's legacy also highlights the crucial importance of ethical and methodological rigor in scientific research.

In recent years, however, there has been a renewed interest and significant research into the therapeutic potential of psilocybin and psychedelic compounds. With modern advancements in neuroscience and psychopharmacology, researchers are now uncovering and assembling the pharmacological properties of action of psilocybin. These insights into the underlying mechanism of action and cellular activation patterns, are shedding light on how psilocybin can be used to treat various psychiatric disorders.

1.5 Psilocybin: Neuropharmacology, Pharmacokinetics, and Dosing

Psilocybin, a naturally occurring psychedelic compound found in certain species of mushrooms, has garnered significant scientific interest due to its profound effects on consciousness, potential therapeutic benefits, and relatively favorable safety profile. Psilocybin is predominantly found in mushrooms belonging to the genus *Psilocybe*, such as *Psilocybe cubensis* and *Psilocybe semilanceata* (12,21). Psilocybin (4-phosphoryloxy-N, N-dimethyltryptamine) is a prodrug that is converted into its active form, psilocin (4-hydroxy-N, N-dimethyltryptamine), after ingestion. Both psilocybin and psilocin share a structural similarity with serotonin (5-hydroxytryptamine, 5-HT), which underlies their psychoactive effects.

Given its significant psychoactive properties and structural similarities to serotonin, researchers have sought to understand the precise mechanisms through which psilocybin carries out its neuropharmacological and psychological effects. In a groundbreaking study conducted in 1999, Swiss researchers were the first to identify the serotonin subtypes of psilocybin directly involved with its pharmacological effects and receptor binding profile. At the time, the interaction and modulating effects between serotonin and dopamine on neurotransmission in the brain were

complex and not fully understood, especially during acute episodes of psychosis. The primary aim of their study was to elucidate the role of serotonin (5-HT) receptors in modulating dopamine neurotransmission, particularly in the context of acute psychotic states. For their study, they investigated the effects of psilocybin on dopamine release in the striatum of healthy volunteers. The research involved seven healthy male volunteers. Each participant underwent two PET scans with [11C]raclopride: one after receiving a placebo and another after ingesting a psilocybin dose of 0.25 mg/kg. The PET scans measured the occupancy of D2-dopamine receptors in the striatum before and after psilocybin administration, providing insight into changes in dopamine levels. Psychopathological ratings were also conducted immediately following the scans.

Psilocybin administration produced a *"psychosis-like syndrome"* characterized by mood changes, disturbances in thinking, illusions, elementary and complex visual hallucinations, and impaired ego functioning. The PET scan results revealed a significant decrease in the binding potential of [11C]raclopride in the caudate nucleus (19%) and putamen (20%), which is indicative of increased dopamine release. This effect was notably correlated with euphoria and depersonalization symptoms. Based on these findings, they suggest that the psychotic effects of psilocybin involve increased striatal dopamine release, mediated by the stimulation of 5-HT_{2A} and 5-HT_{1A} receptors. Building on this understanding of psilocybin's interaction with serotonin and dopamine systems, it is essential to place psilocybin within the broader context of classic psychedelics, which have become now known as *"classic serotonergic psychedelics"*.

As discussed in our review paper on the cellular neurobiology of psychedelics (24), classic serotonergic psychedelics have a chemical structure similar to serotonin 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 β-carbolines and exhibit comparatively less specificity for the 5-HT type 2A receptor (5-HT_{2A}R); in particular, they show significant agonism at 5-HT_{1A}Rs as well as adrenergic and dopaminergic receptors

(24,74,75). The phenylalkylamines (e.g., mescaline, DOM, DOI) bind more specifically to 5-HT_{2A}Rs and 5-HT_{2C}Rs. DOI, in particular, is commonly used in animal studies, but there are no published studies on its effects in human subjects.

Despite their expansive binding profile, the effects of psychedelics in humans strongly correlate with 5-HT_{2A}R occupancy in the central nervous system (CNS) and are almost entirely blocked by 5-HT_{2A} antagonists (66,76–78), thus motivating focus on the 5-HT_{2A}R. Indeed, psychedelics are typically defined as psychoactive agonists of the serotonin 5-HT_{2A} receptor (5-HT_{2A}R). Although, it is important to note, however, that 5-HT_{2A}R antagonists are not perfectly selective either, and there is some evidence suggesting the involvement of other receptors in the neurophysiological effects of psychedelics (79,80). Thus, the involvement of other receptors in the behavioral and therapeutic effects in humans should not be entirely excluded.

Expression of 5-HT_{2A}R mRNA and protein in the brain is widespread. It is most prevalent in excitatory neurons in the *neocortex* but is also expressed in inhibitory interneurons (81,82). Importantly, depression and other mood disorders are associated with increased 5-HT_{2A}R density in the brain, especially in the *prefrontal cortex* (PFC) (83,84), and antidepressant treatments are associated with decreased density (85,86), as is the administration of psychedelics (87).

To better understand the role of this receptor, it is essential to examine its structural characteristics and functionality. The 5-HT_{2A} receptor is a Class A, rhodopsin-like, *G-protein–coupled* receptor (88) for which 5-HT, the endogenous ligand, is a full agonist with a K_d of just over 1 nM (89). Canonically, 5-HT_{2A}Rs couple with the G-protein G_q (**Figure 2**), catalyzing the production of phospholipase C and inositol triphosphate to mobilize intracellular calcium, activate calcineurin and inhibit type 1.2 voltage-gated calcium channels (90,91). Understanding the canonical signaling of the 5-HT_{2A} receptor provides a foundation for exploring its interaction with other compounds. The molecular signaling pathways initiated by the activation of the 5-HT_{2A} receptor by psychedelic drugs are indeed complex and essential for the nuanced effects these

substances have on the brain and perception.

Additionally, the interaction of psychedelics with the 5-HT_{2A} receptor leads to the activation of Gq-family G proteins, the first step in a signaling cascade with wide-ranging effects on cellular activity. Once activated, the Gq-protein stimulates phospholipase C (PLC), an enzyme that catalyzes the conversion of phosphate 4,5-bisphosphate (PIP2) to the second messenger's trisphosphate (IP3) and Diacylglycerol (DAG) (90–93). IP3 is fundamental in increasing intracellular calcium levels by binding to IP3 receptors on the endoplasmic reticulum, causing the release of calcium ions into the cytoplasm (51,92–94). This increase in intracellular calcium plays a significant role in neuronal excitability and the facilitation of neurotransmitter release; both are thought to contribute to the cognitive and sensory alterations characteristic of the psychedelic experience (51,95–97). At the same time, DAG activates protein kinase C (PKC), which phosphorylates several target proteins, resulting in various changes in cellular functions and gene expression. This pathway is essential for neuronal activity modulation and could lead to long-term changes in brain function, which are associated with the lasting effects of psychedelic experiences (98). In addition, 5-HT_{2A}R signaling through G-proteins from the G_i family and subsequent inhibition of cAMP formation have also been observed (99). Either of these pathways can contribute to neural plasticity (100,101). These changes in neural plasticity are further supported by neuropeptide synthesis and release, structural changes to neuronal architecture, and altered expression, localization, and phosphorylation of ionotropic glutamate receptors. Psychedelic-induced, large-scale changes in brain activity and attendant perceptual and cognitive changes in information processing modify the consequences of this neural plasticity. When psychedelic drugs are given in the context of psychedelic-assisted therapy, they can facilitate and support long-term changes in behavior and promotion of mental well-being.

Psychedelic agonists of 5-HT_{2A}R are associated with profound changes in perception and cognition, whereas other ligands such as 5-HT 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 (102). Long-standing evidence indicates that 5-HT_{2A}R signaling efficacy is ligand-dependent and prone to biased agonism across these pathways (103–108). Molecular modeling, site-directed mutagenesis, and x-ray crystallography have identified the distinct psychedelic and nonpsychedelic ligand binding sites that ultimately lead to these differential functional signaling consequences (109–111).

In the case of Gq-dependent signaling, both psychedelic (DOI) and nonpsychedelic (lisuride) agonists of 5-HT_{2A}R activate several shared downstream pathways, including pPLC, pPKC, pERK, and pCREB; however, the magnitude of these Gq-mediated responses is greater for psychedelic ligands (**Figure 2**) (112). Non-Gq pathways likely contribute to psychedelic effects as well. For example, in mice lacking Gq expression, the response to DOI is blunted but not eliminated (113). Furthermore, psychedelic-dependent phosphoproteomic and transcriptomic signatures are pertussis toxin–sensitive (114,115), suggesting the involvement of Gi/o signaling. Notably, Gi/o-dependent outcomes may depend on participation of additional signaling partners, as 5-HT_{2A}Rs do not appear to participate directly in functional coupling with Gi/o (116).

Beyond G-protein–coupled pathways, agonism at 5-HT_{2A}Rs can also engage in β-arrestin signaling (117) via PI3K, SRC, and AKT (**Figure 2**), although the relevance of such β-arrestin–dependent signaling for promoting psychedelic effects is still unclear. For example, while LSD's ability to promote a β-arrestin–biased conformation at 5-HT_{2A}Rs supports its psychedelic effects (116,118), other psychedelics are functionally insensitive to β-arrestin knockout in mice (119,120). Close attention to differences in off-target binding profile (121,122) and time-dependent evolution in signaling (118) is needed for future studies to yield a more comprehensive picture of β-arrestin–

induced effects across the full spectrum of psychedelic ligands.

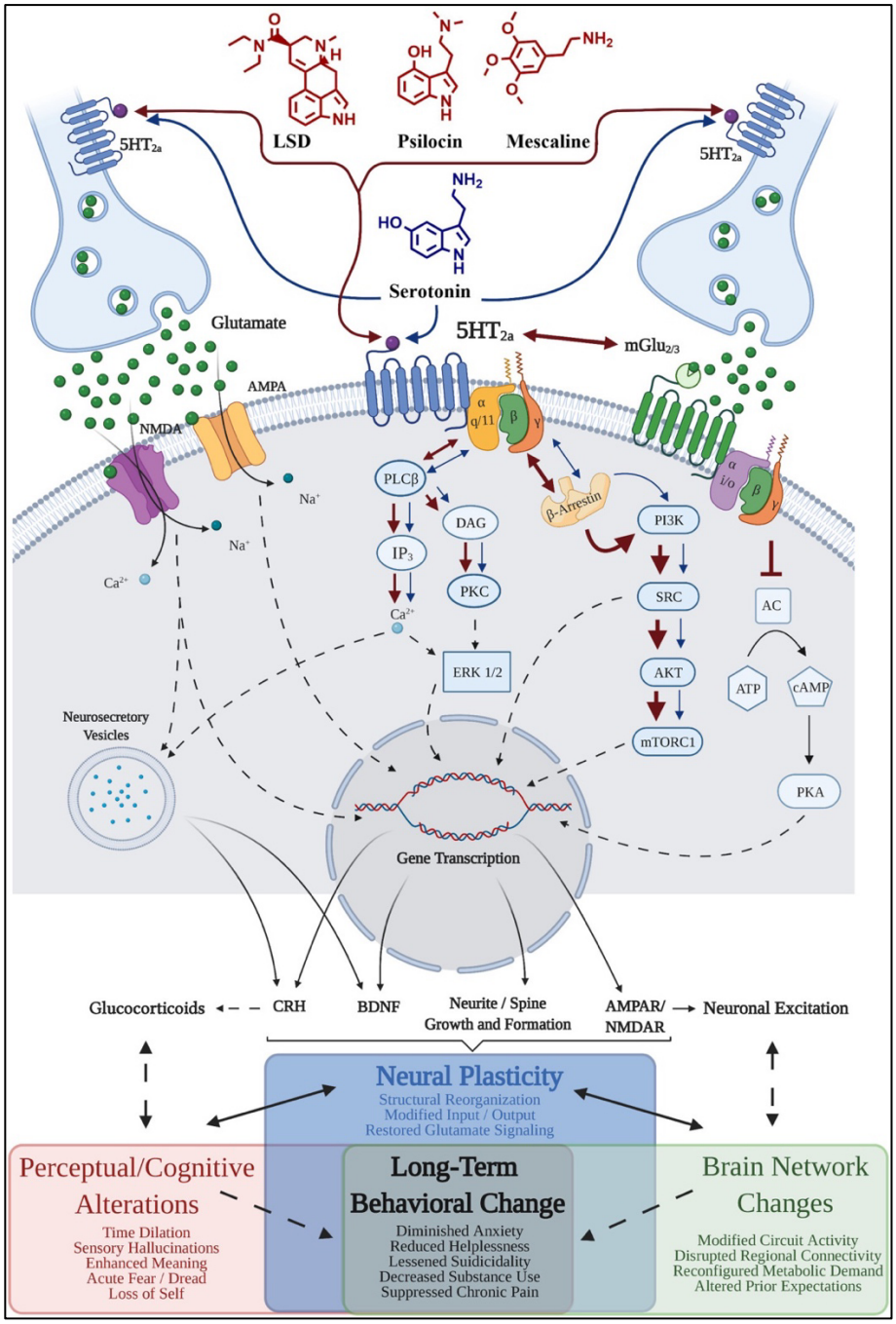

Figure 2: Molecular, cellular, and systems support for psychedelic-induced long-term changes. Psychedelic compounds (LSD, psilocin, mescaline) and serotonin bind with high affinity to serotonin 2A receptors. In mammalian systems, direct (solid arrows) and indirect (dashed arrows) consequences of G-protein and β-arrestin signaling downstream of serotonin 2A receptor activation intersect with glutamate release to yield enhanced neural plasticity. The relative engagement of intracellular

signaling pathways (arrow weight) is distinct for psychedelics (red arrows) vs. serotonin (blue arrows). Banks *et al.,* (2021); 10.1091/mbc.E20-05-0340

In addition to the pharmacological and receptor binding profiles, it is fundamental to study psilocybin's pharmacokinetic profile, especially absorption and dosing. Pre-clinical and clinical studies provide unique insights into these aspects, each contributing to our understanding of how psilocybin exerts its effects. By examining these pharmacokinetic properties, we can gain a more comprehensive understanding of the drug's behavior in the body, which is crucial for optimizing its therapeutic potential and developing alternative prodrugs.

Pharmacokinetics and Dosing:

In clinical studies, psilocybin is typically administered orally at 5 to 30 mg for psychedelic-assisted therapy sessions, translating to 0.1 to 0.4 mg/kg body weight. Upon ingestion, psilocybin is rapidly absorbed by the gastrointestinal tract. The conversion of psilocybin to *'psilocin'*, its psychoactive metabolite, occurs through dephosphorylation, a reaction facilitated by enzymes like alkaline phosphatase. This process is influenced by factors such as the individual's metabolic rate and the presence of other substances that may affect enzyme activity (47,123–125). Moreover, psilocybin is a prodrug that is metabolized into psilocin, its psychoactive metabolite. As seen in **Figure 3**, the detection of psilocin in plasma between 20 to 40 minutes following oral intake points to a rapid absorption and conversion process, with peak plasma concentrations observed within 80 to 105 minutes following ingestion of psilocybin (94,126). This timeframe and the peak concentrations at 8.2 ± 2.8 ng/mL indicate the rapid conversion of psilocybin into psilocin, producing a significant bioavailability of approximately 50% (126). Likewise, intravenous administration reveals a distinct aspect of psilocybin's pharmacokinetics. Intravenous administration peak plasma concentrations occur within approximately 1.9 ± 1.0 minutes post-injection (127,128). This rapid onset might be particularly beneficial in controlled therapeutic settings where timing the onset of effects is critical for synchronizing with therapy sessions.

An open-label study revealed psilocybin's safety profile, underscores its potential as a safe therapeutic agent (129). The absence of psilocybin in plasma and urine after administration, along with the minimal renal clearance of psilocin, highlights the body's efficient metabolism and elimination of these compounds through non-renal pathways. The lack of severe adverse effects, even at high doses (0.6 mg/kg), further supports the potential for psilocybin in therapeutic settings, providing valuable data for establishing dosing guidelines (130).

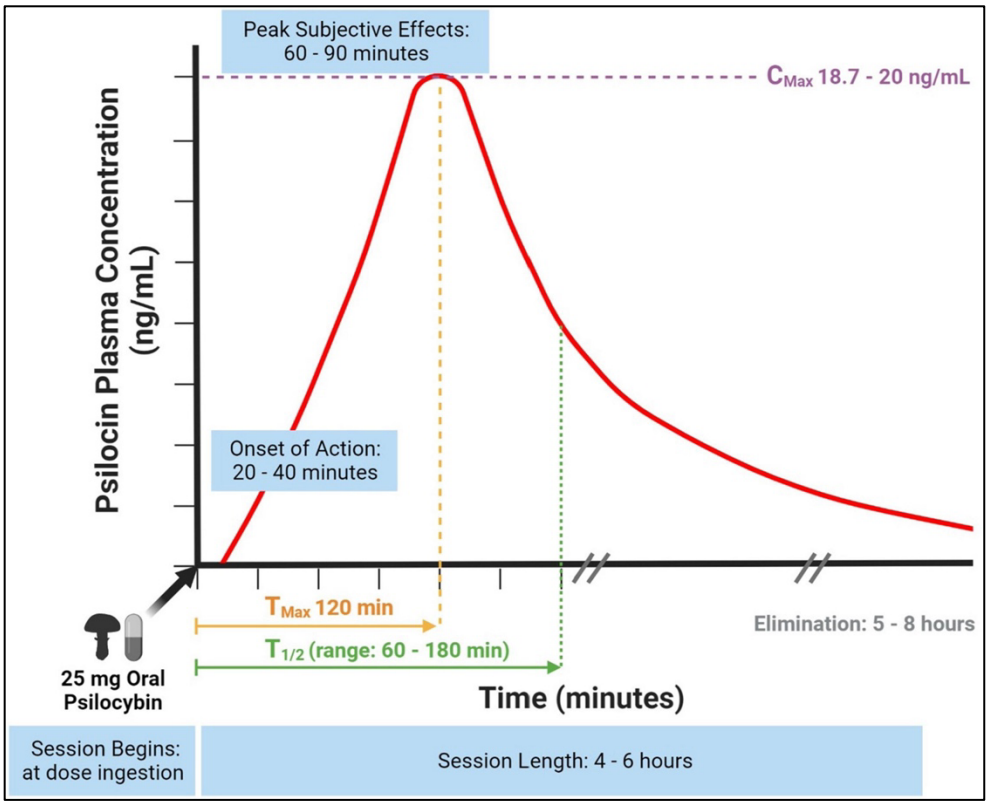

Figure 3: Psilocin concentration to session time curve using a standard psilocybin dose. https://doi.org/10.3389/fpsyt.2022.1040217

Phase-I Metabolism:

Now that we have a time course for psilocybin and peak concentrations, how are they achieved. During phase-I metabolism, psilocin undergoes various biochemical modifications to increase its hydrophilicity. These reactions include oxidation, reduction, and hydrolysis, mediated by liver enzymes, especially from the cytochrome P450 family. The oxidation of psilocin leads to

formation of several metabolites, 4-hydroxyindole-3-acetaldehyde and, subsequently, 4-hydroxyindole-3-acetic acid or alternatively 4-hydroxytryptophol. These transformations make psilocin more water-soluble, facilitating its further metabolism and eventual elimination (131–133).

Phase-II Metabolism:

Moving from phase-1 metabolism, psilocin enters phase-II metabolism, undergoing conjugation reactions. These reactions involve adding endogenous substances like glucuronic acid, making the molecule even more polar and thus more readily excreted in urine. Enzymes such as UDP-glucuronosyltransferases (UGTs) play pivotal roles, particularly UGT1A10 in the small intestine and UGT1A9 in the liver. Conjugation products, including psilocin O-glucuronide, represent the final metabolites ready for elimination from the body (134,135).

Renal Excretion:

Following glucuronidation, psilocin O-Glucuronide, is then ready for renal excretion. The kidneys are the primary organs responsible for eliminating psilocin O-Glucuronide and its metabolites. These water-soluble compounds are efficiently cleared from the bloodstream and excreted in the urine through filtration and tubular secretion. Though, the efficiency of renal excretion is influenced by factors such as the individual's hydration status, kidney function, and urine pH, which can affect the ionization state and solubility of the metabolites (136).

Elimination Half-Life:

Based on the rate of metabolism, glucuronidation, and excretion, calculations for the half-life of psilocin in healthy adults is approximately 3 hours. This metric represents the time it takes for the concentration of the drug in the blood to reduce by half and is a critical determinant of the drug's duration of action and helps guide dosing intervals for therapeutic use. However, it's important to note that the half-life can vary significantly among individuals due to differences in metabolic rates, liver and kidney function, and other physiological factors. Additionally, the route

of administration of psilocybin (e.g., oral, intravenous) can influence the rate at which psilocin reaches the systemic circulation and is subsequently metabolized and excreted (130,137).

Psilocin's Lipophilic Nature and The Blood-Brain Barrier:

Although, before psilocybin is eliminated from the body, it has to carry out its neurological and pharmacological effects. This involves, passage of psilocin across the blood-brain barrier (BBB) into the central nervous system (CNS), a critical event that underpins the psychoactive effects of psilocybin. This process, complex and finely regulated, involves intricate interactions between psilocin and the structural components of the BBB. Understanding this passage requires an appreciation of the BBB's role in brain homeostasis, the properties of psilocin that facilitate its transit, and the subsequent neuropharmacological interactions within the brain. The BBB is a highly selective permeability barrier that separates circulating blood from the brain extracellular fluid in the CNS. It comprises endothelial cells that line the cerebral microvasculature, astrocyte end-feet that enwrap the capillaries, and pericytes embedded within the basement membrane. This complex cellular arrangement is fortified by tight junctions between endothelial cells, which restrict paracellular transport, thereby controlling the brain's internal environment and protecting it from toxins, pathogens, and fluctuations in plasma composition.

Its lipophilic nature significantly influences psilocin's ability to cross the BBB. Lipophilicity, a measure of a compound's affinity for lipids, is a critical determinant of a molecule's ability to penetrate the BBB, which favors the diffusion of non-polar, lipid-soluble substances. Psilocin's molecular structure facilitates its dissolution in the lipid bilayer of endothelial cell membranes, allowing it to traverse the BBB efficiently through passive diffusion. This lipophilic characteristic of psilocin is contrasted with hydrophilic molecules, which, due to their affinity for water, have limited ability to cross the BBB without specific transport mechanisms (51,138). Building on this understanding of psilocin's properties, pioneering studies by Vollenweider *et al.* used positron

emission tomography (PET) to reveal psilocybin's effects after it has crossed the blood-brain barrier and its impact on cerebral metabolism.

1.6 Therapeutic Potential of Psilocybin: Insights from Neuroimaging and Clinical Trials

Furthermore, a pioneering study by Vollenweider *et al.* (1997) used positron emission tomography (PET) to reveal psilocybin's effects on cerebral metabolism. Their findings showed global cerebral excitation, with notable increases in glucose metabolism in the brain's frontomedial and frontolateral cortex, anterior cingulate, and temporomedial cortex. This *"hyperfrontal"* metabolic pattern parallels acute psychotic episodes in chronic schizophrenia, suggesting a shared pathway of 5-HT_{2A} receptor-mediated cortical activation (139). Similarly, Gouzoulis-Mayfrank *et al.'s* (1999) PET study mapped the nuanced neurometabolic landscape under psilocybin's influence, showing increased metabolic rates in specific right hemispheric frontotemporal regions and a dampening effect on the thalamus (140). This dual pattern of activation and deactivation suggests psilocybin's ability to both stimulate and suppress brain activity, orchestrating a delicate balance that underlies the psychedelic state.

Building on these foundational findings, Dr. David Nutt and Dr. Robin Carhart-Harris at Imperial College London's Center for Psychedelic Research have been at the forefront of exploring how psychedelics like psilocybin and LSD impact brain activity. Their notable discovery is the reduction of activity in the brain's default mode network (DMN), which maintains the sense of self. This reduction is linked to *'ego-dissolution,'* potentially resetting maladaptive neural circuits related to mental health disorders like depression (141,142).

In a pivotal 2012 study, their team used task-free functional MRI (fMRI) to observe shifts from a normal to a psychedelic state, employing techniques like arterial spin labeling and blood-oxygen-level-dependent (BOLD) fMRI to track cerebral blood flow and oxygenation. They found that psilocybin led to decreased activity in critical areas such as the thalamus and the anterior

and posterior cingulate cortex (ACC and PCC), correlating with the intensity of reported psychedelic experiences. Further analysis revealed how psilocybin disrupts the brain's network connectivity, particularly diminishing the positive connection between the medial prefrontal cortex (mPFC) and PCC (142). This shift in brain communication patterns suggests that changes in DMN activity and connectivity might underpin the profound alterations in consciousness associated with psychedelic experiences.

Building on these critical studies, these researchers aimed to assess and compare the efficacy of psilocybin-assisted therapy and serotonin reuptake inhibitors. Therefore in 2021, Dr. David Nutt and Dr. Carhart-Harris conducted an important Phase II trial comparing psilocybin-assisted therapy to the antidepressant escitalopram for treating depression, published in the New England Journal of Medicine (143). Despite being one of the most comprehensive studies in the field, the trial's outcomes were inconclusive about psilocybin's superiority over escitalopram, highlighting the need for further, larger-scale research.

However, the study faced several criticisms, especially regarding the inconclusive results. Firstly, critics have pointed out the study's relatively small sample size, involving only 59 participants. This limited number can affect the generalizability of the findings. Furthermore, the study population consisted predominantly of white, highly educated individuals, which is not representative of the broader demographic suffering from depression (144). This lack of diversity raises concerns about the applicability of the results to a wider population. Another significant critique revolves around the selection and choice of outcome measures. The primary measure used was the Quick Inventory of Depressive Symptomatology–Self-Report (QIDS SR-16), which showed no significant difference between psilocybin and escitalopram. However, secondary measures like the Hamilton Depression Rating Scale (HAMD-17) indicated a more favorable outcome for psilocybin. This lends itself to the argument that the primary outcome measure should more closely align with clinically meaningful outcomes to provide a clearer understanding of the

treatment's efficacy. Additionally, the placebo effect also comes under scrutiny. The study used a very low dose of psilocybin (1 mg) as a placebo, which might not be a true inactive and inert placebo. This could have influenced participants' expectations and potentially confounded the results (145) .

Regarding the interpretation of results, the study concluded that psilocybin is non-inferior to escitalopram. However, some critics believe this interpretation is overly simplistic (143,144). A Bayesian reanalysis indicated that while psilocybin outperformed escitalopram on several secondary measures, it did not do so to a clinically meaningful extent on the primary measure, suggesting that a more nuanced interpretation and larger studies are necessary to understand psilocybin's efficacy fully (144). Additionally, the psilocybin-assisted therapy and antidepressant escitalopram groups in the study received psychological support during the trial, which could have significantly contributed to the observed improvements in both groups. This psychological support makes it challenging to isolate the specific, pharmacological, effects of psilocybin compared to escitalopram (143,145). These critiques highlight the need for more and larger-scale studies with diverse populations and rigorous methodologies to understand psilocybin's therapeutic potential better and address the limitations identified in this trial.

Meanwhile, at Johns Hopkins University, Dr. Roland Griffiths has been at the forefront of exploring psilocybin's therapeutic potential, particularly for major depressive disorder (MDD) and existential anxiety in terminally ill cancer patients. His research has shown that psilocybin can lead to significant, rapid, and enduring alleviation of depressive and anxiety symptoms (35). In a key 2016 study, participants with life-threatening cancer diagnoses received controlled doses of psilocybin, resulting in substantial symptom relief that persisted for up to six months following psilocybin-assisted therapy. This study involved administering psilocybin in two sessions, spaced five weeks apart: the first session used a low dose of 1 or 3 mg per 70 kg as a placebo, and the second session provided a moderate to high dose of 22 or 30 mg per 70 kg. Moreover, the

outcomes highlighted psilocybin's effectiveness in significantly reducing depression and anxiety levels while inducing major changes in mood, outlook, overall quality of life, sense of meaning, and optimism among participants. In their studies, the research team used the GRID-Hamilton Depression Rating Scale (GRID-HAMD) and the Hamilton Anxiety Rating Scale (HAM-A) to assess depression and anxiety severity. The implementation of these standardized tools precisely monitors treatment impacts and symptom changes over time. Six months post-treatment, 80% of participants showed sustained reductions in depression and anxiety levels, with 60% reaching symptom remission. Additionally, 83% reported enhanced well-being or life satisfaction, 67% considered their experience among their top five most meaningful, and 70% rated it as one of their top five spiritual events. Despite these positive outcomes, some participants experienced side effects such as nausea, vomiting, psychological discomfort at higher doses, temporary blood pressure increases, heightened levels of acute anxiety, and post-session headaches (35).

Building on this promising therapeutic potential for psilocybin-assisted therapy, in 2020, Johns Hopkins Medicine conducted a study on adults with major depression, demonstrating that two doses of psilocybin with psychotherapy significantly reduced depressive symptoms, with half of the participants in remission by four weeks. Unlike their 2016 study, this 2020 study no longer used exclusively terminally ill cancer patients and moved to treating those suffering from major depression. Furthermore, this study enrolled 24 participants with a two-year history of depression, predominantly white, who underwent a discontinuation of their current antidepressants before receiving psilocybin. Treatment involved two psilocybin doses two weeks apart, in sessions lasting five hours with sensory isolation via eyeshades and music, under clinical supervision. Depression levels, measured using the GRID-Hamilton Depression Rating Scale, initially indicated moderate to severe depression, but remarkably showed striking improvement post-treatment. A significant portion of patients achieved remission by the four-week follow-up, highlighting psilocybin-assisted therapy’s promising therapeutic potential.

Based on these findings that psilocybin-assisted therapy had such a substantial effect on reducing major depression at four-weeks following treatment, the Hopkins psychedelics research team wanted to assess if this effect would last longer. Therefore, in 2022, their researchers found that psilocybin-assisted therapy, can offer long-term antidepressant effects, extending at least a year for some patients. For the study, 24 participants attended all follow-up visits throughout the 12-month period. The study observed significant reductions in GRID-HAMD scores from baseline at each follow-up interval (1, 3, 6, and 12 months). Moreover, at the 12-month mark, 75% of participants achieved a treatment response, defined as a reduction of 50% or more in their GRID-HAMD scores from baseline. Furthermore, 58% of participants reached remission. Importantly, there were no serious adverse events attributed to psilocybin during the long-term follow-up, and none of the participants reported using psilocybin outside the study context.

The cumulative findings from these studies illustrate that psilocybin exerts a profound impact on brain function and holds considerable promise as a therapeutic intervention for mental health disorders, particularly depression. The research consistently shows that psilocybin induces significant cerebral metabolic changes (139), reduces default mode network (DMN) activity (142), and alters brain connectivity. These changes are linked to profound psychological experiences and potential therapeutic benefits. Consequently, these neurophysiological effects are mirrored by substantial clinical outcomes, with psilocybin demonstrating rapid and enduring reductions in depressive and anxiety symptoms for 1 – 12 months following psilocybin-assisted therapy (34,35,125). Given these promising outcomes, there is a growing interest in using psilocybin to treat stress-related disorders such as depression, anxiety, and post-traumatic stress disorder (PTSD). Despite this increasing focus, the interaction between psychiatric disorders, stress, and psychedelics remains an understudied and largely unknown area of research.

1.7 Psilocybin and Its Influence on Cortisol and Stress Hormones via the 5-HT System

As described in our review paper detailing the neurobiology of psychedelics (24), the intersection between psychiatric disorders, stress, and psychedelics is an emerging area of interest (146–151). Chronic stress is a major precipitating factor in the etiology of many psychiatric disorders that psychedelics have shown clinical efficacy in treating (152). Animal models have shown that chronic stress induces behavioral and neural changes that can be reversed by antidepressants (153–160). Therefore, 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 (161–163), and signaling at the 5-HT_{2A}R plays a role in this neural plasticity (146,164–166). Along with this convergence on neuroplastic mechanisms to modify behavior, psychedelics trigger an acute biochemical, drug-induced, stress response consisting of catecholamine and glucocorticoid release (149,167–170), and it has been postulated that this stress response is critical for the transformative nature of the psychedelic experience (147).

However, the mechanism underlying this stress response is unclear, but there are two obvious (and not necessarily mutually exclusive) possibilities. First, psychedelics may act directly at 5-HT_{2A}Rs in the hypothalamus to induce expression and/or release of corticotrophin-releasing hormone, elevating plasma concentrations of stress-associated glucocorticoids (171,172). This possibility is consistent with the established regulatory role of 5-HT in the hypothalamic–pituitary–adrenal (HPA) axis, the primary system involved in stress regulation (167,173). As seen in **Figure 4**, the HPA axis controls the body's reaction to stress, with cortisol release being one of its end products. Furthermore, when an individual perceives a situation as stressful, the hypothalamus releases corticotropin-releasing hormone (CRH), signaling the pituitary gland to secrete adrenocorticotropic hormone (ACTH), which stimulates cortisol release from the adrenal glands

(**Figure 5**). Elevated cortisol levels impact various bodily functions, preparing the individual to respond to stress through the *"fight or flight"* response. Although a critical aspect is that the HPA axis is regulated via negative feedback mechanisms. Elevated cortisol levels can inhibit further CRH and ACTH release, maintaining homeostasis. Importantly, chronic stress or dysregulation of this axis can lead to altered cortisol levels, affecting mood, cognitive functions, and susceptibility to mental health disorders (156,163).

To understand the complexities of this regulatory system, it is essential to explore the underlying neurochemical pathways involved. One such pathway is the mechanistic link between 5-HT_{2A} receptor activation and glutamate release in the hypothalamus. This process is characterized by a sophisticated interplay of neurotransmitter systems and intracellular signaling pathways. The 5-HT_{2A} receptors, which are G protein-coupled receptors predominantly expressed in the central nervous system, play a pivotal role in modulating neuronal activity (174). Upon activation by serotonin, these receptors initiate a cascade of intracellular events through the Gq/11 protein pathway, which is essential for various cellular responses (175). Activation of the 5-HT_{2A} receptor stimulates phospholipase C (PLC), leading to the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into two secondary messengers: inositol trisphosphate (IP3) and diacylglycerol (DAG) (91,176). IP3 binds to its receptors on the endoplasmic reticulum, causing the release of calcium ions into the cytoplasm. The increase in intracellular calcium concentration is crucial for various cellular processes, including the exocytosis of neurotransmitter-containing vesicles (177). Concurrently, DAG remains in the plasma membrane and activates protein kinase C (PKC), which further modulates numerous downstream targets involved in neurotransmission (177).

The rise in intracellular calcium levels is particularly significant for the release of glutamate, the primary excitatory neurotransmitter in the brain. Calcium ions facilitate the fusion of glutamate-containing vesicles with the presynaptic membrane, thereby promoting the release of glutamate

into the synaptic cleft (178). This process is vital for synaptic transmission and plasticity, influencing numerous physiological functions regulated by the hypothalamus, such as stress response, feeding behavior, and thermoregulation (179). In addition to the direct pathway, 5-HT_{2A} receptor activation modulates glutamatergic activity through indirect mechanisms. For instance, it influences other neurotransmitter systems, such as GABAergic and dopaminergic pathways, which can subsequently affect glutamate release (180). GABAergic neurons, which release the inhibitory neurotransmitter GABA, can be inhibited by 5-HT_{2A} receptor activation, leading to a disinhibition of glutamatergic neurons and increased glutamate release (181) Similarly, dopaminergic modulation by 5-HT_{2A} receptors can influence glutamate release through complex feedback mechanisms involving dopaminergic and glutamatergic neurons (182).

This intricate regulation underscores the role of 5-HT_{2A} receptors in maintaining hypothalamic neurotransmitter homeostasis. Dysregulation of 5-HT_{2A} receptor signaling and glutamate release has been implicated in various neuropsychiatric disorders, including schizophrenia, depression, and anxiety (183–186). Therefore, understanding the mechanistic link between 5-HT_{2A} receptor activation and glutamate release not only provides insights into fundamental brain functions but also highlights potential therapeutic targets for treating disorders associated with dysregulated glutamate transmission (180,187).

The mechanistic link between 5-HT_{2A} receptor activation and glutamate release in the hypothalamus not only underscores fundamental neurophysiological processes but also plays a critical role in the body's stress response. The hypothalamus, a key brain region involved in maintaining homeostasis, orchestrates the stress response through the hypothalamic-pituitary-adrenal (HPA) axis (**Figure 4**). Activation of 5-HT_{2A} receptors in the hypothalamus influences this process by modulating glutamate release, which in turn affects the secretion of corticotropin-releasing hormone (CRH) and subsequent HPA axis activity (163,188–190).

When 5-HT_{2A} receptors are activated, the resultant increase in glutamate release enhances the excitatory input to CRH-producing neurons in the paraventricular nucleus (PVN) of the hypothalamus (191). Glutamate, acting through its ionotropic receptors, such as NMDA and AMPA receptors, depolarizes these neurons, thereby increasing CRH transcription and release (190,192). CRH then travels through the hypothalamic-hypophyseal portal system to the anterior pituitary gland, where it stimulates the secretion of adrenocorticotropic hormone (ACTH) into the bloodstream (**Figure 4**) (193). ACTH subsequently acts on the adrenal cortex, promoting the release of glucocorticoids, primarily cortisol in humans. These glucocorticoids exert widespread effects on various tissues, preparing the body to respond to stress by mobilizing energy reserves, modulating immune function, and influencing brain function to enhance alertness and cognition (192). The increase in glucocorticoids also exerts a negative feedback effect on the hypothalamus and pituitary gland, helping to terminate the stress response and restore homeostasis once the stressor is removed (194).

Additionally, the modulation of other neurotransmitter systems by 5-HT_{2A} receptor activation, such as the inhibitory GABAergic and dopaminergic systems, provides a fine-tuned regulation of the stress response. For example, the inhibition of GABAergic interneurons can lead to a disinhibition of CRH neurons, further amplifying the stress response (195). Conversely, dopaminergic modulation can either enhance or inhibit CRH neuron activity depending on the receptor subtypes involved and the specific brain regions affected (196). Thus, the activation of 5-HT_{2A} receptors and the subsequent increase in glutamate release in the hypothalamus constitute a crucial mechanism in the initiation and regulation of the stress response. This pathway highlights the intricate neurochemical interactions that underpin the body's ability to adapt to and cope with stress, emphasizing the importance of 5-HT_{2A} receptors as potential therapeutic targets for stress-related disorders (183)

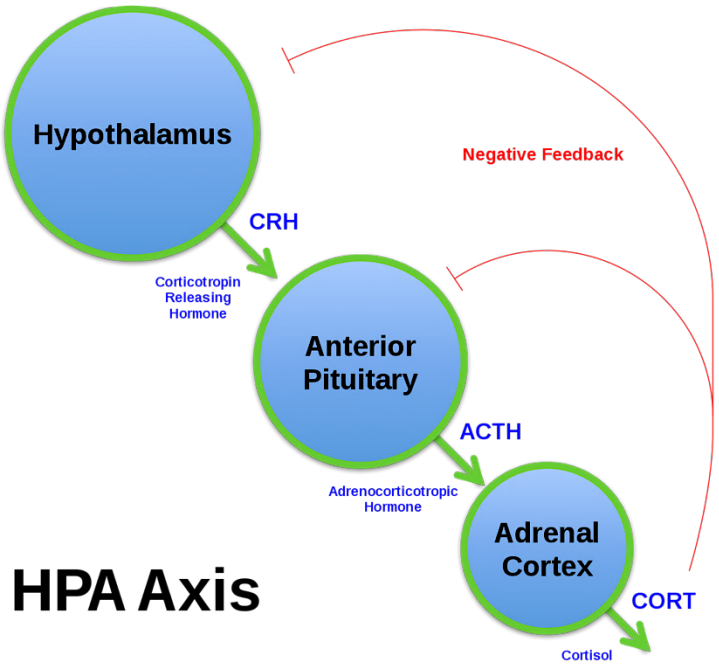

Figure 4: Schematic of the HPA axis.

https://en.wikipedia.org/wiki/Hypothalamic%E2%80%93pituitary%E2%80%93adrenal_axis

Alternatively, the psychedelic-induced altered state of consciousness itself may trigger the acute stress response, as such states frequently include challenging components such as fear and anxiety. This raises the possibility that the pro-neuroplastic effects of psychedelics depend in part on the acute stress response that they induce, but whether the stressful components of the psychedelic experience are hindrances to or foundational for therapeutic benefit is a matter of debate (30,197–199). In an effort to better understand this novel interaction between stress and psychedelics Hasler *et al.* (2004) conducted a double-blind, placebo-controlled study involving healthy volunteers (168). They found that psilocybin-assisted therapy dose-dependently increased scores on all core dimensions of the Altered States of Consciousness (5D-ASC) rating scale, especially at medium and high doses. These dimensions include *"oceanic boundlessness"* (OB), *"visionary restructuralization"* (VR), and *"anxious ego dissolution"* (AED), with the latter just missing statistical significance. Prominently, subjective effects of psilocybin included changes in mood, sensory perception such as visual illusions and hallucinations, and altered perceptions of

time and self. While higher doses (MD and HD) were generally associated with positive retrospective experiences, only one subject experienced transient anxiety at the highest dose (168).

Regarding physiological effects, high doses of psilocybin led to a transient and moderate increase in mean arterial blood pressure (MAP), particularly noticeable 60 minutes post-administration. However, neither electrocardiogram (EKG) parameters nor body temperature were significantly affected by any dose of psilocybin. Intriguingly, one notable physiological finding was the significant elevation in ACTH and cortisol plasma levels during the peak effects of high-dose psilocybin. In more detail, these hormones were measured 105 minutes post-administration of psilocybin and found ACTH levels increased significantly following the high dose (315 µg/kg). In **Table 2** and **Figure 5**, plasma levels rose from a baseline mean of 12 ng/l to 50 ng/l. Similarly, cortisol levels significantly increased at high doses, rising from a baseline mean of 385 nmol/l to 567 nmol/l (168).

Neuroendocrine Effects of Psilocybin: Dose-Dependent Plasma Concentrations

	Placebo	Psilocybin VLD (45 µg/kg)	Psilocybin LD (115 µg/kg)	Psilocybin MD (215 µg/kg)	Psilocybin HD (315 µg/kg)
Sampling 1 (baseline, $t_{0-20\ min}$)					
Thyroid-stimulating hormone (TSH [mU/l])	1.30±0.15	1.62±0.13	1.54±0.13	1.40±0.16	1.27±0.14
Prolactin (PRL [µg/l])	16.7±5.5	16.0±5.4	14.6±4.3	15.5±5.7	14.8±2.8
Adrenocorticotropic hormone (ACTH [ng/l])	21±6	17±5	17±4	18±6	12±2
Cortisol (CORT [nmol/l])	432±59	336±54	378±77	382±50	385±71
Sampling 2 ($t_{0+105\ min}$)					
Thyroid-stimulating hormone (TSH [mU/l])	1.24±0.16	1.41±0.09	1.50±0.13	1.54±0.16	1.66±0.22**
Prolactin (PRL [µg/l])	9.5±2.0	12.8±4.6	14.5±5.3	22.4±7.7**	28.0±7.5***
Adrenocorticotropic hormone (ACTH [ng/l])	20±5	19±4	25±5	42±14	50±12**
Cortisol (CORT [nmol/l])	348±50	353±85	443±47	522±82	567±74*
Sampling 3 ($t_{0+300\ min}$)					
Thyroid-stimulating hormone (TSH [mU/l])	1.16±0.15	1.38±0.19	1.15±0.09	1.20±0.13	1.18±0.18
Prolactin (PRL [µg/l])	12.4±2.7	9.0±1.6	9.1±1.7	9.7±2.0	12.9±2.9
Adrenocorticotropic hormone (ACTH [ng/l])	18±4	15±3	15±2	14±2	15±2
Cortisol (CORT [nmol/l])	353±54	306±64	289±60	294±57	344±61

Table 2: Neuroendocrine effects of psilocybin. Dose-dependent plasma concentrations of hormones (mean ± SEM, n=8). From Hasler *et al.,* (2004).

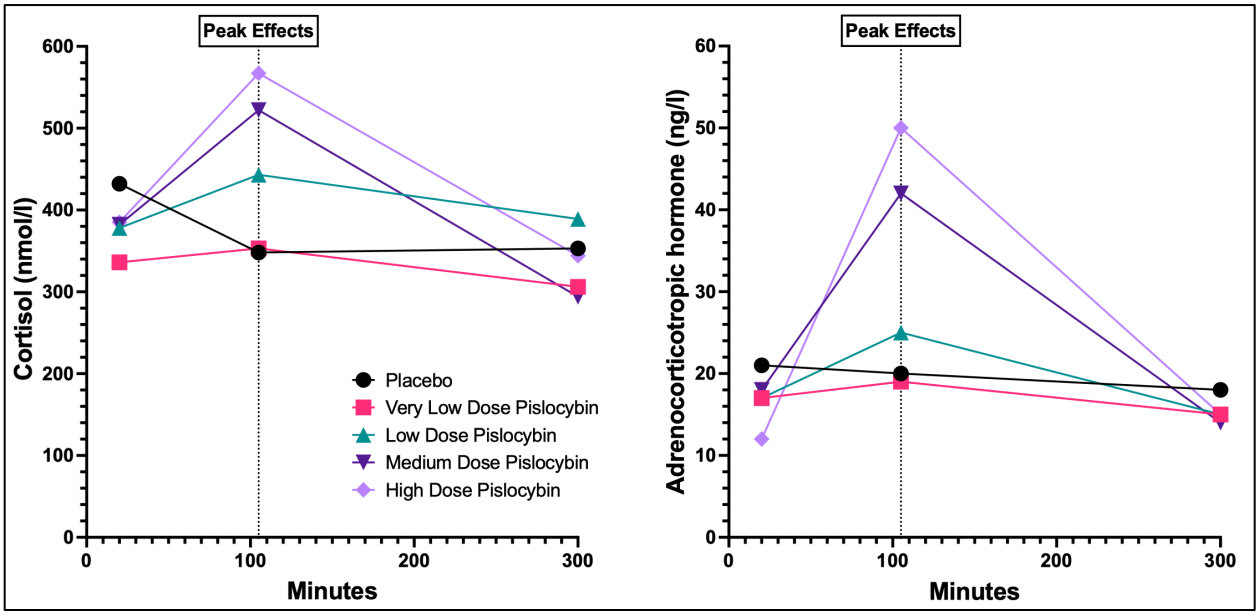

Figure 5: Graphical Representation for the 'Neuroendocrine Effects of Psilocybin'. Data-Table from Hasler *et al.,* (2004)

The increase in ACTH and cortisol levels suggests that psilocybin activates the HPA axis, likely through 5-HT_{2A} receptor stimulation as discussed previously. Moreover, the study concluded that psilocybin, even at high doses, is not hazardous to healthy subjects. However, the transient increase in blood pressure at high doses indicates that individuals with cardiovascular conditions should avoid its use. Additionally, the psychological effects, though profound, were generally well-tolerated and integrated by the subjects. This emphasizes the importance of a controlled clinical setting to manage any potential adverse reactions, particularly anxiety. Based on these findings, psilocybin activates the HPA axis, resulting in increased levels of ACTH and cortisol.

Building on these findings, a different study found that both LSD and psilocybin can induce a temporary increase in cortisol levels. Interestingly, the increase in cortisol may be a direct result of the heightened sensory and emotional experiences that characterize the psychedelic state, which the body maybe interpreting as a form of stress. To further investigate this question, the study from Holze *et al.* (2022) offers comprehensive insights into the subjective, autonomic, and endocrine effects for both LSD and psilocybin (169). Conducted with a double-blind, randomized, placebo-controlled, crossover design in 28 healthy subjects, this study provides robust evidence for the comparative effects of these psychedelics.

The study found that LSD (at doses of 100 μg and 200 μg) and psilocybin (at a dose of 15 and 30 mg) produced comparable subjective effects. Notably, the 200 μg dose of LSD induced higher ratings of ego-dissolution, impairments in control and cognition, and anxiety than the 100 μg dose. Both doses of LSD had a significantly longer duration of effects compared to psilocybin, which suggests a more prolonged alteration of consciousness. Both LSD and psilocybin exhibited dose-proportional pharmacokinetics and first-order elimination. In terms of the pharmacokinetics, the elimination half-life of LSD was approximately 4 hours, while psilocybin's active metabolite, psilocin, had a half-life of around 2.5 hours. In addition, they found the 15 mg dose of psilocybin produced weaker subjective effects compared to both doses of LSD and the 30 mg dose of

psilocybin (169). The study also highlighted that ego-dissolution and ineffability were significantly higher with the 200 μg dose of LSD compared to the 30 mg dose of psilocybin.

Regarding endocrine responses, their supplementary information (**Figure 6** and **Table 3**) presents a detailed analysis of plasma concentrations of cortisol, prolactin, oxytocin, and BDNF over time following the administration of LSD (100 μg or 200 μg), psilocybin (15 mg or 30 mg), or a placebo (169). The data reveal significant insights into the dose-dependent effects of these substances on stress and endocrine responses. Both doses of LSD (100 μg and 200 μg) result in a significant increase in cortisol levels over time, indicating a drug-induced stress response, with the 200 μg dose slightly higher than the 100 μg dose. Similarly, both doses of psilocybin (15 mg and 30 mg) increase cortisol levels, with the 30 mg dose causing a more substantial rise than the 15 mg dose. In contrast, the placebo group shows a decrease in cortisol levels, serving as a baseline and indicating no stress response. Furthermore, LSD administration also increased prolactin levels, with the 200 μg dose showing a more pronounced effect than the 100 μg dose, suggesting a dose-dependent response. Psilocybin similarly elevated prolactin levels, with the 30 mg dose having a higher impact than the 15 mg dose (169). The placebo group, however, showed a slight decrease in prolactin levels, indicating no significant effect.

Although not measured in this study, ACTH measurements would further confirm the HPA axis activation and its dose-dependent response to these substances. Corresponding increases in ACTH levels likely mediate the observed increases in cortisol levels following LSD and psilocybin administration. The increase in cortisol and prolactin levels following the administration of LSD and psilocybin underscores the role of these hormones in the stress response and HPA-axis activation.

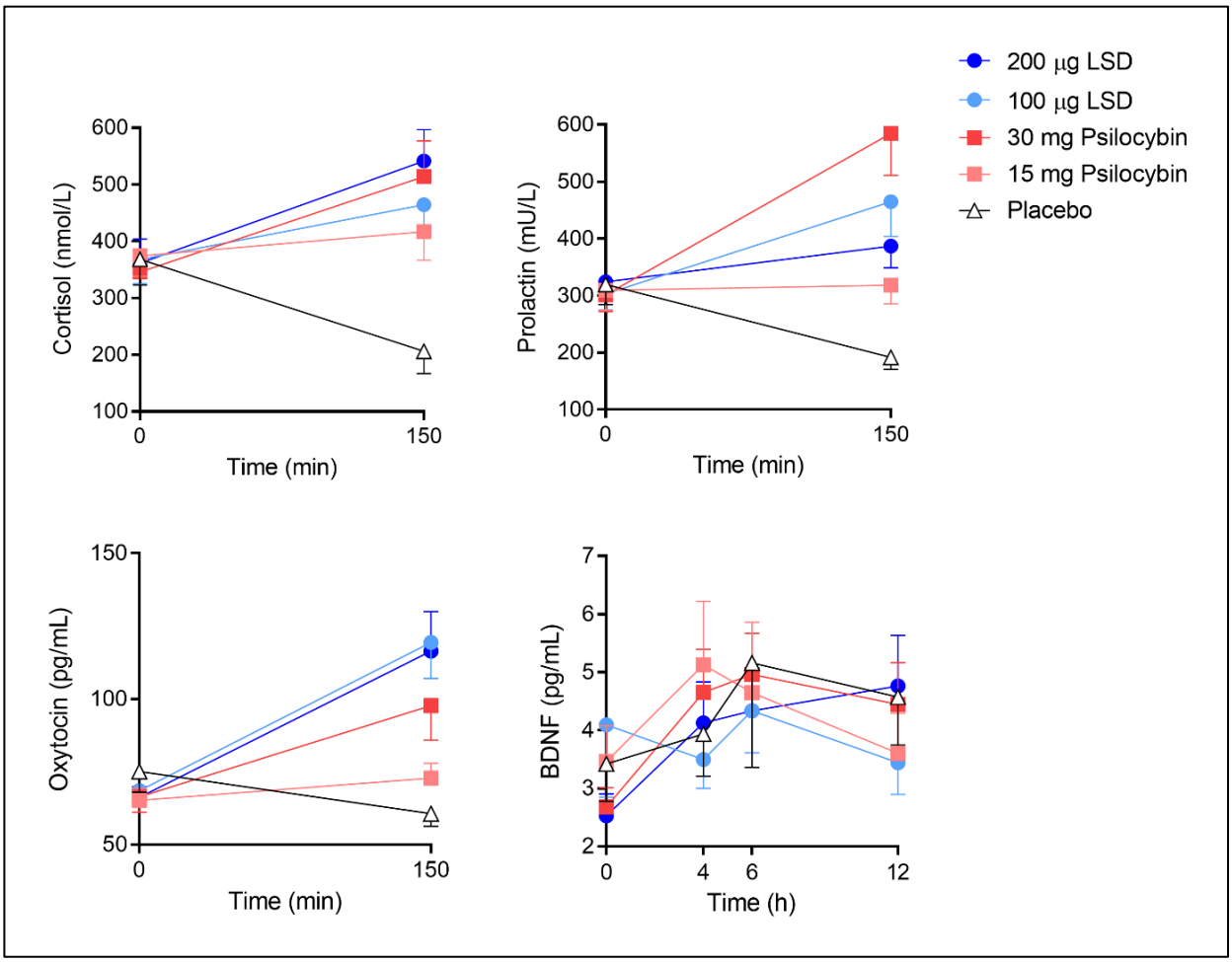

Figure 6: Plasma concentrations of cortisol, prolactin, oxytocin, and brain-derived neurotrophic factor (BDNF). The data are expressed as mean ± SEM. LSD (100 or 200 *µ*g), psilocybin (15 or 30 mg), or placebo was administered at t = 0 h. The correspond maximal effects and statistics are shown in Supplementary Table S5. From Holze *et al.,* 2022; "Figure S5."

Table S5. Mean values and statistics for the acute autonomic and endorine effects of LSD, psilocybin, and placebo

		Placebo (mean ± SEM)	15 mg Psilocybin (mean ± SEM)	30 mg Psilocybin (mean ± SEM)	100 µg LSD (mean ± SEM)	200 µg LSD (mean ± SEM)	$F_{4,108}$	P=	Pla - 15 mg Psilo	Pla - 30 mg Psilo	Pla - 100 µg LSD	Pla - 200 µg LSD	15 mg Psilo - 30 mg Psilo	15 mg Psilo - 100 µg LSD	15 mg Psilo - 200 µg LSD	30 mg Psilo - 100 µg LSD	30 mg Psilo - 200 µg LSD	100 µg LSD - 200 µg LSD
Autonomic Effects																		
Systolic blood pressure (mmHg)	E_{max}	131 ± 2.0	140 ± 2.2	146 ± 2.7	138 ± 2.4	141 ± 2.5	17.4	<0.001	***	***	**	***	*	NS	NS	***	*	NS
Diastolic blood pressure (mmHg)	E_{max}	82 ± 1.1	89 ± 1.6	93 ± 1.6	86 ± 1.6	87 ± 1.7	23.4	<0.001	***	***	*	***	*	*	NS	***	***	NS
Mean arterial pressure (mmHg)	E_{max}	98 ± 1.4	106 ± 1.5	110 ± 1.8	102 ± 1.7	104 ± 1.9	25.3	<0.001	***	***	**	***	*	*	NS	***	***	NS
Heart rate (beats/min)	E_{max}	74 ± 2.0	78 ± 2.1	82 ± 3.1	83 ± 2.5	90 ± 3.2	14.8	<0.001	NS	**	***	***	NS	NS	***	NS	**	*
Rate pressure product (mmHg x bpm)	E_{max}	9334 ± 327	10443 ± 372	11344 ± 523	11119 ± 399	12097 ± 470	15.2	<0.001	*	***	***	***	NS	NS	***	NS	NS	(*)
Body temperature (°C)	E_{max}	37.1 ± 0.1	37.6 ± 0.07	37.9 ± 0.09	37.6 ± 0.05	37.5 ± 0.08	25.1	<0.001	***	***	***	***	**	NS	NS	**	**	NS
Pupil dilation (mm)	E_{max}	5.8 ± 0.2	6.5 ± 0.2	6.6 ± 0.2	6.6 ± 0.2	6.7 ± 0.2	53.0	<0.001	***	***	***	***	NS	NS	NS	NS	NS	NS
	E_{min}	4.0 ± 0.1	4.9 ± 0.2	5.4 ± 0.2	5.0 ± 0.2	5.1 ± 0.2	62.4	<0.001	***	***	***	***	***	NS	NS	***	*	NS
Pupil contratction (mm)	E_{min}	1.5 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	10.1	<0.001	NS	***	NS	*	**	NS	NS	***	*	NS
List of Complaints (LC Score)																		
Acute adverse effects	0-12 h	1.9 ± 0.6	8.9 ± 1.4	13 ± 1.6	12 ± 2.0	15 ± 2.0	22.07	<0.001	***	***	***	***	(*)	NS	**	NS	NS	NS
Subacute adverse effects	12-24 h	1.3 ± 0.3	2.4 ± 0.5	4.1 ± 0.8	3.1 ± 0.6	4.8 ± 0.8	6.53	<0.001	NS	**	NS	***	NS	NS	*	NS	NS	NS
Adverse Effects	E_{max}	2.1 ± 0.6	9.0 ± 1.4	14 ± 1.6	13 ± 1.9	15 ± 2.0	23.04	<0.001	***	***	***	***	*	NS	***	NS	NS	NS
Hormones and Markers																		
Cortisol (nmol/L)	$\Delta C_{baseline}$	-162 ± 22	43 ± 28.8	168 ± 36.3	96 ± 22	180 ± 36	30.28	<0.001	***	***	***	***	**	NS	**	NS	NS	NS
Prolactin (mU/L)	$\Delta C_{baseline}$	-128 ± 38	8.7 ± 42	283 ± 72	162 ± 67	63 ± 51	11.10	<0.001	NS	***	***	*	***	NS	NS	NS	**	NS
Oxytocin (pg/mL)	$\Delta C_{baseline}$	-14 ± 8	8.3 ± 4.9	31 ± 12	51 ± 13	50 ± 13	8.16	<0.001	NS	**	***	***	NS	*	*	NS	NS	NS
BDNF (pg/mL)	ΔC_{max}	4.2 ± 1.8	3.4 ± 1.0	4.0 ± 0.8	1.6 ± 0.6	4.5 ± 0.9	0.45	NS	-	-	-	-	-	-	-	-	-	-

(*)P<0.1, *P<0.05, **P<0.01, ***P<0.001; NS, not significant; $\Delta c_{baseline}$, concentration difference from baseline; N=28; BDNF, Brain-Derived Neurotrophic Factor.

Table 3: Mean values and statistics for the acute autonomic and endocrine effects of LSD, psilocybin, and placebo. From Holze *et al.,* (2022).

Moreover, this study provides noteworthy evidence that the acute effects of LSD and psilocybin are largely similar in terms of subjective experiences, with differences in effect duration being a primary distinguishing factor. In addition, both substances were found to induce a stress response as evidenced by increased cortisol levels in a dose-dependent manner (**Figure 6** and **Table 3**). These findings contribute valuable information for dose finding and understanding the therapeutic potential of these psychedelics in clinical research and treatment contexts.

Building in these findings, a study by Mason et al. (2023), explored the effects of psilocybin on immune function, stress response, and psychological outcomes (170). This research aimed to elucidate the mechanisms by which psilocybin exerts its therapeutic effects, particularly focusing on its interaction with the immune system and the HPA axis. They found that acutely, psilocybin administration led to a significant increase in cortisol concentrations, peaking around 80 minutes post-administration. As seen in **Figure 7**, cortisol levels in the psilocybin group increased from a baseline mean of 12.89 ng/mL to a peak of 17.15 ng/mL approximately 80 minutes post-administration. This indicates an increase of 4.26 ng/mL in cortisol levels following psilocybin

administration. In contrast, the placebo group showed a baseline mean cortisol level of 12.01 ng/mL, which decreased to 9.45 ng/mL at the same time, indicating a 2.56 ng/mL decrease (170). Therefore, the data demonstrates a more pronounced cortisol response in the psilocybin group, highlighting its HPA axis activation.

To further this novel intersection between stress and psychedelics, the study evaluated participants' reactions to a psychosocial stressor seven days post-administration. This involved a stress-induction protocol designed to elicit a robust stress response, including cortisol, blood pressure, and heart rate measurements. Furthermore, the results showed that psilocybin did not significantly alter these physiological stress markers compared to the placebo group (**Figure 7**). Both groups exhibited similar increases in cortisol, blood pressure, and heart rate in response to the stressor. However, a notable finding emerged in the subjective experience of stress. Remarkably, participants who received psilocybin did not report increased anxiety following the stress test, unlike those in the placebo group (170). This suggests that psilocybin may have an anxiolytic effect, reducing the subjective experience of anxiety even if physiological stress markers do not show significant differences.

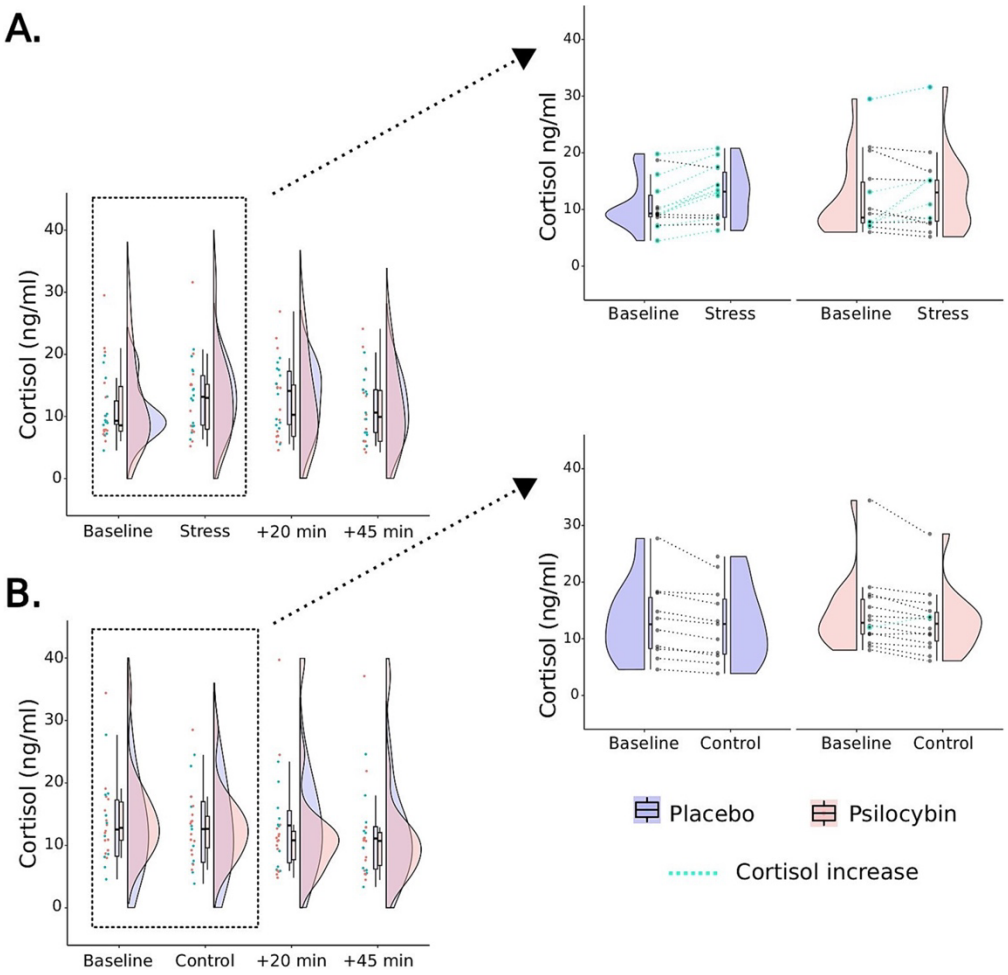

Figure 7: Neuroendocrine response (cortisol values) before, during, and after the stress. (A) or the control (B) protocol, in those who received psilocybin or placebo. The left panel displays the cortisol response across all time points. After the stress condition, both those who received psilocybin or placebo showed a significant increase in cortisol up to 45 min after the stress test. There were no significant changes in cortisol after the control condition. The right panel zooms in, displaying cortisol concentrations before the stress/control protocol and during the stress/control protocol. The connecting lines demonstrate how individual participant's cortisol concentrations changed over these two time points and are separated by drug treatment condition (placebo or psilocybin). Blue lines indicate a cortisol increase. Although numerically more people in the placebo group showed increased cortisol concentrations after stress compared to psilocybin, the group difference was not significant. From Holze *et al.,* (2023).

This novel interaction between stress and psychedelics could be significant for therapeutic applications, indicating that psilocybin might help individuals manage stress more effectively by activating the HPA axis and mitigating anxiety responses. Furthermore, while psilocybin acutely activates the HPA axis, as evidenced by increased cortisol levels, it does not appear to have a lasting impact on increased cortisol concentration or physiological stress responses. Instead, its potential anxiolytic effects could provide stress resilience and a recalibration of the HPA axis and glucocorticoid levels.

These studies indicate that the psilocybin-induced stress response and increase in cortisol could be a critical factor driving reduction in depression and anxiety. While correlations between drug-induced cortisol elevation, self-reported anxiety, and treatment outcomes have been reported for human studies during psilocybin-assisted therapy, the mechanistic relationship between psychedelic-associated alterations in plasma glucocorticoid responses and the time course of anxious responsiveness remains unclear. Current research on psilocybin has primarily focused on its psychological effects and therapeutic potential in treating various mental health disorders. However, the specific interaction between psilocybin and the drug-induced stress response mediated by the HPA axis remains poorly understood. This represents a significant knowledge gap for the novel interaction between stress and psychedelics, as investigating this relationship could further clarify the mechanisms underlying psilocybin's therapeutic benefits and help optimize its use in clinical settings. More research is needed to fully understand its interaction with the stress response. To address this gap in knowledge, my graduate thesis research has provided valuable insights on the interaction between psilocybin and the stress response through animal studies. My research explored how psilocybin influences glucocorticoid levels and anxiety-like behaviors, providing evidence for a novel mechanism of action underlying its anxiolytic effects.

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*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, 32(12), 1135–1144. https://doi.org/10.1091/mbc.E20-05-0340*

REFERENCES

1. Mental Illness. *National Institute of Mental Health* (2023)
2. WHO. Depression and Other Common Mental Disorders - Global Health Estimates. *Geneva: World Health Organization* (2017)
3. Major Depression. *National Institute of Mental Health* (2023)
4. Abuse S. “Key substance use and mental health indicators in the United States: Results from the 2021 National Survey on Drug Use and Health (HHS Publication No. PEP22-07-01-005, NSDUH Series H-57.,” *Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration*. (2022)
5. Pratt LA, Brody DJ, Gu Q. Antidepressant Use among Persons Aged 12 and Over: United States, 2011-2014. NCHS Data Brief. Number 283. *National Center for Health Statistics* (2017)
6. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, Niederehe G, Thase ME, Lavori PW, Lebowitz BD, et al. Acute and Longer-Term Outcomes in Depressed Outpatients Requiring One or Several Treatment Steps: A STAR*D Report. *American Journal of Psychiatry* (2006) 163:1905–1917. doi: 10.1176/ajp.2006.163.11.1905
7. Hill C, Hospital MG. What Did STAR ∗ D Teach Us ? Results From a Large-Scale , Practical , Clinical Trial for Patients With Depression. (2009) 60:
8. Bosman RC, Waumans RC, Jacobs GE, Oude Voshaar RC, Muntingh ADT, Batelaan NM, Van Balkom AJLM. Failure to Respond after Reinstatement of Antidepressant Medication: A Systematic Review. *Psychother Psychosom* (2018) 87:268–275. doi: 10.1159/000491550
9. Cipriani A, Furukawa TA, Salanti G, Chaimani A, Atkinson LZ, Ogawa Y, Leucht S, Ruhe HG, Turner EH, Higgins JPT. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *The Lancet* (2018) 391:1357–1366.
10. Thase ME, Mahableshwarkar AR, Dragheim M, Loft H, Vieta E. A meta-analysis of randomized, placebo-controlled trials of vortioxetine for the treatment of major depressive disorder in adults. *European Neuropsychopharmacology* (2016) 26:979–993.

11. Daly EJ, Trivedi MH, Janik A, Li H, Zhang Y, Li X, Lane R, Lim P, Duca AR, Hough D. Efficacy of esketamine nasal spray plus oral antidepressant treatment for relapse prevention in patients with treatment-resistant depression: a randomized clinical trial. *JAMA Psychiatry* (2019) 76:893–903.

12. Gotvaldová K, Hájková K, Borovička J, Jurok R, Cihlářová P, Kuchař M. Stability of psilocybin and its four analogs in the biomass of the psychotropic mushroom Psilocybe cubensis. *Drug Test Anal* (2021) 13:439–446.

13. Mahmoudi E, Faizi M, Hajiaghaee R, Razmi A. Alteration of Depressive-like Behaviors by Psilocybe cubensis Alkaloid Extract in Mice: the Role of Glutamate Pathway. *Research Journal of Pharmacognosy* (2018) 5:17–24. doi: 10.22127/RJP.2018.58486

14. dos Santos RG, Hallak JEC. Ayahuasca, an ancient substance with traditional and contemporary use in neuropsychiatry and neuroscience. *Epilepsy and Behavior* (2019)106300. doi: 10.1016/j.yebeh.2019.04.053

15. McKenna DJ. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. *Pharmacol Ther* (2004) 102:111–129.

16. Harner MJ. *Hallucinogens and shamanism.* Oxford, England: Oxford U. Press. (1973). 200, xv, 200–xv p.

17. Labate BC, Cavnar C. *Ayahuasca shamanism in the Amazon and beyond*. Oxford University Press, USA. (2014).

18. De Rios MD. A modern-day shamanistic healer in the Peruvian Amazon: pharmacopoeia and trance. *J Psychoactive Drugs* (1989) 21:91–99.

19. de Rios MD. *Hallucinogens, Cross-cultural Perspectives*. University of New Mexico Press. (1984). https://books.google.com/books?id=IdSAAAAAMAAJ

20. de Rios MD. María Sabina: Her Life and Chants. (1982)

21. Borowiak KS, Ciechanowski K, Waloszczyk P. Psilocybin mushroom (Psilocybe semilanceata) intoxication with myocardial infarction. *J Toxicol Clin Toxicol* (1998) 36:47–49.

22. Ross S, Agrawal M, Griffiths RR, Grob C, Berger A, Henningfield JE. Psychedelic-assisted psychotherapy to treat psychiatric and existential distress in life-threatening medical

illnesses and palliative care. *Neuropharmacology* (2022) 216:109174. doi: https://doi.org/10.1016/j.neuropharm.2022.109174

23. Nichols DE. Psychedelics. *Pharmacol Rev* (2016) 68:264–355.

24. Banks MI, Zahid Z, Jones NT, Sultan ZW, Wenthur CJ. Catalysts for change: the cellular neurobiology of psychedelics. *Mol Biol Cell* (2021) 32:1135–1144. doi: 10.1091/mbc.e20-05-0340

25. Nutt D, Erritzoe D, Carhart-Harris R. Psychedelic Psychiatry's Brave New World. *Cell* (2020) 181:24–28. doi: 10.1016/j.cell.2020.03.020

26. Sessa B. Can psychedelics have a role in psychiatry once again? *Br J Psychiatry* (2005) 186:457–458. https://api.semanticscholar.org/CorpusID:854916

27. Sessa B. Shaping the renaissance of psychedelic research. *The Lancet* (2012) 380:200–201.

28. Nicholas CR, Henriquez KM, Gassman MC, Cooper KM, Muller D, Hetzel S, Brown RT, Cozzi N V, Thomas C, Hutson PR. High dose psilocybin is associated with positive subjective effects in healthy volunteers. *Journal of psychopharmacology* (2018) 32:770–778.

29. Nicholas CR, Henriquez KM, Gassman MC, Cooper KM, Muller D, Hetzel S, Brown RT, Cozzi N V, Thomas C, Hutson PR. High dose psilocybin is associated with positive subjective effects in healthy volunteers. *Journal of psychopharmacology* (2018) 32:770–778.

30. Carhart-Harris RL, Roseman L, Haijen E, Erritzoe D, Watts R, Branchi I, Kaelen M. Psychedelics and the essential importance of context. *Journal of Psychopharmacology* (2018) 32:725–731. doi: 10.1177/0269881118754710

31. Leger RF, Unterwald EM. Assessing the effects of methodological differences on outcomes in the use of psychedelics in the treatment of anxiety and depressive disorders: A systematic review and meta-analysis. *Journal of Psychopharmacology* (2021) doi: 10.1177/02698811211044688

32. Pearson CS, Siegel JS, Gold JA. Psilocybin-assisted psychotherapy for depression: Emerging research on a psychedelic compound with a rich history. *J Neurol Sci* (2021) 434: https://api.semanticscholar.org/CorpusID:245258099

33. Roseman L, Nutt DJ, Carhart-Harris RL. Quality of acute psychedelic experience predicts therapeutic efficacy of psilocybin for treatment-resistant depression. *Front Pharmacol* (2018) 8: doi: 10.3389/fphar.2017.00974

34. Gukasyan N, Davis AK, Barrett FS, Cosimano MP, Sepeda ND, Johnson MW, Griffiths RR. Efficacy and safety of psilocybin-assisted treatment for major depressive disorder: Prospective 12-month follow-up. *Journal of Psychopharmacology* (2022) 36:151–158. doi: 10.1177/02698811211073759

35. Griffiths RR, Johnson MW, Carducci MA, Umbricht A, Richards WA, Richards BD, Cosimano MP, Klinedinst MA. Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial. *Journal of Psychopharmacology* (2016) 30:1181–1197. doi: 10.1177/0269881116675513

36. Davis AK, Barrett FS, May DG, Cosimano MP, Sepeda ND, Johnson MW, Finan PH, Griffiths RR. Effects of Psilocybin-Assisted Therapy on Major Depressive Disorder: A Randomized Clinical Trial. *JAMA Psychiatry* (2021) 78:481–489. doi: 10.1001/jamapsychiatry.2020.3285

37. Doss MK, Považan M, Rosenberg MD, Sepeda ND, Davis AK, Finan PH, Smith GS, Pekar JJ, Barker PB, Griffiths RR, et al. Psilocybin therapy increases cognitive and neural flexibility in patients with major depressive disorder. *Transl Psychiatry* (2021) 11:1–10. doi: 10.1038/s41398-021-01706-y

38. Muttoni S, Ardissino M, John C. Classical psychedelics for the treatment of depression and anxiety: A systematic review. *J Affect Disord* (2019) 258:11–24. doi: 10.1016/j.jad.2019.07.076

39. Goldberg SB, Pace BT, Nicholas CR, Raison CL, Hutson PR. The experimental effects of psilocybin on symptoms of anxiety and depression: A meta-analysis. *Psychiatry Res* (2020) 284:112749. doi: 10.1016/j.psychres.2020.112749

40. Davis AK, Barrett FS, Griffiths RR. Psychological flexibility mediates the relations between acute psychedelic effects and subjective decreases in depression and anxiety. *J Contextual Behav Sci* (2020) 15:39–45.

41. Mitchell JM, Bogenschutz M, Lilienstein A, Harrison C, Kleiman S, Parker-Guilbert K, Ot'alora G M, Garas W, Paleos C, Gorman I. MDMA-assisted therapy for severe PTSD: a

randomized, double-blind, placebo-controlled phase 3 study. *Focus (Madison)* (2023) 21:315–328.

42. Smith KW, Sicignano DJ, Hernandez A V, White CM. MDMA-assisted psychotherapy for treatment of posttraumatic stress disorder: A systematic review with meta-analysis. *The Journal of Clinical Pharmacology* (2022) 62:463–471.

43. Illingworth BJG, Lewis DJ, Lambarth AT, Stocking K, Duffy JMN, Jelen LA, Rucker JJ. A comparison of MDMA-assisted psychotherapy to non-assisted psychotherapy in treatment-resistant PTSD: A systematic review and meta-analysis. *Journal of psychopharmacology* (2021) 35:501–511.

44. Morgan C, McAndrew A, Stevens T, Nutt D, Lawn W. Tripping up addiction: the use of psychedelic drugs in the treatment of problematic drug and alcohol use. *Curr Opin Behav Sci* (2017) 13:71–76.

45. Koslowski M, Johnson MW, Gründer G, Betzler F. Novel treatment approaches for substance use disorders: therapeutic use of psychedelics and the role of psychotherapy. *Curr Addict Rep* (2021)1–11.

46. Argento E, Tupper KW, Socias ME. The tripping point: The potential role of psychedelic-assisted therapy in the response to the opioid crisis. *International Journal of Drug Policy* (2019) 66:80–81. doi: 10.1016/j.drugpo.2018.11.006

47. Griffiths RR, Johnson MW, Carducci MA, Umbricht A, Richards WA, Richards BD, Cosimano MP, Klinedinst MA. Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial. *Journal of Psychopharmacology* (2016) 30:1181–1197. doi: 10.1177/0269881116675513

48. Hofmann A. *LSD, My Problem Child: Reflections on Sacred Drugs, Mysticism, and Science*. Multidisciplinary Assoc. for Psychedelic Studies. (2005). https://books.google.com/books?id=vq0kAQAAMAAJ

49. Hofmann A. How LSD originated. *J Psychedelic Drugs* (1979) 11:53–60.

50. Hofmann A. The discovery of LSD and subsequent investigations on naturally occurring hallucinogens. *Discoveries in biological psychiatry* (1970)91–106.

51. Geiger H, Wurst M, Daniels RN. DARK Classics in Chemical Neuroscience: Psilocybin. *ACS Chem Neurosci* (2018) 9 10:2438–2447. https://api.semanticscholar.org/CorpusID:49591766

52. Huxley A. *The Doors Of Perception*. HarperCollins Canada. (2014). https://books.google.com/books?id=bRjtAQAAQBAJ

53. Huxley A. *Brave new world*. DigiCat. (2022).

54. Meckier J. Aldous huxley: Satire and structure. *H Bloom (ed)* (2010) 2010:31–40.

55. Tanne JH. Humphry osmond. *BMJ: British Medical Journal* (2004) 328:713.

56. Osmond H, Smythies J. Schizophrenia: a new approach. *Journal of Mental Science* (1952) 98:309–315.

57. Siegler M, Osmond H, Newell S. Models of alcoholism. *Q J Stud Alcohol* (1968) 29:571–591.

58. Leary T, Metzner R, Weil GM. *The Psychedelic Reader: Classic Selections from the Psychedelic Review, the Revolutionary 1960's Forum of Psychopharmacological Substances*. Citadel. (1965).

59. Timothy L, Ralph M, Madison P, Gunther W, Ralph S, Sara K. A new behavior change program using psilocybin. *Psychotherapy: Theory, Research & Practice* (1965) 2:61.

60. Leary T. The effectsof consciousness-expanding drugs on prisoner rehabilitation. *Psychedelic Rev* (1969) 10:29–45.

61. Leary T. *Flashbacks, an Autobiography*. J.P. Tarcher. (1983). https://books.google.com/books?id=be99AAAAMAAJ

62. Leary T, Litwin GH, Metzner R. Reactions to psilocybjn administered in a supportive environment. *J Nerv Ment Dis* (1963) 137:561–573.

63. Metzner R. Reflections on the concord prison project and the follow-up study. *J Psychoactive Drugs* (1998) 30:427–428.

64. Doblin R. Pahnke's "Good Friday experiment": A long-term follow-up and methodological critique. *Journal of Transpersonal Psychology* (1991) 23:1–28.

65. Neitzke-Spruill L. Psychedelics and desistance from crime: Lessons from the concord prison experiment. *J Humanist Psychol* (2022)00221678221136233.

66. Vollenweider FX, Vollenweider-Scherpenhuyzen MFI, Bäbler A, Vogel H, Hell D. Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport* (1998) 9:3897–3902.

67. Rajpal H, Mediano PAM, Rosas FE, Timmermann CB, Brugger S, Muthukumaraswamy S, Seth AK, Bor D, Carhart-Harris RL, Jensen HJ. Psychedelics and schizophrenia: Distinct alterations to Bayesian inference. doi: 10.1101/2022.01.31.478484

68. Moreno JL, Miranda-Azpiazu P, García-Bea A, Younkin J, Cui M, Kozlenkov A, Ben-Ezra A, Voloudakis G, Fakira AK, Baki L, et al. Allosteric signaling through an mGlu2 and 5-HT2A heteromeric receptor complex and its potential contribution to schizophrenia. *Sci Signal* (2016) 9:1–19. doi: 10.1126/scisignal.aab0467

69. Greenfield R. *Timothy Leary: A Biography*. Harcourt, Incorporated. (2006). https://books.google.com/books?id=of4J2dbKvMoC

70. Lattin D. *The Harvard Psychedelic Club: How Timothy Leary, Ram Dass, Huston Smith, and Andrew Weil Killed the Fifties and Ushered in a New Age for America*. HarperCollins. (2011). https://books.google.com/books?id=yNylsx9HD28C

71. Grady C. Institutional review boards: Purpose and challenges. *Chest* (2015) 148:1148–1155.

72. Khan I. Convention on psychotropic substances, 1971: The Role and Responsibilities of the World Health Organization. *Prog Neuropsychopharmacol* (1979) 3:11–14. doi: https://doi.org/10.1016/0364-7722(79)90064-X

73. Beswerchij A, Sisti D. From Underground to Mainstream: Establishing a Medical Lexicon for Psychedelic Therapy. *Front Psychiatry* (2022) 13: https://www.frontiersin.org/journals/psychiatry/articles/10.3389/fpsyt.2022.870507

74. Halberstadt AL, Geyer MA. Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. *Neuropharmacology* (2011) 61:364–381.

75. Rickli A, Moning OD, Hoener MC, Liechti ME. Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. *European Neuropsychopharmacology* (2016) 26:1327–1337.

76. Valle M, Maqueda AE, Rabella M, Rodríguez-Pujadas A, Antonijoan RM, Romero S, Alonso JF, Mañanas MÀ, Barker S, Friedlander P. Inhibition of alpha oscillations through

serotonin-2A receptor activation underlies the visual effects of ayahuasca in humans. *European Neuropsychopharmacology* (2016) 26:1161–1175.

77. Madsen MK, Fisher PM, Burmester D, Dyssegaard A, Stenbæk DS, Kristiansen S, Johansen SS, Lehel S, Linnet K, Svarer C. Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels. *Neuropsychopharmacology* (2019) 44:1328–1334.

78. Preller KH, Duerler P, Burt JB, Ji JL, Adkinson B, Stämpfli P, Seifritz E, Repovš G, Krystal JH, Murray JD. Psilocybin induces time-dependent changes in global functional connectivity. *Biol Psychiatry* (2020) 88:197–207.

79. Halberstadt AL, Geyer MA. Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. *Neuropharmacology* (2011) 61:364–381.

80. Pokorny T, Preller KH, Kraehenmann R, Vollenweider FX. Modulatory effect of the 5-HT1A agonist buspirone and the mixed non-hallucinogenic 5-HT1A/2A agonist ergotamine on psilocybin-induced psychedelic experience. *European Neuropsychopharmacology* (2016) 26:756–766.

81. Jakab RL, Goldman-Rakic PS. 5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proceedings of the National Academy of Sciences* (1998) 95:735–740.

82. Weber ET, Andrade R. Htr2a gene and 5-HT2A receptor expression in the cerebral cortex studied using genetically modified mice. *Front Neurosci* (2010) 4:36.

83. Meyer JH, McMain S, Kennedy SH, Korman L, Brown GM, DaSilva JN, Wilson AA, Blak T, Eynan-Harvey R, Goulding VS. Dysfunctional attitudes and 5-HT2 receptors during depression and self-harm. *American journal of Psychiatry* (2003) 160:90–99.

84. Bhagwagar Z, Hinz R, Taylor M, Fancy S, Cowen P, Grasby P. Increased 5-HT 2A receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [11 C] MDL 100,907. *American Journal of Psychiatry* (2006) 163:1580–1587.

85. Yatham LN, Liddle PF, Dennie J, Shiah I-S, Adam MJ, Lane CJ, Lam RW, Ruth TJ. 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) 56:705–711.

86. Meyer JH, Kapur S, Eisfeld B, Brown GM, Houle S, DaSilva J, Wilson AA, Rafi-Tari S, Mayberg HS, Kennedy SH. The effect of paroxetine on 5-HT2A receptors in depression: an [18F] setoperone PET imaging study. *American Journal of Psychiatry* (2001) 158:78–85.

87. Buckholtz NS, Zhou D, Freedman DX. Serotonin2 agonist administration down-regulates rat brain serotonin2 receptors. *Life Sci* (1988) 42:2439–2445.

88. López-Giménez JF, González-Maeso J. Hallucinogens and serotonin 5-HT 2A receptor-mediated signaling pathways. *Behavioral neurobiology of psychedelic drugs* (2018)45–73.

89. Sleight AJ, Stam NJ, Mutel V, Vanderheyden PML. Radiolabelling of the human 5-HT2A receptor with an agonist, a partial agonist and an antagonist: effects on apparent agonist affinities. *Biochem Pharmacol* (1996) 51:71–76.

90. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* (1994) 46:157–203.

91. Day M, Olson PA, Platzer J, Striessnig J, Surmeier DJ. Stimulation of 5-HT2 receptors in prefrontal pyramidal neurons inhibits Cav1. 2 L-type Ca2+ currents via a PLCβ/IP3/calcineurin signaling cascade. *J Neurophysiol* (2002) 87:2490–2504.

92. Berridge MJ. The Inositol Trisphosphate/Calcium Signaling Pathway in Health and Disease. *Physiol Rev* (2016) 96:1261–1296. doi: 10.1152/physrev.00006.2016

93. Kamato D, Thach L, Bernard R, Chan V, Zheng W, Kaur H, Brimble M, Osman N, Little PJ. Structure, Function, Pharmacology, and Therapeutic Potential of the G Protein, Gα/q,11. *Front Cardiovasc Med* (2015) 2:1–11. doi: 10.3389/fcvm.2015.00014

94. Passie T, Seifert J, Schneider U, Emrich HM. The pharmacology of psilocybin. *Addiction Biology* (2002) 7:357–364. doi: https://doi.org/10.1080/1355621021000005937

95. Kometer M, Vollenweider FX. “Serotonergic Hallucinogen-Induced Visual Perceptual Alterations.,” In: Halberstadt AL, Vollenweider FX, Nichols DE, editors. *Behavioral Neurobiology of Psychedelic Drugs*. Berlin, Heidelberg: Springer Berlin Heidelberg (2018). p. 257–282 doi: 10.1007/7854_2016_461

96. Valle M, Maqueda AE, Rabella M, Rodríguez-Pujadas A, Antonijoan RM, Romero S, Alonso JF, Mañanas MÀ, Barker S, Friedlander P, et al. Inhibition of alpha oscillations through serotonin-2A receptor activation underlies the visual effects of ayahuasca in humans. *European Neuropsychopharmacology* (2016) 26:1161–1175. doi: https://doi.org/10.1016/j.euroneuro.2016.03.012

97. Passie T, Seifert J, Schneider U, Emrich HM. The pharmacology of psilocybin. *Addiction Biology* (2002) 7:357–364. doi: https://doi.org/10.1080/1355621021000005937

98. Ray TS. Psychedelics and the Human Receptorome. *PLoS One* (2010) 5:e9019. doi: 10.1371/journal.pone.0009019

99. Garnovskaya MN, Nebigil CG, Arthur JM, Spurney RF, Raymond JR. 5-Hydroxytryptamine2A receptors expressed in rat renal mesangial cells inhibit cyclic AMP accumulation. *Mol Pharmacol* (1995) 48:230–237.

100. Tedford HW, Zamponi GW. Direct G protein modulation of Cav2 calcium channels. *Pharmacol Rev* (2006) 58:837–862.

101. Betke KM, Wells CA, Hamm HE. GPCR mediated regulation of synaptic transmission. *Prog Neurobiol* (2012) 96:304–321.

102. Kenakin TP. Biased signalling and allosteric machines: new vistas and challenges for drug discovery. *Br J Pharmacol* (2012) 165:1659–1669.

103. Roth BL, Choudhary MS, Khan N, Uluer AZ. High-affinity agonist binding is not sufficient for agonist efficacy at 5-hydroxytryptamine2A receptors: evidence in favor of a modified ternary complex model. *Journal of Pharmacology and Experimental Therapeutics* (1997) 280:576–583.

104. Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P, Clarke WP. Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol Pharmacol* (1998) 54:94–104.

105. Fitzgerald LW, Conklin DS, Krause CM, Marshall AP, Patterson JP, Tran DP, Iyer G, Kostich WA, Largent BL, Hartig PR. 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) 72:2127–2134.

106. Egan C, Grinde E, Dupre A, Roth BL, Hake M, Teitler M, Herrick-Davis K. Agonist high and low affinity state ratios predict drug intrinsic activity and a revised Ternary complex mechanism at serotonin 5-HT2A and 5-HT2C receptors. *Synapse* (2000) 35:144–150.

107. Cussac D, Boutet-Robinet E, Ailhaud M-C, Newman-Tancredi A, Martel J-C, Danty N, Rauly-Lestienne I. 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) 594:32–38.

108. López-Giménez JF, González-Maeso J. Hallucinogens and serotonin 5-HT 2A receptor-mediated signaling pathways. *Behavioral neurobiology of psychedelic drugs* (2018)45–73.

109. Wang C-D, Gallaher TK, Shih JC. Site-directed mutagenesis of the serotonin 5-hydroxytrypamine2 receptor: identification of amino acids necessary for ligand binding and receptor activation. *Mol Pharmacol* (1993) 43:931–940.

110. Shapiro DA, Kristiansen K, Kroeze WK, Roth BL. Differential modes of agonist binding to 5-hydroxytryptamine2A serotonin receptors revealed by mutation and molecular modeling of conserved residues in transmembrane region 5. *Mol Pharmacol* (2000) 58:877–886.

111. Kanagarajadurai K, Malini M, Bhattacharya A, Panicker MM, Sowdhamini R. Molecular modeling and docking studies of human 5-hydroxytryptamine 2A (5-HT2A) receptor for the identification of hotspots for ligand binding. *Mol Biosyst* (2009) 5:1877–1888.

112. Banerjee AA, Vaidya VA. Differential signaling signatures evoked by DOI versus lisuride stimulation of the 5-HT2A receptor. *Biochem Biophys Res Commun* (2020) 531:609–614.

113. Garcia EE, Smith RL, Sanders-Bush E. Role of Gq protein in behavioral effects of the hallucinogenic drug 1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane. *Neuropharmacology* (2007) 52:1671–1677.

114. González-Maeso J, Weisstaub N V, Zhou M, Chan P, Ivic L, Ang R, Lira A, Bradley-Moore M, Ge Y, Zhou Q. Hallucinogens recruit specific cortical 5-HT2A receptor-mediated signaling pathways to affect behavior. *Neuron* (2007) 53:439–452.

115. Karaki S, Becamel C, Murat S, La Cour CM, Millan MJ, Prezeau L, Bockaert J, Marin P, Vandermoere F. Quantitative phosphoproteomics unravels biased phosphorylation of serotonin 2A receptor at Ser280 by hallucinogenic versus nonhallucinogenic agonists. *Molecular & Cellular Proteomics* (2014) 13:1273–1285.

116. Kim K, Che T, Panova O, DiBerto JF, Lyu J, Krumm BE, Wacker D, Robertson MJ, Seven AB, Nichols DE. Structure of a hallucinogen-activated Gq-coupled 5-HT2A serotonin receptor. *Cell* (2020) 182:1574–1588.

117. Pottie E, Dedecker P, Stove CP. Identification of psychedelic new psychoactive substances (NPS) showing biased agonism at the 5-HT2AR through simultaneous use of β-arrestin 2 and miniGαq bioassays. *Biochem Pharmacol* (2020) 182:114251.

118. Wacker D, Wang S, McCorvy JD, Betz RM, Venkatakrishnan AJ, Levit A, Lansu K, Schools ZL, Che T, Nichols DE. Crystal structure of an LSD-bound human serotonin receptor. *Cell* (2017) 168:377–389.

119. Schmid CL, Raehal KM, Bohn LM. Agonist-directed signaling of the serotonin 2A receptor depends on β-arrestin-2 interactions in vivo. *Proceedings of the National Academy of Sciences* (2008) 105:1079–1084.

120. Schmid CL, Bohn LM. Serotonin, but not N-methyltryptamines, activates the serotonin 2A receptor via a β-arrestin2/Src/Akt signaling complex in vivo. *Journal of Neuroscience* (2010) 30:13513–13524.

121. Rickli A, Moning OD, Hoener MC, Liechti ME. Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. *European Neuropsychopharmacology* (2016) 26:1327–1337.

122. Halberstadt AL, Geyer MA. Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. *Neuropharmacology* (2011) 61:364–381.

123. Garcia-Romeu A, Barrett FS, Carbonaro TM, Johnson MW, Griffiths RR. Optimal dosing for psilocybin pharmacotherapy: Considering weight-adjusted and fixed dosing approaches. *Journal of Psychopharmacology* (2021) 35:353–361. doi: 10.1177/0269881121991822

124. MacCallum CA, Lo LA, Pistawka CA, Deol JK. Therapeutic use of psilocybin: Practical considerations for dosing and administration. *Front Psychiatry* (2022) 13: https://www.frontiersin.org/journals/psychiatry/articles/10.3389/fpsyt.2022.1040217

125. Davis AK, Barrett FS, May DG, Cosimano MP, Sepeda ND, Johnson MW, Finan PH, Griffiths RR. Effects of Psilocybin-Assisted Therapy on Major Depressive Disorder: A

Randomized Clinical Trial. *JAMA Psychiatry* (2020)1–9. doi: 10.1001/jamapsychiatry.2020.3285

126. Hasler F, Bourquin D, Brenneisen R, Bär T, Vollenweider FX. Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. *Pharm Acta Helv* (1997) 72:175–184.

127. Turton S, Nutt DJ, Carhart-Harris RL. A qualitative report on the subjective experience of intravenous psilocybin administered in an FMRI environment. *Curr Drug Abuse Rev* (2014) 7:117–127.

128. Hasler F, Bourquin D, Brenneisen R, Bär T, Vollenweider FX. Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. *Pharm Acta Helv* (1997) 72:175–184.

129. Carhart-Harris RL, Bolstridge M, Rucker J, Day CMJ, Erritzoe D, Kaelen M, Bloomfield M, Rickard JA, Forbes B, Feilding A. Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. *Lancet Psychiatry* (2016) 3:619–627.

130. Brown RT, Nicholas CR, Cozzi N V, Gassman MC, Cooper KM, Muller D, Thomas CD, Hetzel SJ, Henriquez KM, Ribaudo AS, et al. Pharmacokinetics of Escalating Doses of Oral Psilocybin in Healthy Adults. *Clin Pharmacokinet* (2017) 56:1543–1554. doi: 10.1007/s40262-017-0540-6

131. Dinis-Oliveira RJ. Metabolism of psilocybin and psilocin: clinical and forensic toxicological relevance. *Drug Metab Rev* (2017) 49:84–91. https://api.semanticscholar.org/CorpusID:7656157

132. Fricke J, Sherwood A, Kargbo R, Orry A, Blei F, Naschberger A, Rupp B, Hoffmeister D. Enzymatic route toward 6-methylated baeocystin and psilocybin. *ChemBioChem* (2019) 20:2824–2829.

133. Irvine W, Tyler M, Delgoda R. In silico characterization of the psilocybin biosynthesis pathway. *Comput Biol Chem* (2023) 104:107854. doi: https://doi.org/10.1016/j.compbiolchem.2023.107854

134. Meyer MR, Maurer HH. Absorption, distribution, metabolism and excretion pharmacogenomics of drugs of abuse. *Pharmacogenomics* (2011) 12:215–233. doi: 10.2217/pgs.10.171

135. Manevski N, Kurkela M, Höglund C, Mauriala T, Court MH, Yli-Kauhaluoma J, Finel M. Glucuronidation of Psilocin and 4-Hydroxyindole by the Human UDP-Glucuronosyltransferases. *Drug Metabolism and Disposition* (2010) 38:386. doi: 10.1124/dmd.109.031138

136. Hasler F, Bourquin D, Brenneisen R, Vollenweider FX. Renal excretion profiles of psilocin following oral administration of psilocybin: a controlled study in man. *J Pharm Biomed Anal* (2002) 30:331–339.

137. Holze F, Becker AM, Kolaczynska KE, Duthaler U, Liechti ME. Pharmacokinetics and Pharmacodynamics of Oral Psilocybin Administration in Healthy Participants. *Clin Pharmacol Ther* (2023) 113:822–831. doi: 10.1002/cpt.2821

138. Rautio J, Laine K, Gynther M, Savolainen J. Prodrug Approaches for CNS Delivery. *AAPS J* (2008) 10:92–102. doi: 10.1208/s12248-008-9009-8

139. Vollenweider FX, Leenders KL, Scharfetter C, Maguire P, Stadelmann O, Angst J. Positron Emission Tomography and Fluorodeoxyglucose Studies of Metabolic Hyperfrontality and Psychopathology in the Psilocybin Model of Psychosis. *Neuropsychopharmacology* (1997) 16:357–372. doi: 10.1016/S0893-133X(96)00246-1

140. Gouzoulis-Mayfrank E, Schreckenberger M, Sabri O, Arning C, Thelen B, Spitzer M, Kovar K-A, Hermle L, Büll U, Sass H. Neurometabolic Effects of Psilocybin, 3,4-Methylenedioxyethylamphetamine (MDE) and d-Methamphetamine in Healthy Volunteers A Double-Blind, Placebo-Controlled PET Study with [18F]FDG. *Neuropsychopharmacology* (1999) 20:565–581. doi: 10.1016/S0893-133X(98)00089-X

141. Mason NL, Kuypers KPC, Müller F, Reckweg J, Tse DHY, Toennes SW, Hutten NRPW, Jansen JFA, Stiers P, Feilding A, et al. Me, myself, bye: regional alterations in glutamate and the experience of ego dissolution with psilocybin. *Neuropsychopharmacology* (2020) 45:2003–2011. doi: 10.1038/s41386-020-0718-8

142. Carhart-Harris RL, Erritzoe D, Williams T, Stone JM, Reed LJ, Colasanti A, Tyacke RJ, Leech R, Malizia AL, Murphy K, et al. Neural correlates of the psychedelic state as determined by fMRI studies with psilocybin. *Proceedings of the National Academy of Sciences* (2012) 109:2138–2143. doi: 10.1073/pnas.1119598109

143. Robin C-H, Bruna G, Rosalind W, Michelle B-J, Ashleigh M-B, Roberta M, Jonny M, Allan B, David E, J ND. Trial of Psilocybin versus Escitalopram for Depression. *New England Journal of Medicine* (2021) 384:1402–1411. doi: 10.1056/NEJMoa2032994

144. Nayak SM, Bari BA, Yaden DB, Spriggs MJ, Rosas FE, Peill JM, Giribaldi B, Erritzoe D, Nutt DJ, Carhart-Harris R. A Bayesian Reanalysis of a Trial of Psilocybin Versus Escitalopram for Depression. *Psychedelic Medicine* (2023) 1:18–26. doi: 10.1089/psymed.2022.0002

145. Szigeti B, Weiss B, Rosas FE, Erritzoe D, Nutt D, Carhart-Harris R. Assessing expectancy and suggestibility in a trial of escitalopram v. psilocybin for depression. *Psychol Med* (2024)1–8. doi: DOI: 10.1017/S0033291723003653

146. Murnane KS. Serotonin 2A receptors are a stress response system: implications for post-traumatic stress disorder. *Behavioural pharmacology* (2019) 30:151–162.

147. Brouwer A, Carhart-Harris RL. Pivotal mental states. *Journal of Psychopharmacology* (2021) 35:319–352.

148. Jones NT, Zahid Z, Grady SM, Sultan ZW, Zheng Z, Razidlo J, Banks MI, Wenthur CJ. Transient Elevation of Plasma Glucocorticoids Supports Psilocybin-Induced Anxiolysis in Mice. *ACS Pharmacol Transl Sci* (2023) 6:1221–1231. doi: 10.1021/acsptsci.3c00123

149. Galvão AC de M, De Almeida RN, Silva EADS, Freire FAM, Palhano-Fontes F, Onias H, Arcoverde E, Maia-de-Oliveira JP, De Araujo DB, Lobão-Soares B. Cortisol modulation by ayahuasca in patients with treatment resistant depression and healthy controls. *Front Psychiatry* (2018) 9:185.

150. Wegman-Points L, Pope B, Zobel-Mask A, Winter L, Wauson E, Duric V, Yuan L-L. Corticosterone as a Potential Confounding Factor in Delineating Mechanisms Underlying Ketamine's Rapid Antidepressant Actions. doi: 10.3389/fphar.2020.590221

151. Sowa J, Kusek M, Bobula B, Hess G, Tokarski K. Ketamine Administration Reverses Corticosterone-Induced Alterations in Excitatory and Inhibitory Transmission in the Rat Dorsal Raphe Nucleus. *Neural Plast* (2019) doi: 10.1155/2019/3219490

152. McEwen BS. 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) 1032:1–7.

153. Campos AC, Fogaça M V, Aguiar DC, Guimaraes FS. Animal models of anxiety disorders and stress. *Brazilian Journal of Psychiatry* (2013) 35:S101–S111.

154. Moda-Sava RN, Murdock MH, Parekh PK, Fetcho RN, Huang BS, Huynh TN, Witztum J, Shaver DC, Rosenthal DL, Alway EJ. Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. *Science (1979)* (2019) 364:eaat8078.

155. Planchez B, Surget A, Belzung C. Animal models of major depression: drawbacks and challenges. *J Neural Transm* (2019) 126:1383–1408.

156. Vyas S, Rodrigues AJ, Silva JM, Tronche F, Almeida OFX, Sousa N, Sotiropoulos I. Chronic Stress and Glucocorticoids: From Neuronal Plasticity to Neurodegeneration. *Neural Plast* (2016) 2016:6391686. doi: 10.1155/2016/6391686

157. Garcia LSB, Comim CM, Valvassori SS, Réus GZ, Stertz L, Kapczinski F, Gavioli EC, Quevedo J. Ketamine treatment reverses behavioral and physiological alterations induced by chronic mild stress in rats. *Prog Neuropsychopharmacol Biol Psychiatry* (2009) 33:450–455. doi: 10.1016/j.pnpbp.2009.01.004

158. Johnston JN, Thacker JS, Desjardins C, Kulyk BD, Romay-Tallon R, Kalynchuk LE, Caruncho HJ. Ketamine Rescues Hippocampal Reelin Expression and Synaptic Markers in the Repeated-Corticosterone Chronic Stress Paradigm. *Front Pharmacol* (2020) 11:1–13. doi: 10.3389/fphar.2020.559627

159. Wang W, Liu L, Yang X, Gao H, Tang Q-K, Yin L-Y, Yin X-Y, Hao J-R, Geng D-Q, Gao C. Ketamine improved depressive-like behaviors via hippocampal glucocorticoid receptor in chronic stress induced- susceptible mice. *Behavioural Brain Research* (2019) 364:75–84. doi: https://doi.org/10.1016/j.bbr.2019.01.057

160. Dutton M, Can AT, Lagopoulos J, Hermens DF. Stress, mental disorder and ketamine as a novel, rapid acting treatment. *European Neuropsychopharmacology* (2022) 65:15–29. doi: https://doi.org/10.1016/j.euroneuro.2022.09.006

161. Huang C-C, Yang C-H, Hsu K-S. Do stress and long-term potentiation share the same molecular mechanisms? *Mol Neurobiol* (2005) 32:223–235.

162. Cadle CE, Zoladz PR. Stress time-dependently influences the acquisition and retrieval of unrelated information by producing a memory of its own. *Front Psychol* (2015) 6:146433.

163. Pittenger C, Duman RS. Stress, Depression, and Neuroplasticity: A Convergence of Mechanisms. *Neuropsychopharmacology Reviews* (2008) 33:88–109. doi: 10.1038/sj.npp.1301574

164. Calder AE, Hasler G. Towards an understanding of psychedelic-induced neuroplasticity. *Neuropsychopharmacology* (2023) 48:104–112. doi: 10.1038/s41386-022-01389-z

165. Aleksandrova LR, Phillips AG. Neuroplasticity as a convergent mechanism of ketamine and classical psychedelics. *Trends Pharmacol Sci* (2021) 42:929–942. doi: 10.1016/j.tips.2021.08.003

166. Vargas M V, Dunlap LE, Dong C, Carter SJ, Tombari RJ, Jami SA, Cameron LP, Patel SD, Hennessey JJ, Saeger HN, et al. Psychedelics promote neuroplasticity through the activation of intracellular 5-HT2A receptors. *Science (1979)* (2023) 379:700–706. doi: 10.1126/science.adf0435

167. Hemrick-Luecke SK, Evans DC. 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-HT2A receptor mediation of the HPA axis. *Neuropharmacology* (2002) 42:162–169.

168. Hasler F, Grimberg U, Benz MA, Huber T, Vollenweider FX. Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose–effect study. *Psychopharmacology (Berl)* (2004) 172:145–156.

169. Holze F, Ley L, Müller F, Becker AM, Straumann I, Vizeli P, Kuehne SS, Roder MA, Duthaler U, Kolaczynska KE, et al. Direct comparison of the acute effects of lysergic acid diethylamide and psilocybin in a double-blind placebo-controlled study in healthy subjects. *Neuropsychopharmacology* (2022) 47:1180–1187. doi: 10.1038/s41386-022-01297-2

170. Mason NL, Szabo A, Kuypers KPC, Mallaroni PA, de la Torre Fornell R, Reckweg JT, Tse DHY, Hutten NRPW, Feilding A, Ramaekers JG. Psilocybin induces acute and persisting alterations in immune status in healthy volunteers: An experimental, placebo-controlled study. *Brain Behav Immun* (2023) 114:299–310. doi: https://doi.org/10.1016/j.bbi.2023.09.004

171. Van de Kar LD, Javed A, Zhang Y, Serres F, Raap DK, Gray TS. 5-HT2A receptors stimulate ACTH, corticosterone, oxytocin, renin, and prolactin release and activate

hypothalamic CRF and oxytocin-expressing cells. *Journal of Neuroscience* (2001) 21:3572–3579.

172. Jørgensen H, Knigge U, Kjaer A, Møller M, Warberg J. Serotonergic stimulation of corticotropin-releasing hormone and pro-opiomelanocortin gene expression. *J Neuroendocrinol* (2002) 14:788–795.

173. Contesse V, Lefebvre H, Lenglet S, Kuhn J-M, Delarue C, Vaudry H. 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) 78:967–983.

174. Bockaert J, Claeysen S, Compan V, Dumuis A. 5-HT4 receptors: history, molecular pharmacology and brain functions. *Neuropharmacology* (2008) 55:922–931.

175. Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* (2002) 71:533–554.

176. Nishizuka Y. Intracellular Signaling by Hydrolysis of Phospholipids and Activation of Protein Kinase C. *Science (1979)* (1992) 258:607–614. doi: 10.1126/science.1411571

177. Exton JH. Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins. *Annu Rev Pharmacol Toxicol* (1996) 36:481–509.

178. Nicholls DG. The glutamatergic nerve terminal. *Eur J Biochem* (1993) 212:613–631.

179. Swanson LW. Cerebral hemisphere regulation of motivated behavior. *Brain Res* (2000) 886:113–164.

180. Marek GJ, Wright RA, Schoepp DD, Monn JA, Aghajanian GK. Physiological antagonism between 5-hydroxytryptamine2A and group II metabotropic glutamate receptors in prefrontal cortex. *Journal of Pharmacology and Experimental Therapeutics* (2000) 292:76–87.

181. Benes FM, Vincent SL, Todtenkopf M. The density of pyramidal and nonpyramidal neurons in anterior cingulate cortex of schizophrenic and bipolar subjects. *Biol Psychiatry* (2001) 50:395–406.

182. Seamans JK, Gorelova N, Durstewitz D, Yang CR. Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. *Journal of Neuroscience* (2001) 21:3628–3638.

183. Millan MJ. Serotonin 5-HT2C Receptors as a Target for the Treatment of Depressive and Anxious States: Focus on Novel Therapeutic Strategies. *Therapies* (2005) 60:441–460. doi: https://doi.org/10.2515/therapie:2005065

184. Mathews DC, Henter ID, Zarate CA. Targeting the Glutamatergic System to Treat Major Depressive Disorder Rationale and Progress to Date.

185. Aghajanian GK, Marek GJ. Serotonin model of schizophrenia: emerging role of glutamate mechanisms. *Brain Res Rev* (2000) 31:302–312. doi: 10.1016/S0165-0173(99)00046-6

186. Popoli M, Yan Z, McEwen BS, Sanacora G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat Rev Neurosci* (2012) 13:22–37. doi: 10.1038/nrn3138

187. Benvenga MJ, Chaney SF, Baez M, Britton TC, Hornback WJ, Monn JA, Marek GJ. Metabotropic glutamate2 receptors play a key role in modulating head twitches induced by a serotonergic hallucinogen in mice. *Front Pharmacol* (2018) 9:1–12. doi: 10.3389/fphar.2018.00208

188. Barden N. Implication of the hypothalamic-pituitary-adrenal axis in the physiopathology of depression. (2004).

189. Vollmayr B, Henn FA. Stress models of depression. *Clin Neurosci Res* (2003) 3:245–251.

190. McEwen BS. Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain. *Physiol Rev* (2007) 87:873–904. doi: 10.1152/physrev.00041.2006

191. Jankord R, Herman JP. Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. *Ann N Y Acad Sci* (2008) 1148:64–73.

192. Herman JP, Prewitt CM-F, Cullinan WE. Neuronal circuit regulation of the hypothalamo-pituitary-adrenocortical stress axis. *Crit Rev Neurobiol* (1996) 10:

193. Wolfson B, Manning RW, Davis LG, Arentzen R, Baldino F. Co-localization of corticotropin releasing factor and vasopressin mRNA in neurones after adrenalectomy. *Nature* (1985) 315:59–61. doi: 10.1038/315059a0

194. Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry* (2005) 29:1201–1213.

195. Herman JP, Cullinan WE, Ziegler DR, Tasker JG. Role of the paraventricular nucleus microenvironment in stress integration. *European Journal of Neuroscience* (2002) 16:381–385.

196. Piazza PVincenzo, Deminière J-Marie, Moal Michel Le, Simon Hervé. Factors That Predict Individual Vulnerability to Amphetamine Self-Administration. *Science (1979)* (1989) 245:1511–1513. doi: 10.1126/science.2781295

197. Carbonaro TM, Bradstreet MP, Barrett FS, MacLean KA, Jesse R, Johnson MW, Griffiths RR. Survey study of challenging experiences after ingesting psilocybin mushrooms: Acute and enduring positive and negative consequences. *Journal of psychopharmacology* (2016) 30:1268–1278.

198. Roseman L, Nutt DJ, Carhart-Harris RL. Quality of acute psychedelic experience predicts therapeutic efficacy of psilocybin for treatment-resistant depression. *Front Pharmacol* (2018) 8:309463.

199. Wolff M, Evens R, Mertens LJ, Koslowski M, Betzler F, Gründer G, Jungaberle H. Learning to let go: a cognitive-behavioral model of how psychedelic therapy promotes acceptance. *Front Psychiatry* (2020) 11:501786.

200. Jones NT, Zahid Z, Grady SM, Sultan ZW, Zheng Z, Razidlo J, Banks MI, Wenthur CJ. Transient elevation of plasma glucocorticoids supports psilocybin-induced anxiolysis in mice. *ACS Pharmacol Transl Sci* (2023) 6:1221–1231.

201. Sharma P, Nguyen QA, Matthews SJ, Carpenter E, Mathews DB, Patten CA, Hammond CJ. Psilocybin history, action and reaction: A narrative clinical review. *Journal of Psychopharmacology* (2023) 37:849–865.

202. Hartogsohn I. Constructing drug effects: a history of set and setting. Drug science, Policy and Law 3. *Link: https://bit ly/2Op2JGI* (2017)

203. Khan I. Convention on psychotropic substances, 1971: the role and responsibilities of the World Health Organization. *Prog Neuropsychopharmacol* (1979) 3:11–14.

204. Penedos S, Ramos C, Miguel M, Alves M, Paulino L, Azevedo A, Magalhães M, Moreno L, Ribeiro N, Fonseca I, et al. P.0729 Highlights of psychedelic history and current research on psilocybin application for treatment of depression – a comprehensive literature review.

European Neuropsychopharmacology (2021) 53:S532–S533. doi: https://doi.org/10.1016/j.euroneuro.2021.10.797

205. Sharma P, Nguyen QA, Matthews SJ, Carpenter E, Mathews DB, Patten CA, Hammond CJ. Psilocybin history, action and reaction: A narrative clinical review. *Journal of Psychopharmacology* (2023) 37:849–865. https://api.semanticscholar.org/CorpusID:261395845

206. Hasler F, Bourquin D, Brenneisen R, Bär T, Vollenweider FX. Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. *Pharm Acta Helv* (1997) 72:175–184.

207. Horita A, Weber LJ. The enzymic dephosphorylation and oxidation of psilocybin and pscilocin by mammalian tissue homogenates. *Biochem Pharmacol* (1961) 7:47–54.

208. Pham DNK, Chadeayne AR, Golen JA, Manke DR. Psilacetin derivatives: fumarate salts of the methyl–ethyl, methyl–allyl and diallyl variants of the psilocin prodrug. *Acta Crystallogr E Crystallogr Commun* (2021) 77:101–106.

209. Jones, Nathan T. Wagner, Laura Scarlett, Cameron 0. Hanh, Molly C. Wenthur CJ. In Vivo Validation of Psilacetin as a Prodrug Yielding Modestly Lower Peripheral Psilocin Exposure than Psilocybin. *Front Psychiatry* (2023) 14: doi: doi: 10.3389/fpsyt.2023.1303365

210. Nichols DE, Frescas S. Improvements to the synthesis of psilocybin and a facile method for preparing the O-acetyl prodrug of psilocin. *Synthesis (Stuttg)* (1999) 1999:935–938.

211. Chadeayne AR, Golen JA, Manke DR. Bis(4-acetoxy-N,N-dimethyltryptammonium) fumarate: a new crystalline form of psilacetin, an alternative to psilocybin as a psilocin prodrug. *Acta Crystallogr E Crystallogr Commun* (2019) 75:900–902. https://api.semanticscholar.org/CorpusID:191146517

212. Elliott SP, Holdbrook T, Brandt SD. Prodrugs of New Psychoactive Substances (NPS): A New Challenge. *J Forensic Sci* (2020) 65: https://api.semanticscholar.org/CorpusID:210335207

213. Rautio J, Laine K, Gynther M, Savolainen J. Prodrug Approaches for CNS Delivery. *AAPS J* (2008) 10:92–102. doi: 10.1208/s12248-008-9009-8

214. Sherwood AM, Meisenheimer P, Tarpley G, Kargbo RB. An improved, practical, and scalable five-step synthesis of psilocybin. *Synthesis (Stuttg)* (2020) 52:688–694.

215. Geiger HA, Wurst MG, Daniels RN. DARK classics in chemical neuroscience: psilocybin. *ACS Chem Neurosci* (2018) 9:2438–2447.

216. Bogenschutz MP, Forcehimes AA, Pommy JA, Wilcox CE, Barbosa PCR, Strassman RJ. Psilocybin-assisted treatment for alcohol dependence: a proof-of-concept study. *Journal of psychopharmacology* (2015) 29:289–299.

217. Davis AK, Barrett FS, May DG, Cosimano MP, Sepeda ND, Johnson MW, Finan PH, Griffiths RR. Effects of psilocybin-assisted therapy on major depressive disorder: a randomized clinical trial. *JAMA Psychiatry* (2021) 78:481–489.

218. Mertens LJ, Wall MB, Roseman L, Demetriou L, Nutt DJ, Carhart-Harris RL. Therapeutic mechanisms of psilocybin: changes in amygdala and prefrontal functional connectivity during emotional processing after psilocybin for treatment-resistant depression. *Journal of Psychopharmacology* (2020) 34:167–180.

219. Roseman L, Demetriou L, Wall MB, Nutt DJ, Carhart-Harris RL. Increased amygdala responses to emotional faces after psilocybin for treatment-resistant depression. *Neuropharmacology* (2018) 142:263–269.

220. Doss MK, Považan M, Rosenberg MD, Sepeda ND, Davis AK, Finan PH, Smith GS, Pekar JJ, Barker PB, Griffiths RR. Psilocybin therapy increases cognitive and neural flexibility in patients with major depressive disorder. Transl Psychiatry 11 (1): 574. (2021)

221. Goldberg SB, Pace BT, Nicholas CR, Raison CL, Hutson PR. The experimental effects of psilocybin on symptoms of anxiety and depression: A meta-analysis. *Psychiatry Res* (2020) 284:112749.

222. Carhart-Harris RL, Bolstridge M, Day CMJ, Rucker J, Watts R, Erritzoe DE, Kaelen M, Giribaldi B, Bloomfield M, Pilling S. Psilocybin with psychological support for treatment-resistant depression: six-month follow-up. *Psychopharmacology (Berl)* (2018) 235:399–408.

223. Tylš F, Páleníček T, Horáček J. Psilocybin–summary of knowledge and new perspectives. *European Neuropsychopharmacology* (2014) 24:342–356.

224. Brown RT, Nicholas CR, Cozzi N V, Gassman MC, Cooper KM, Muller D, Thomas CD, Hetzel SJ, Henriquez KM, Ribaudo AS. Pharmacokinetics of escalating doses of oral psilocybin in healthy adults. *Clin Pharmacokinet* (2017) 56:1543–1554.

225. Kolaczynska KE, Liechti ME, Duthaler U. Development and validation of an LC-MS/MS method for the bioanalysis of psilocybin's main metabolites, psilocin and 4-hydroxyindole-3-acetic acid, in human plasma. *Journal of Chromatography B* (2021) 1164:122486.

226. Holze F, Ley L, Müller F, Becker AM, Straumann I, Vizeli P, Kuehne SS, Roder MA, Duthaler U, Kolaczynska KE. Direct comparison of the acute effects of lysergic acid diethylamide and psilocybin in a double-blind placebo-controlled study in healthy subjects. *Neuropsychopharmacology* (2022) 47:1180–1187.

227. Nichols DE, Nichols CD, Mckenna DJ, Mangini M, Grigsby J, Lsd C 8., Panik K, Presti DE, Lancelotta R, Davis AK, et al. Handbook of MEDICAL HALLUCINOGENS. (2021) https://api.semanticscholar.org/CorpusID:261210049

228. Pearson C, Siegel J, Gold JA. Psilocybin-assisted psychotherapy for depression: Emerging research on a psychedelic compound with a rich history. *J Neurol Sci* (2022) 434:120096.

229. Hofmann Albert TF. C07F9/5728 Five-membered rings condensed with carbocyclic rings or carbocyclic ring systems. (1963) https://patents.google.com/patent/US3075992A/en?oq=US3075992#patentCitations

230. Chadeayne AR, Golen JA, Manke DR. Bis (4-acetoxy-N, N-dimethyltryptammonium) fumarate: a new crystalline form of psilacetin, an alternative to psilocybin as a psilocin prodrug. *Acta Crystallogr E Crystallogr Commun* (2019) 75:900–902.

231. Chadeayne AR, Golen JA, Manke DR. The crystal structure of 4-AcO-DMT fumarate. *Psychedelic Sci Rev* (2019)

232. Sessa B. Can psychedelics have a role in psychiatry once again? *The British Journal of Psychiatry* (2005) 186:457–458.

233. United Nations AddressNew York, NY 10017 US. Convention on Psychotropic Substances, 1971. (1977). https://www.ojp.gov/ncjrs/virtual-library/abstracts/convention-psychotropic-substances-1971

234. Glatfelter GC, Manke DR, Chadayne AR, Baumann MH. Receptor binding profiles and behavioral effects of psilocybin analogs. *The FASEB Journal* (2022) 36:

235. Sessa B, Glatfelter GC, Pottie E, Partilla JS, Sherwood AM, Kaylo K, Pham DNK, Naeem M, Sammeta VR, DeBoer S, et al. Structure–Activity Relationships for Psilocybin,

Baeocystin, Aeruginascin, and Related Analogues to Produce Pharmacological Effects in Mice. *ACS Pharmacol Transl Sci* (2022) 5:1181–1196. doi: 10.1021/acsptsci.2c00177

236. Sanacora G, Yan Z, Popoli M, Carhart-Harris RL, Nutt DJ, Cao D, Yu J, Wang H, Luo Z, Liu X, et al. Structure-based discovery of nonhallucinogenic psychedelic analogs. *Journal of Psychopharmacology* (2022) 38:370–384. doi: 10.1016/j.jad.2022.01.104

237. Kargbo RB, Sherwood AM, Walker A, Cozzi N V, Dagger RE, Sable JH, O'Hern K, Kaylo KW, Patterson T, Tarpley G, et al. Direct Phosphorylation of Psilocin Enables Optimized cGMP Kilogram-Scale Manufacture of Psilocybin. *ACS Omega* (2020) 5:16959–16966. https://api.semanticscholar.org/CorpusID:220599227

238. Klein AK, Chatha M, Laskowski LJ, Anderson EI, Brandt SD, Chapman SJ, McCorvy JD, Halberstadt AL. Investigation of the structure–activity relationships of psilocybin analogues. *ACS Pharmacol Transl Sci* (2020) 4:533–542.

239. Meyer MR, Caspar A, Brandt SD, Maurer HH. A qualitative/quantitative approach for the detection of 37 tryptamine-derived designer drugs, 5 β-carbolines, ibogaine, and yohimbine in human urine and plasma using standard urine screening and multi-analyte approaches. *Anal Bioanal Chem* (2014) 406:225–237.

240. Palma-Conesa ÁJ, Ventura M, Galindo L, Fonseca F, Grifell M, Quintana P, Fornís I, Gil C, Farré M, Torrens M. Something new about something old: a 10-year follow-up on classical and new psychoactive tryptamines and results of analysis. *J Psychoactive Drugs* (2017) 49:297–305.

241. Palamar JJ, Acosta P. A qualitative descriptive analysis of effects of psychedelic phenethylamines and tryptamines. *Human Psychopharmacology: Clinical and Experimental* (2020) 35:e2719.

242. Palamar JJ, Barratt MJ, Ferris JA, Winstock AR. Correlates of new psychoactive substance use among a self-selected sample of nightclub attendees in the United States. *Am J Addict* (2016) 25:400–407.

243. Kargbo RB, Sherwood A, Walker A, Cozzi N V, Dagger RE, Sable J, O'Hern K, Kaylo K, Patterson T, Tarpley G. Direct phosphorylation of psilocin enables optimized cGMP kilogram-scale manufacture of psilocybin. *ACS Omega* (2020) 5:16959–16966.

244. Dinis-Oliveira RJ. Metabolism of psilocybin and psilocin: clinical and forensic toxicological relevance. *Drug Metab Rev* (2017) 49:84–91.

245. Higgins GA, Carroll NK, Brown M, MacMillan C, Silenieks LB, Thevarkunnel S, Izhakova J, Magomedova L, DeLannoy I, Sellers EM. Low Doses of Psilocybin and Ketamine Enhance Motivation and Attention in Poor Performing Rats: Evidence for an Antidepressant Property. *Front Pharmacol* (2021) 12: doi: 10.3389/fphar.2021.640241

246. Kamata T, Katagi M, Tsuchihashi H. Metabolism and toxicological analyses of hallucinogenic tryptamine analogues being abused in Japan. *Forensic Toxicol* (2010) 28:1–8. doi: 10.1007/s11419-009-0087-9

247. Manevski N, Kurkela M, Höglund C, Mauriala T, Court MH, Yli-Kauhaluoma J, Finel M. Glucuronidation of Psilocin and 4-Hydroxyindole by the Human UDP-Glucuronosyltransferases. *Drug Metabolism and Disposition* (2010) 38:386. doi: 10.1124/dmd.109.031138

248. Anderson BT, Danforth A, Grob CS. Psychedelic medicine: safety and ethical concerns. *Lancet Psychiatry* (2020) 7 10:829–830. https://api.semanticscholar.org/CorpusID:221807015

249. Hill SL, Thomas SHL, Klein AK, Chatha MR, Laskowski LJ, Anderson EI, Brandt SD, Chapman SJ, McCorvy JD, Halberstadt AL, et al. Human hallucinogen research: guidelines for safety. *Drug Test Anal* (2020) 49:457–458. doi: 10.1021/acsptsci.2c00177

250. Family N, Maillet EL, Williams LTJ, Krediet E, Carhart-Harris RL, Williams TM, Nichols CD, Goble DJ, Raz S. Safety, tolerability, pharmacokinetics, and pharmacodynamics of low dose lysergic acid diethylamide (LSD) in healthy older volunteers. *Psychopharmacology (Berl)* (2020) 237:841–853.

251. Mocanu V, Mackay L, Christie D, Argento E. Safety considerations in the evolving legal landscape of psychedelic-assisted psychotherapy. *Subst Abuse Treat Prev Policy* (2022) 17:37.

252. Gasser P, Holstein D, Michel Y, Doblin R, Yazar-Klosinski B, Passie T, Brenneisen R. Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. *J Nerv Ment Dis* (2014) 202:513–520.

253. D'Souza DC, Syed SA, Flynn LT, Safi-Aghdam H, Cozzi N V, Ranganathan M. Exploratory study of the dose-related safety, tolerability, and efficacy of dimethyltryptamine (DMT) in healthy volunteers and major depressive disorder. *Neuropsychopharmacology* (2022) 47:1854–1862.

254. Brown RT, Nicholas CR, Cozzi N V, Gassman MC, Cooper KM, Muller D, Thomas CD, Hetzel SJ, Henriquez KM, Ribaudo AS. Pharmacokinetics of escalating doses of oral psilocybin in healthy adults. *Clin Pharmacokinet* (2017) 56:1543–1554.

255. Goodwin GM, Aaronson ST, Alvarez O, Arden PC, Baker A, Bennett JC, Bird C, Blom RE, Brennan C, Brusch D. Single-dose psilocybin for a treatment-resistant episode of major depression. *New England Journal of Medicine* (2022) 387:1637–1648.

256. Griffiths RR, Richards WA, McCann U, Jesse R. Psilocybin can occasion mystical-type experiences having substantial and sustained personal meaning and spiritual significance. *Psychopharmacology (Berl)* (2006) 187:268–283. doi: 10.1007/s00213-006-0457-5

257. Vogt SB, Ley L, Erne L, Straumann I, Becker AM, Klaiber A, Holze F, Vandersmissen A, Mueller L, Duthaler U. Acute effects of intravenous DMT in a randomized placebo-controlled study in healthy participants. *Transl Psychiatry* (2023) 13:172.

258. Gallimore AR, Strassman RJ. A model for the application of target-controlled intravenous infusion for a prolonged immersive DMT psychedelic experience. *Front Pharmacol* (2016) 7:205781.

259. Lawrence DW, Carhart-Harris R, Griffiths R, Timmermann C. Phenomenology and content of the inhaled N, N-dimethyltryptamine (N, N-DMT) experience. *Sci Rep* (2022) 12:8562.

260. Carbonaro TM, Gatch MB. Neuropharmacology of N, N-dimethyltryptamine. *Brain Res Bull* (2016) 126:74–88.

261. Good M, Joel Z, Benway T, Routledge C, Timmermann C, Erritzoe D, Weaver R, Allen G, Hughes C, Topping H. Pharmacokinetics of N, N-dimethyltryptamine in humans. *Eur J Drug Metab Pharmacokinet* (2023) 48:311–327.

262. Cameron LP, Olson DE. Dark classics in chemical neuroscience: N, N-dimethyltryptamine (DMT). *ACS Chem Neurosci* (2018) 9:2344–2357.

263. Holze F, Becker AM, Kolaczynska KE, Duthaler U, Liechti ME. Pharmacokinetics and pharmacodynamics of oral psilocybin administration in healthy participants. *Clin Pharmacol Ther* (2023) 113:822–831.

264. Dolder PC, Schmid Y, Haschke M, Rentsch KM, Liechti ME. Pharmacokinetics and concentration-effect relationship of oral LSD in humans. *International Journal of Neuropsychopharmacology* (2016) 19:pyv072.

265. Good M, Joel Z, Benway T, Routledge C, Timmermann C, Erritzoe D, Weaver R, Allen G, Hughes C, Topping H. Pharmacokinetics of N, N-dimethyltryptamine in humans. *Eur J Drug Metab Pharmacokinet* (2023) 48:311–327.

266. Shen H-W, Jiang X-L, C Winter J, Yu A-M. Psychedelic 5-methoxy-N, N-dimethyltryptamine: metabolism, pharmacokinetics, drug interactions, and pharmacological actions. *Curr Drug Metab* (2010) 11:659–666.

267. Liechti ME, Dolder PC, Schmid Y. Alterations of consciousness and mystical-type experiences after acute LSD in humans. *Psychopharmacology (Berl)* (2017) 234:1499–1510.

268. Schmid Y, Liechti ME. Long-lasting subjective effects of LSD in normal subjects. *Psychopharmacology (Berl)* (2018) 235:535–545.

269. Savage C, Savage E, Fadiman J, Harman W. LSD: Therapeutic effects of the psychedelic experience. *Psychol Rep* (1964) 14:111–120.

270. Gallimore AR, Strassman RJ. A model for the application of target-controlled intravenous infusion for a prolonged immersive DMT psychedelic experience. *Front Pharmacol* (2016) 7:205781.

271. Timmermann C, Roseman L, Williams L, Erritzoe D, Martial C, Cassol H, Laureys S, Nutt D, Carhart-Harris R. DMT models the near-death experience. *Front Psychol* (2018) 9:395026.

272. Shen H-W, Jiang X-L, C Winter J, Yu A-M. Psychedelic 5-methoxy-N, N-dimethyltryptamine: metabolism, pharmacokinetics, drug interactions, and pharmacological actions. *Curr Drug Metab* (2010) 11:659–666.

273. James E, Robertshaw TL, Hoskins M, Sessa B. Psilocybin occasioned mystical-type experiences. *Human Psychopharmacology: Clinical and Experimental* (2020) 35:e2742. doi: https://doi.org/10.1002/hup.2742

274. MacLean KA, Leoutsakos JMS, Johnson MW, Griffiths RR. Factor Analysis of the Mystical Experience Questionnaire: A Study of Experiences Occasioned by the Hallucinogen Psilocybin. *J Sci Study Relig* (2012) 51:721–737. doi: 10.1111/j.1468-5906.2012.01685.x

275. Koslowski M, Johnson MW, Gründer G, Betzler F. Novel treatment approaches for substance use disorders: therapeutic use of psychedelics and the role of psychotherapy. *Curr Addict Rep* (2021)1–11.

276. Schenberg EE. Psychedelic-assisted psychotherapy: a paradigm shift in psychiatric research and development. *Front Pharmacol* (2018) 9:323606.

277. Reiff CM, Richman EE, Nemeroff CB, Carpenter LL, Widge AS, Rodriguez CI, Kalin NH, McDonald WM, Work Group on Biomarkers and Novel Treatments a D of the APAC of R. Psychedelics and psychedelic-assisted psychotherapy. *American Journal of Psychiatry* (2020) 177:391–410.

278. Reiff CM, Richman EE, Nemeroff CB, Carpenter LL, Widge AS, Rodriguez CI, Kalin NH, McDonald WM. Psychedelics and psychedelic-assisted psychotherapy. *American Journal of Psychiatry* (2020) 177:391–410. doi: 10.1176/appi.ajp.2019.19010035

279. McNamee S, Devenot N, Buisson M. Studying harms is key to improving psychedelic-assisted therapy—participants call for changes to research landscape. *JAMA Psychiatry* (2023) 80:411–412.

280. Wolfgang AS, Hoge CW. Psychedelic-Assisted Therapy in Military and Veterans Healthcare Systems: Clinical, Legal, and Implementation Considerations. *Curr Psychiatry Rep* (2023) 25:513–532.

281. McCrone P, Fisher H, Knight C, Harding R, Schlag AK, Nutt DJ, Neill JC. Cost-effectiveness of psilocybin-assisted therapy for severe depression: exploratory findings from a decision analytic model. *Psychol Med* (2023) 53:7619–7626.

282. Marseille E, Bertozzi S, Kahn JG. The economics of psychedelic-assisted therapies: A research agenda. *Front Psychiatry* (2022) 13:1025726.

283. Luoma JB, Chwyl C, Bathje GJ, Davis AK, Lancelotta R. A meta-analysis of placebo-controlled trials of psychedelic-assisted therapy. *J Psychoactive Drugs* (2020) 52:289–299.

284. Hoener S, Wolfgang A, Nissan D, Howe E. Ethical considerations for psychedelic-assisted therapy in military clinical settings. *J Med Ethics* (2024) 50:258–262.

285. Johnston CB, Mangini M, Grob C, Anderson B. The safety and efficacy of psychedelic-assisted therapies for older adults: knowns and unknowns. *The American Journal of Geriatric Psychiatry* (2023) 31:44–53.

286. Bouchet L, Sager Z, Yrondi A, Nigam KB, Anderson BT, Ross S, Petridis PD, Beaussant Y. Older adults in psychedelic-assisted therapy trials: A systematic review. *Journal of Psychopharmacology* (2024)02698811231215420.

287. Beaussant Y, Nigam K. Expending perspectives on the potential for psychedelic-assisted therapies to improve the experience of aging. *The American Journal of Geriatric Psychiatry* (2023) 31:54–57.

288. Davis AK, Levin AW, Nagib PB, Armstrong SB, Lancelotta RL. Study protocol of an open-label proof-of-concept trial examining the safety and clinical efficacy of psilocybin-assisted therapy for veterans with PTSD. *BMJ Open* (2023) 13:e068884.

289. Davis AK, Averill LA, Sepeda ND, Barsuglia JP, Amoroso T. Psychedelic treatment for trauma-related psychological and cognitive impairment among US special operations forces veterans. *Chronic Stress* (2020) 4:2470547020939564.

290. Maia LO, Beaussant Y, Garcia ACM. The therapeutic potential of psychedelic-assisted therapies for symptom control in patients diagnosed with serious illness: a systematic review. *J Pain Symptom Manage* (2022) 63:e725–e738.

291. Iwata N, Ishigooka J, Kim W-H, Yoon B-H, Lin S-K, Sulaiman AH, Cosca R, Wang L, Suchkov Y, Agarkov A. Efficacy and safety of blonanserin transdermal patch in patients with schizophrenia: a 6-week randomized, double-blind, placebo-controlled, multicenter study. *Schizophr Res* (2020) 215:408–415.

292. Citrome L, Walling DP, Zeni CM, Starling BR, Terahara T, Kuriki M, Park AS, Komaroff M. Efficacy and safety of HP-3070, an asenapine transdermal system, in patients with schizophrenia: a phase 3, randomized, placebo-controlled study. *J Clin Psychiatry* (2020) 82:1417.

293. Citrome L, Zeni CM, Correll CU. Patches: established and emerging transdermal treatments in psychiatry. *J Clin Psychiatry* (2019) 80:21174.

294. Carrithers B, El-Mallakh RS. Transdermal asenapine in schizophrenia: a systematic review. *Patient Prefer Adherence* (2020)1541–1551.

295. Frampton JE. Rotigotine transdermal patch: a review in Parkinson's disease. *CNS Drugs* (2019) 33:707–718.

296. Sanford M, Scott LJ. Rotigotine transdermal patch: a review of its use in the treatment of Parkinson's disease. *CNS Drugs* (2011) 25:699–719.

297. Schnitzler A, Leffers K-W, Häck H-J. High compliance with rotigotine transdermal patch in the treatment of idiopathic Parkinson's disease. *Parkinsonism Relat Disord* (2010) 16:513–516.

298. Winblad B, Machado JC. Use of rivastigmine transdermal patch in the treatment of Alzheimer's disease. *Expert Opin Drug Deliv* (2008) 5:1377–1386.

299. Larkin HD. First donepezil transdermal patch approved for Alzheimer disease. *JAMA* (2022) 327:1642.

300. Kurz A, Farlow M, Lefevre G. Pharmacokinetics of a novel transdermal rivastigmine patch for the treatment of Alzheimer's disease: a review. *Int J Clin Pract* (2009) 63:799–805.

301. Lefevre G, Sędek G, Jhee SS, Leibowitz MT, Huang H, Enz A, Maton S, Ereshefsky L, Pommier F, Schmidli H. Pharmacokinetics and pharmacodynamics of the novel daily rivastigmine transdermal patch compared with twice-daily capsules in Alzheimer's disease patients. *Clin Pharmacol Ther* (2008) 83:106–114.

302. Abelin T, Müller P, Buehler A, Vesanen K, Imhof PR. Controlled trial of transdermal nicotine patch in tobacco withdrawal. *The Lancet* (1989) 333:7–10.

303. Mulligan SC, Masterson JG, Devane JG, Kelly JG. Clinical and pharmacokinetic properties of a transdermal nicotine patch. *Clin Pharmacol Ther* (1990) 47:331–337.

304. Davaran S, Rashidi MR, Khandaghi R, Hashemi M. Development of a novel prolonged-release nicotine transdermal patch. *Pharmacol Res* (2005) 51:233–237.

305. HURT RD, LAUGER GG, OFFORD KP, KOTTKE TE, DALE LC. Nicotine-replacement therapy with use of a transdermal nicotine patch—a randomized double-blind placebo-controlled trial. *Mayo Clinic Proceedings*. Elsevier (1990). p. 1529–1537

306. George TP, Ziedonis DM, Feingold A, Pepper WT, Satterburg CA, Winkel J, Rounsaville BJ, Kosten TR. Nicotine transdermal patch and atypical antipsychotic medications for smoking cessation in schizophrenia. *American Journal of Psychiatry* (2000) 157:1835–1842.

307. Cameron LP, Nazarian A, Olson DE. Psychedelic microdosing: prevalence and subjective effects. *J Psychoactive Drugs* (2020) 52:113–122.

308. Polito V, Stevenson RJ. A systematic study of microdosing psychedelics. *PLoS One* (2019) 14: doi: 10.1371/journal.pone.0211023

309. Polito V, Liknaitzky P. The emerging science of microdosing: A systematic review of research on low dose psychedelics (1955–2021) and recommendations for the field. *Neurosci Biobehav Rev* (2022) 139:104706.

310. Fadiman J, Korb S. Might microdosing psychedelics be safe and beneficial? An initial exploration. *J Psychoactive Drugs* (2019) 51:118–122.

311. Hutten NRPW, Mason NL, Dolder PC, Kuypers KPC. Self-rated effectiveness of microdosing with psychedelics for mental and physical health problems among microdosers. *Front Psychiatry* (2019) 10:481074.

312. Ona G, Bouso JC. Potential safety, benefits, and influence of the placebo effect in microdosing psychedelic drugs: A systematic review. *Neurosci Biobehav Rev* (2020) 119:194–203.

313. Kuypers KPC. The therapeutic potential of microdosing psychedelics in depression. *Ther Adv Psychopharmacol* (2020) 10:2045125320950567.

314. Pastore MN, Kalia YN, Horstmann M, Roberts MS. Transdermal patches: history, development and pharmacology. *Br J Pharmacol* (2015) 172:2179–2209.

315. Citrome L, Zeni CM, Correll CU. Patches: established and emerging transdermal treatments in psychiatry. *J Clin Psychiatry* (2019) 80:21174.

316. Courtenay AJ, McAlister E, McCrudden MTC, Vora L, Steiner L, Levin G, Levy-Nissenbaum E, Shterman N, Kearney M-C, McCarthy HO. Hydrogel-forming microneedle arrays as a therapeutic option for transdermal esketamine delivery. *Journal of controlled release* (2020) 322:177–186.

317. Scheindlin S. Transdermal drug delivery: past, present, future. *Mol Interv* (2004) 4:308.

318. Tanner T, Marks R. Delivering drugs by the transdermal route: review and comment. *Skin Research and Technology* (2008) 14:249–260.

319. Rapoport AM, Freitag F, Pearlman SH. Innovative delivery systems for migraine: the clinical utility of a transdermal patch for the acute treatment of migraine. *CNS Drugs* (2010) 24:929–940.

320. Rifkin BD, Maraver MJ, Colzato LS. Microdosing psychedelics as cognitive and emotional enhancers. *Psychology of Consciousness: Theory, Research, and Practice* (2020) 7:316.

321. Olson DE. Psychoplastogens: a promising class of plasticity-promoting neurotherapeutics. *J Exp Neurosci* (2018) 12:1179069518800508.

322. Nichols DE, Walter H. The history of psychedelics in psychiatry. *Pharmacopsychiatry* (2021) 54:151–166.

323. Goodwin GM, Aaronson ST, Alvarez O, Atli M, Bennett JC, Croal M, DeBattista C, Dunlop BW, Feifel D, Hellerstein DJ, et al. Single-dose psilocybin for a treatment-resistant episode of major depression: Impact on patient-reported depression severity, anxiety, function, and quality of life. *J Affect Disord* (2023) 327:120–127. doi: https://doi.org/10.1016/j.jad.2023.01.108

324. Goodwin G, Aaronson S, Alvarez Bobo O, Arden P, Baker A, Bennett J, Bird C, Blom R, Brennan C, Brusch D, et al. Single-Dose Psilocybin for a Treatment-Resistant Episode of Major Depression. *New England Journal of Medicine* (2022) 387:1637–1648. doi: 10.1056/NEJMoa2206443

325. Palhano-Fontes F, Andrade KC, Tofoli LF, Santos AC, Crippa JAS, Hallak JEC, Ribeiro S, De Araujo DB. The psychedelic state induced by ayahuasca modulates the activity and connectivity of the default mode network. *PLoS One* (2015) 10:e0118143.

326. Turner EH. Esketamine for treatment-resistant depression: seven concerns about efficacy and FDA approval. *Lancet Psychiatry* (2019) 6:977 – 979. doi: 10.1016/S2215-0366(19)30394-3

327. Good M, Joel Z, Benway T, Routledge C, Timmermann C, Erritzoe D, Weaver R, Allen G, Hughes C, Topping H. Pharmacokinetics of N, N-dimethyltryptamine in humans. *Eur J Drug Metab Pharmacokinet* (2023) 48:311–327.

328. Szára St. Dimethyltryptamin: Its metabolism in man; the relation of its psychotic effect to the serotonin metabolism. *Experientia* (1956) 12:441 – 442. doi: 10.1007/BF02157378

329. Turner WJ, Merlis S. Effect of some indolealkylamines on man. *AMA Arch Neurol Psychiatry* (1959) 81:121–129.

330. Frecska E, Bokor P, Winkelman M. The therapeutic potentials of ayahuasca: possible effects against various diseases of civilization. *Front Pharmacol* (2016) 7:35.

331. Palhano-Fontes F, Barreto D, Onias H, Andrade KC, Novaes MM, Pessoa JA, Mota-Rolim SA, Osório FL, Sanches R, dos Santos RG, et al. Rapid antidepressant effects of the psychedelic ayahuasca in treatment-resistant depression: a randomized placebo-controlled trial. *Psychol Med* (2019) 49:655–663. doi: DOI: 10.1017/S0033291718001356

332. Barker SA, Borjigin J, Lomnicka I, Strassman R. LC/MS/MS analysis of the endogenous dimethyltryptamine hallucinogens, their precursors, and major metabolites in rat pineal gland microdialysate. *Biomedical Chromatography* (2013) 27:1690–1700.

333. Barker SA, McIlhenny EH, Strassman R. A critical review of reports of endogenous psychedelic N, N-dimethyltryptamines in humans: 1955–2010. *Drug Test Anal* (2012) 4:617–635.

334. Vargas M V, Dunlap LE, Dong C, Carter SJ, Tombari RJ, Jami SA, Cameron LP, Patel SD, Hennessey JJ, Saeger HN, et al. Psychedelics promote neuroplasticity through the activation of intracellular 5-HT2A receptors. *Science (1979)* (2023) 379:700 – 706. doi: 10.1126/science.adf0435

335. James E, Erritzoe D, Benway T, Joel Z, Timmermann C, Good M, Agnorelli C, Weiss BM, Barba T, Campbell G. Safety, tolerability, pharmacodynamic and wellbeing effects of SPL026 (dimethyltryptamine fumarate) in healthy participants: a randomized, placebo-controlled phase 1 trial. *Front Psychiatry* (2024) 14:1305796.

336. Strassman RJ, Qualls CR. Dose-Response Study of N, N-Dimethyltryptamine in Humans: I. Neuroendocrine, Autonomic, and Cardiovascular Effects. *Arch Gen Psychiatry* (1994) 51:85 – 97. doi: 10.1001/archpsyc.1994.03950020009001

337. Thomas G, Lucas P, Capler NR, Tupper KW, Martin G. Ayahuasca-assisted therapy for addiction: Results from a preliminary observational study in Canada. *Curr Drug Abuse Rev* (2013) 6:30 – 42. doi: 10.2174/15733998113099990003

338. Canal CE, Morgan D. 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) 4:556–576.

339. Ly C, Greb AC, Cameron LP, Wong JM, Barragan E V, Wilson PC, Burbach KF, Zarandi SS, Sood A, Paddy MR. Psychedelics promote structural and functional neural plasticity. *Cell Rep* (2018) 23:3170–3182.

340. Nardai S, László M, Szabó A, Alpár A, Hanics J, Zahola P, Merkely B, Frecska E, Nagy Z. N, N-dimethyltryptamine reduces infarct size and improves functional recovery following transient focal brain ischemia in rats. *Exp Neurol* (2020) 327:113245.

341. Szabó Í, Varga VÉ, Dvorácskó S, Farkas AE, Körmöczi T, Berkecz R, Kecskés S, Menyhárt Á, Frank R, Hantosi D, et al. N,N-Dimethyltryptamine attenuates spreading depolarization and restrains neurodegeneration by sigma-1 receptor activation in the ischemic rat brain. *Neuropharmacology* (2021) 192: doi: 10.1016/j.neuropharm.2021.108612

342. Cameron LP, Benson CJ, DeFelice BC, Fiehn O, Olson DE. Chronic, intermittent microdoses of the psychedelic N, N-dimethyltryptamine (DMT) produce positive effects on mood and anxiety in rodents. *ACS Chem Neurosci* (2019) 10:3261–3270.

343. Cheng D, Lei Z-G, Chu K, Lam OJH, Chiang CY, Zhang Z-J. N, N-Dimethyltryptamine, a natural hallucinogen, ameliorates Alzheimer's disease by restoring neuronal Sigma-1 receptor-mediated endoplasmic reticulum-mitochondria crosstalk. *Alzheimers Res Ther* (2024) 16:95.

344. Morales-Garcia JA, Calleja-Conde J, Lopez-Moreno JA, Alonso-Gil S, Sanz-SanCristobal M, Riba J, Perez-Castillo A. N, N-dimethyltryptamine compound found in the hallucinogenic tea ayahuasca, regulates adult neurogenesis in vitro and in vivo. *Transl Psychiatry* (2020) 10:331.

345. Kiilerich KF, Lorenz J, Scharff MB, Speth N, Brandt TG, Czurylo J, Xiong M, Jessen NS, Casado-Sainz A, Shalgunov V. Repeated low doses of psilocybin increase resilience to stress, lower compulsive actions, and strengthen cortical connections to the paraventricular thalamic nucleus in rats. *Mol Psychiatry* (2023) 28:3829–3841.

346. Bodkin JA, Amsterdam JD. Transdermal selegiline in major depression: a double-blind, placebo-controlled, parallel-group study in outpatients. *American Journal of Psychiatry* (2002) 159:1869–1875.

347. El-Tokhy FS, Abdel-Mottaleb MMA, El-Ghany EA, Geneidi AS. Transdermal delivery of second-generation antipsychotics for management of schizophrenia; disease overview, conventional and nanobased drug delivery systems. *J Drug Deliv Sci Technol* (2021) 61:102104.

348. Jiménez JH, Bouso JC. Significance of mammalian N, N-dimethyltryptamine (DMT): A 60-year-old debate. *Journal of Psychopharmacology* (2022) 36:905–919.

349. Cozzi N V, Daley PF. Synthesis and characterization of high-purity N, N-dimethyltryptamine hemifumarate for human clinical trials. *Drug Test Anal* (2020) 12:1483–1493.

350. Haq A, Goodyear B, Ameen D, Joshi V, Michniak-Kohn B. Strat-M® synthetic membrane: Permeability comparison to human cadaver skin. *Int J Pharm* (2018) 547:432–437.

351. McIlhenny EH, Riba J, Barbanoj MJ, Strassman R, Barker SA. Methodology for and the determination of the major constituents and metabolites of the Amazonian botanical medicine ayahuasca in human urine. *Biomedical Chromatography* (2011) 25:970–984.

352. Lane ME. Skin penetration enhancers. *Int J Pharm* (2013) 447:12–21.

353. Alper K, Cange J, Sah R, Schreiber-Gregory D, Sershen H, Vinod KY. Psilocybin sex-dependently reduces alcohol consumption in C57BL/6J mice. *Front Pharmacol* (2023) 13:1074633.

354. Polito V, Stevenson RJ. A systematic study of microdosing psychedelics. *PLoS One* (2019) 14: doi: 10.1371/journal.pone.0211023

355. Li L, Vlisides PE. Ketamine: 50 years of modulating the mind. *Front Hum Neurosci* (2016) 10:612.

356. Mion G. History of anaesthesia: The ketamine story–past, present and future. *European Journal of Anaesthesiologyl EJA* (2017) 34:571–575.

357. Aan Het Rot M, Zarate Jr CA, Charney DS, Mathew SJ. Ketamine for depression: where do we go from here? *Biol Psychiatry* (2012) 72:537–547.

358. Corriger A, Pickering G. Ketamine and depression: a narrative review. *Drug Des Devel Ther* (2019)3051–3067.

359. Wan L-B, Levitch CF, Perez AM, Brallier JW, Iosifescu D V, Chang LC, Foulkes A, Mathew SJ, Charney DS, Murrough JW. Ketamine safety and tolerability in clinical trials for treatment-resistant depression. *J Clin Psychiatry* (2014) 76:10121.

360. Schwartz J, Murrough JW, Iosifescu D V. Ketamine for treatment-resistant depression: recent developments and clinical applications. *BMJ Ment Health* (2016) 19:35–38.

361. Oliver PA, Snyder AD, Feinn R, Malov S, McDiarmid G, Arias AJ. Clinical effectiveness of intravenous racemic ketamine infusions in a large community sample of patients with treatment-resistant depression, suicidal ideation, and generalized anxiety symptoms: a retrospective chart review. *J Clin Psychiatry* (2022) 83:42811.

362. Idvall J, Ahlgren I, Aronsen KF, Stenberg P. Ketamine infusions: pharmacokinetics and clinical effects. *Br J Anaesth* (1979) 51:1167–1173.

363. Johnston CJ, Fitzgerald PJ, Gewarges JS, Watson BO, Spencer-Segal JL. Ketamine decreases HPA axis reactivity to a novel stressor in male but not female mice. *bioRxiv* (2021)2021–2026.

364. Dutton M, Can AT, Lagopoulos J, Hermens DF. Stress, mental disorder and ketamine as a novel, rapid acting treatment. *European Neuropsychopharmacology* (2022) 65:15–29.

365. Besnier E, Clavier T, Tonon M-C, Selim J, Lefevre-Scelles A, Morin F, Tamion F, Dureuil B, Castel H, Compere V. Ketamine and etomidate down-regulate the hypothalamic–pituitary–adrenal axis in an endotoxemic mouse model. *Anesthesiology* (2017) 127:347–354.

366. Garcia LSB, Comim CM, Valvassori SS, Réus GZ, Stertz L, Kapczinski F, Gavioli EC, Quevedo J. Ketamine treatment reverses behavioral and physiological alterations induced by chronic mild stress in rats. *Prog Neuropsychopharmacol Biol Psychiatry* (2009) 33:450–455.

367. Wang W, Liu L, Yang X, Gao H, Tang Q-K, Yin L-Y, Yin X-Y, Hao J-R, Geng D-Q, Gao C. Ketamine improved depressive-like behaviors via hippocampal glucocorticoid receptor in chronic stress induced-susceptible mice. *Behavioural brain research* (2019) 364:75–84.

368. Birnie MT, Eapen A V, Kershaw YM, Lodge D, Collingridge GL, Conway-Campbell BL, Lightman SL. Time of day influences stress hormone response to ketamine. *J Neuroendocrinol* (2022) 34:e13194.

369. Khalili-Mahani N, Martini CH, Olofsen E, Dahan A, Niesters M. Effect of subanaesthetic ketamine on plasma and saliva cortisol secretion. *Br J Anaesth* (2015) 115:68–75.

370. Chiba S, Numakawa T, Ninomiya M, Richards MC, Wakabayashi C, Kunugi H. Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. *Prog Neuropsychopharmacol Biol Psychiatry* (2012) 39:112–119. doi: 10.1016/j.pnpbp.2012.05.018

371. Hammen CL. Stress and depression: old questions, new approaches. *Curr Opin Psychol* (2015) 4:80–85.

372. Tafet GE, Bernardini R. Psychoneuroendocrinological links between chronic stress and depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2003) 27:893–903.

373. Breslau N, Davis GC. Chronic stress and major depression. *Arch Gen Psychiatry* (1986) 43:309–314.

374. Monroe S, Harkness K. Life Stress, the “Kindling” Hypothesis, and the Recurrence of Depression: Considerations From a Life Stress Perspective. *Psychol Rev* (2005) 112:417–445. doi: 10.1037/0033-295X.112.2.417

375. Feder A, Nestler EJ, Charney DS. Psychobiology and molecular genetics of resilience. *Nat Rev Neurosci* (2009) 10:446–457. doi: 10.1038/nrn2649

376. Birnie MT, Eapen A V, Kershaw YM, Lodge D, Collingridge GL, Conway-Campbell BL, Lightman SL. Time of day influences stress hormone response to ketamine. *J Neuroendocrinol* (2022) 34:e13194. doi: https://doi.org/10.1111/jne.13194

377. Gould T, Georgiou P. Inconvenient truths and the usefulness of identifying unknown unknowns. *Nat Neurosci* (2022) 25:1122–1123. doi: 10.1038/s41593-022-01147-w

378. Georgiou P, Zanos P, Mou T-CM, An X, Gerhard DM, Dryanovski DI, Potter LE, Highland JN, Jenne CE, Stewart BW, et al. Experimenters’ sex modulates mouse behaviors and neural responses to ketamine via corticotropin releasing factor. *Nat Neurosci* (2022) 25:1191–1200. doi: 10.1038/s41593-022-01146-x

379. Witowski CG, Hess MR, Jones NT, Pellitteri Hahn MC, Razidlo J, Bhavsar R, Beer C, Gonzalez-Velazquez N, Scarlett CO, Wenthur CJ, et al. Novel extended-release transdermal formulations of the psychedelic N,N-dimethyltryptamine (DMT). *European Journal of Pharmaceutical Sciences* (2024) 199:106803. doi: https://doi.org/10.1016/j.ejps.2024.106803

Chapter 2: Transient Elevation of Plasma Glucocorticoids Supports Psilocybin-Induced Anxiolysis in Mice

2.1 Introduction:

Investigation into the clinical use of psilocybin-assisted psychotherapy has shown positive results in the treatment of substance use disorders, end-of-life anxiety, major depressive disorder, and treatment-resistant depression. (1–14) Although results from these clinical studies have shown positive effects, the underlying mechanisms supporting these long-lasting effects are unclear. To date, all clinical studies on psilocybin-assisted therapy have evaluated the comprehensive effects of both compound action and psychotherapy interventions, encompassing rigorous psychological preparation, debriefing sessions, guided support, and environmental manipulations during the administration session. (4) In addition, nonpharmacological factors have been shown to play a crucial role in determining the quality and nature of the subjective experiences associated with psilocybin use in humans, as well as the potential therapeutic outcomes arising from such use. (5–7) These factors have been referred to as "set and setting," where "set" refers to mindset and includes psychological and emotional components, such as expectations, mood, personality, and past experiences, and "setting" includes physical components such as the location, lighting, and comfort of the environment, as well as social components such as the presence of other people and their relationship to the individual. (8) Consequentially, including these nonpharmacological variables poses a significant challenge to the isolation of the biological and pharmacological effects of psilocybin alone through human studies. (9,10)

Animal models are one way to isolate these molecular effects of psychedelics without additional psychological support. Studies assessing antidepressant-like and anxiolytic-like effects following treatment with psychedelic 5-HT_{2A} agonists in rodent behavioral tests like the forced

swim test (FST), open field test (OFT), and elevated plus maze (EPM) have recently emerged. (11–18) These studies have often identified results consistent with human trials, such as demonstrating antidepressant-like or anxiolytic-like activity through reductions in FST immobility time or increased open arm time in the EPM. However, some results have been inconsistent with this interpretation, such as those demonstrating no effect on FST in Flinders' Sensitive Rats for up to a week after single or repeated psilocybin dosing. (16) Notably, psilocybin is a prodrug that is dephosphorylated by alkaline phosphatases to produce its active metabolite, psilocin, which acts as a psychedelic compound through agonism at the 5-HT_{2A} receptor subtype. (1–3) However, some of the rapidly occurring antianhedonic behavioral effects of psilocybin in mice have been proposed to be $5HT_{2A}$R-independent, suggesting that additional mechanisms may be relevant for the behavioral profile induced by this compound in rodents. (19)

When considering additional or alternative biological modifiers of psilocybin's variable effects, potential targets of interest include stress-associated hormones, such as cortisol and other glucocorticoids, that are released following administration of psilocybin in humans. (20) Recent work has uncovered correlations between acute cortisol release, psychological reports of anxiety, and alterations of long-term therapeutic efficacy, but there is not yet consensus regarding the role of acute stress-associated hormone release in long-term therapeutic outcomes associated with psilocybin or other serotonergic psychedelics. (21) Multiple possible causal relationships between these factors can be formulated; elevated plasma glucocorticoid concentrations may lead directly to manifestation of psychological anxiety; they may exclusively act as downstream biomarkers of an independently initiated psychological process; or there may be mutually reinforcing feedback between these physiological and psychological processes.

Previous animal studies have made headway in assessing the impact of non-psychedelic-induced acute increases in plasma glucocorticoids on subsequent behavior in tasks associated with anxious-like responses. For example, "stress inoculation" effects have been observed in

preclinical studies of rodent anxious responses, where repeated acute stressors reduce subsequent anxious responses. (22,23) Likewise, animal research using ketamine, another rapidly acting antidepressant (RAAD) with marked psychoactive effects, identified rapid transient elevations in plasma and brain corticosterone following its administration to awake rodents. (24) This was suggested to be a supportive mechanism for ketamine's behavioral effects and an important confounding factor to consider in interpreting antidepressant-like outcomes in animal models. Furthermore, ketamine's rapid antidepressant-like effects in rodents were recently reported to be mediated by hypothalamic–pituitary–adrenal (HPA) axis activation, where activation of corticotropin-releasing factor-expressing neurons specifically in response to the scent of male experimenters was essential for ketamine to reduce immobility time in the FST. (25) Together, these observations indicate a better understanding of the relationship between RAAD-induced glucocorticoid release and subsequent behavioral changes is needed. In this work, we test the **hypothesis** *that the drug-induced release of plasma glucocorticoids is a necessary factor for the post-acute (4 h) and long-term (7 days) anxiolytic-like effects of psilocybin in the novelty-suppressed feeding assay (NSF) and OFT.*

2.2 Materials and Methods:

Animals and Husbandry

All experimental procedures were approved by the University of Wisconsin, Madison Animal Care and Use Committee (IACUC) and completed in full accordance with Research Animal Resources and Compliance (RARC) guidelines. All mice used in this work were acclimated to the University of Wisconsin vivarium conditions for at least seven days before handling or experimentation. Unless otherwise noted, food pellets (LabDiet) and water (Inno-Vive) were available ad libitum. All 554 C57Bl6/J mice used (male; 6–8 weeks old; The Jackson

Laboratory, ME, USA) were housed in groups of three or four under a 12 h artificial, reversed light/dark cycle. The room temperature remained constant between 22 and 24 °C.

Drugs

All controlled substances were handled by authorized users on the Schedule I and Schedule II–V DEA (Drug Enforcement Administration) research licenses and WI Special Use Authorizations held by 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 doses between 0.3 and 3 mg/kg. Ketamine hydrochloride (Spectrum Chemical Mfg. Corp.; Gardena, CA) was diluted in 0.9% sterile saline, passed through a 0.2 µm filter, and administered at a dose of 30 mg/kg IP. All IP injections were given at a volume of 10 mL/kg. No mouse was given more than one injection of psilocybin or ketamine or re-used following a washout period. Corticosterone (Sigma-Aldrich) was diluted in 4.5% (2-hydroxypropyl)-beta-cyclodextrin (Biosynth-Carbosynth) in water, vortexed for 1 min, and then sonicated for 3 min at 22 °C, before being diluted to 0.45% beta-cyclodextrin in the animals' drinking water. For experimental batteries requiring chronic corticosterone exposure, mice were given ad libitum access to either corticosterone water (0–80 µg/mL) or the vehicle [0.45% (2-hydroxypropyl)-beta-cyclodextrin] in their home cage for 28-days. (26–28) Both vehicle and corticosterone bottles were refreshed every 7 days for the 28-day period.

Tandem Mass Spectrometry

Tandem mass spectrometry (MS/MS) was performed by the Analytical Instrumentation Center (AIC) in the UW-School of Pharmacy. Instrumentation consisted of a Waters Acquity UPLC system coupled with an AB/Sciex Q-Trap 5500 for traditional triple quadrupole quantitation. Briefly, all standards, quality controls, samples, and blanks were prepared with a 50 ng/mL

concentration of d_{10}-Psilocin (Cerilliant; Round Rock) before being subjected to acetonitrile precipitation and filtration through a Sirocco plate (Waters Corp; Milford, MA). All samples were run on a Kinetex Core–Shell Technology column (Phenomenex; Torrance, CA) with a 2.5 mM aqueous ammonium formate/acetonitrile gradient. Samples were analyzed for both psilocybin and psilocin transitions using the area under the curve for these analytes relative to the internal standard via MultiQuant analysis software (SCIEX; Framingham, MA).

For full experimental details, the instrumentation consisted of a Waters Acquity UPLC system coupled with an AB/Sciex Q-Trap 5500 for traditional triple quadrupole quantitation. All reagents used, including water, were optima grade. Tandem mass spectrometry (LC-MS/MS) began with mixing psilocybin powder with optima-grade methanol to obtain a concentration of 1.0 mg/mL. Dilutions of psilocybin (Usona Institute; Madison, WI) were prepared in ice-cold methanol from 0.5 ng/mL to 600 ng/mL. The psilocin (Usona Institute; Madison, WI) stock solution used for this analysis was diluted in ice-cold methanol at a concentration of 1 mg/mL. The d10-Psilocin (Cerilliant; Round Rock, TX P-099- 1ML) stock solution at a concentration of 100 µg/mL was diluted to 50 ng/mL (1:80). This dilution was utilized as the internal standard. Next, 60 μL of blank mouse serum was pipetted into the calibration and quality control (QC) tubes. The psilocybin dilutions were then spiked, 2.5 μL, into the calibration and QC-labeled tubes. Experimental mouse samples were pipetted into the Sirocco plate (Waters Corp; Milford, MA). After adding the samples, 150 μl of precipitation mix (2 μL d10-Psilocin-, 1.5 μL formic acid-, 146.5 μL acetonitrile- per sample) was pipetted into all the Sirocco wells.

Samples precipitated for 2 minutes before being pushed through the plate at 7 – 12 PSI (Waters; Positive Pressure-96 Processor). The samples were then transferred to 96-Well, 1 ml plates and dried. Samples were resuspended in 98% 2.5 mM Ammonium formate in water/ 2% acetonitrile/ 0.1% formic acid. Two running solvents were used. Solvent A; 2.5 mM Ammonium formate in water/ 0.1% formic acid. Solvent B: acetonitrile/0.1% formic acid. For this analysis, a

Kinetex Core-Shell Technology (Phenomenex; Torrance, CA) column was installed and used on the I-Class UPLC (Waters Acquity UPLC system) coupled with AB/Sciex Q-Trap 5500. Ten blank injections were set up in a sequence to equilibrate the column. The column temperature was held at 28°C, and a flow rate of 0.35 mL/min was used. The numbered data acquisition files were submitted randomized to avoid instrument performance bias. The samples were injected using a 3-minute gradient starting at 25% and going to 61% B in 1.4 minutes, then to 95% over 0.15 min with a 0.6-minute hold at 95%, then back to 25% in 0.15 min and a re-equilibration hold for 0.25 minutes. Samples were injected in triplicate. Two samples were grouped, and each group underwent six injections with six blanks. Transitions analyzed were Psilocybin parent ion – m/z 285.057, daughter ions – m/z 240, m/z 205.1, and m/z 115. Psilocin parent ion – m/z 205.123, daughter ions – m/z 160.1, m/z 115, and m/z 89. d10-Psilocin parent ion – m/z 215.90, daughter ions – m/z 164.1, m/z 117, and m/z 91. The standard curves all had R2 values > 0.995. The MultiQuant analysis software (SCIEX; Framingham, MA) was used to process data obtained from the LC-MS/MS run. A calibration curve was created using standards diluted in blank serum (0.5 to 600 ng/mL). Analyte concentrations in subject plasma were determined using the area under the curve (AUC) of analytes relative to internal standards. The calibration curve was created using a quadratic model with 1/x weighting. Any calibration curve points that showed more than 15% variation in accuracy were removed.

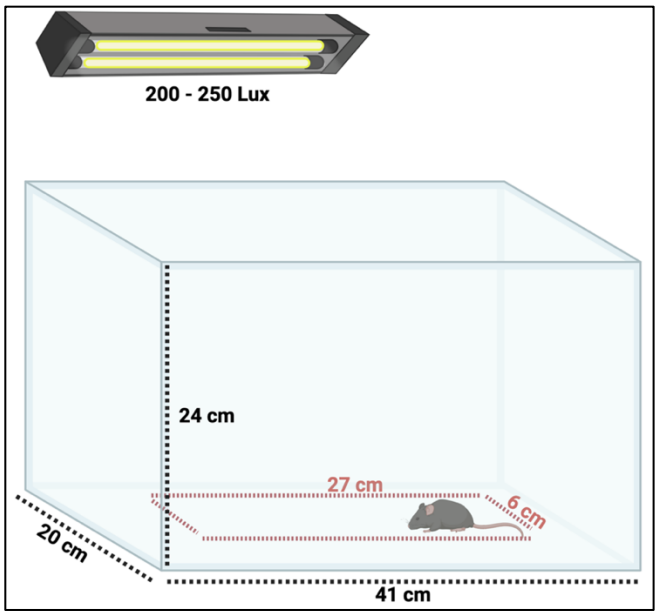
Head Twitch Response and Surgery

To measure acute unconditioned responses to psilocybin, head twitch responses (HTRs) were detected automatically via fluctuations in magnetic field strength signals from magnets cemented to the animals' skulls using slight modifications from previously reported methods. (29) In this study, mice were implanted with skull screws and anesthetized with isoflurane (1.5–2%) to attach a neodymium magnet to the skull screw. Following an at least 7-day recovery period,

individual animals were placed into a clear acrylic cylinder (15.24 cm height × 15.24 cm diameter) wrapped with ~300 rotations of 30-gauge copper magnet wire (Essex, Fort Wayne, IN) inside a dark sound-attenuating chamber. Magnetometer signals were amplified near the source with a homemade custom circuit, and the signal was routed to an RZ5D (filtered at 0.2–1000 Hz and then digitized at 3051.8 Hz). After this time, the animals were administered with 3 mg/kg IP psilocybin and recorded for an additional 2 h. Changes in the local magnetic field induced by head twitches (~60–90 Hz signal) were assessed using custom MATLAB software. Automated results were compared to visually observed HTRs for internal validation. All HTR responses were recorded between 1 and 4 PM.

Open Field Test

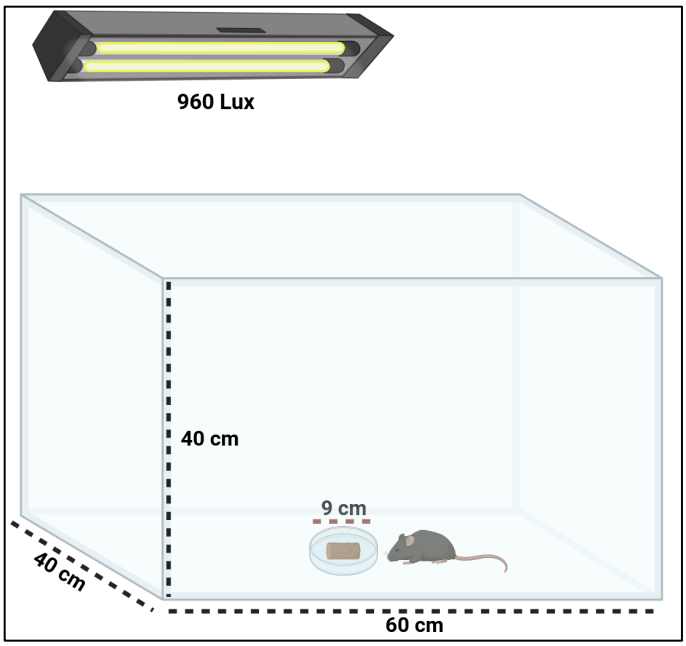
To test for drug-induced changes in locomotor, exploratory, and anxious behavior, mice were assessed in the OFT. Mice were injected IP with psilocybin (0–3 mg/kg) and then individually placed into a corner within an open-field apparatus (41 × 20 × 24 cm), within 5 min–7 days after injection. The center zone was defined as the arena's middle one-third (6 × 27 cm). The apparatus was illuminated at ~200–250 lux. Mice could explore freely for 10 min in acute experiments and 150 min in long-term experiments. Time spent in the center and total distance traveled were automatically quantified using Any-Maze software. Each apparatus was cleaned before and after each test with Trifectant. All OFT measurements were run between 1 and 4 PM. Where repeated measurements were taken, data were normalized to show % of saline average activity at each time point, with repeated measurement in the OFT at 15 min and 4 h. This was done to put both time points on the same scale and highlight dose-dependent interactions that are independent of repeated testing effects.



Scheme 1: Open Field Test (OFT) apparatus with corresponding dimensions and set-up.

Novelty Suppressed Feeding

Mice were assessed in the NSF assay to measure animal behavior in a task combining motivated behavior with environmentally mediated anxiety. Animals underwent a 16-hour period of food deprivation. Mice then received an IP injection of saline or drug 4-h before performing the NSF test. For the test, a food pellet soaked in 50% sucrose solution was placed into a glass petri dish that served as the center zone (9 cm diameter) and centered within a novel cage environment (60 × 40 × 40 cm) illuminated at 960 lux. Mice were then placed into a corner of the apparatus and allowed to explore for 10 min. Latency to the first feed was recorded by a trained and blinded observer, and movement and distance traveled were monitored by Any-Maze software. Pellet weight was also obtained immediately before and after each test. After testing, the mice were returned to their housing and given normal food and water. Each apparatus was cleaned before and after each test with Trifectant. All NSF measurements were run between 1 and 4 PM.



Scheme 2: Novelty Suppressed Feeding (NSF) apparatus with corresponding dimensions and set-up.

Blood Sample Collection and Corticosterone ELISA

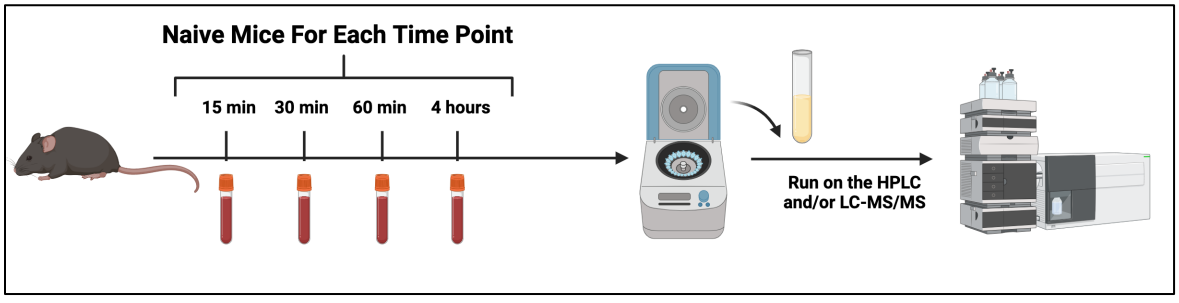
To investigate the effects of psilocybin on corticosterone levels in C57BL/6 mice, repeated retro-orbital bleeds were collected from each mouse at all time points other than the terminal time point, where blood was collected via decapitation. Animals were anesthetized with isoflurane before blood collection at all time points. After collection, the samples were centrifuged at 10,000 rpm (11,292*g*) for 10 min at 4 °C (Eppendorf-Centrifuge 5430 R). The serum fraction was separated and stored at –80 °C. Upon thawing, the plasma corticosterone concentrations were assessed using a colorimetric ELISA analysis (Enzo-Life Sciences, Corticosterone ELISA Kit) following the provided and established protocol. All independent biological samples were run in technical duplicate or triplicate, and calculated concentrations were corrected for assay dilution (40×).

Statistical Analyses

Statistical analyses were performed using GraphPad Prism, version 9 (San Diego, CA). All tests were run as two-tailed analyses, setting $p < 0.05$ as the threshold for significance. Data analyzed across time were assessed using repeated-measures approaches; all samples were otherwise considered independent for analysis purposes. All data were tested for normality and processed using nonparametric tests where appropriate. Post-hoc tests for ANOVA and survival analyses are reported with *p*-values corrected for multiple comparisons when follow-up tests were run to assess differences between specific conditions.

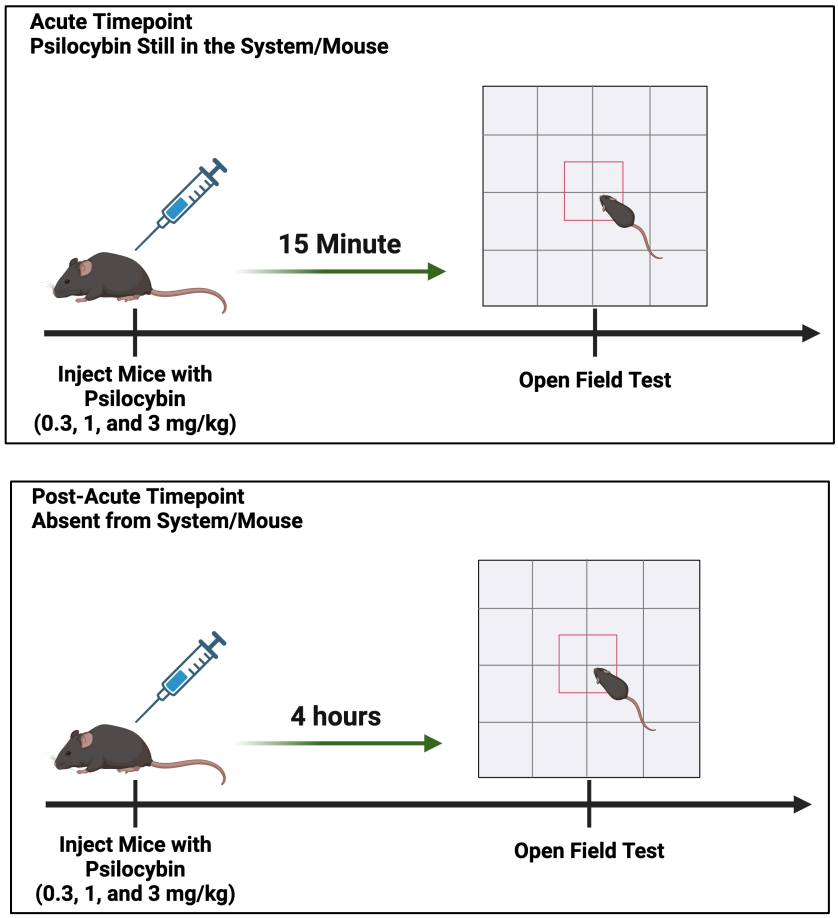
2.3 Results:

To identify a dose of psilocybin that could reliably demonstrate behavioral effects following a single drug exposure, 6–8-week-old male C57Bl/6 mice were given IP injections with 0–3 mg/kg psilocybin, covering the usual range reported in previous animal experiments. (11–18) In these animals, plasma psilocybin and psilocin concentrations at 15 min exhibited a robust dose-dependent plasma exposure profile across this dose range, as measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS; Figure 1A). At the highest dose, plasma concentrations of psilocybin returned to baseline by 60 min, and psilocin returned to baseline by 240 min (Figure 1B). The calculated half-life for psilocybin was 8 ± 1 min, and that for psilocin was 35 ± 4 min.



Scheme 3: Determine psilocybin and psilocin time-course and half-life via blood collections.

With this pharmacokinetic data to guide relevant time-point selection for acute exposure and post-acute drug clearance, an OFT was next used to assess locomotion and time spent in the center of the arena as an ethological test of approach-avoidance behavior. (30) Measurement of these behaviors was assessed at the time of maximal measured plasma concentrations (15 min) and at the time of drug clearance from the plasma (4 h).



Scheme 4: Using the Open Field Test (OFT) characterize psilocybin's locomotor and anxiolytic effects. (Top) Acute 15 minutes. (Bottom) Post-acute 4 hours.

Regarding distance traveled, while there was a significant dose × time interaction, with higher doses trending toward a greater distance traveled at 15 min, no single dose was found to have significantly different effects from saline at either time point (Figure 1C). Regarding time in

the center of the arena, there was also a significant dose × time interaction for psilocybin (Figure 1D). A negative relationship between the dose and the time spent in the center of the open field was seen at 15 min, with 3 mg/kg of psilocybin demonstrating significant suppression as compared to 0.3 mg/kg. In contrast, a positive relationship between the dose and the time spent in the center of the open field was observed at 4 h, with 3 mg/kg psilocybin demonstrating significant enhancement of this measure compared to 0.3 mg/kg. Overall, it was observed that increasing doses of psilocybin supported acute (15 min) anxiogenic-like and post-acute anxiolytic-like behavior in this assay, both when accounting for repeated exposure effects (as in Figure 1) and when using raw measures (Figure S1).

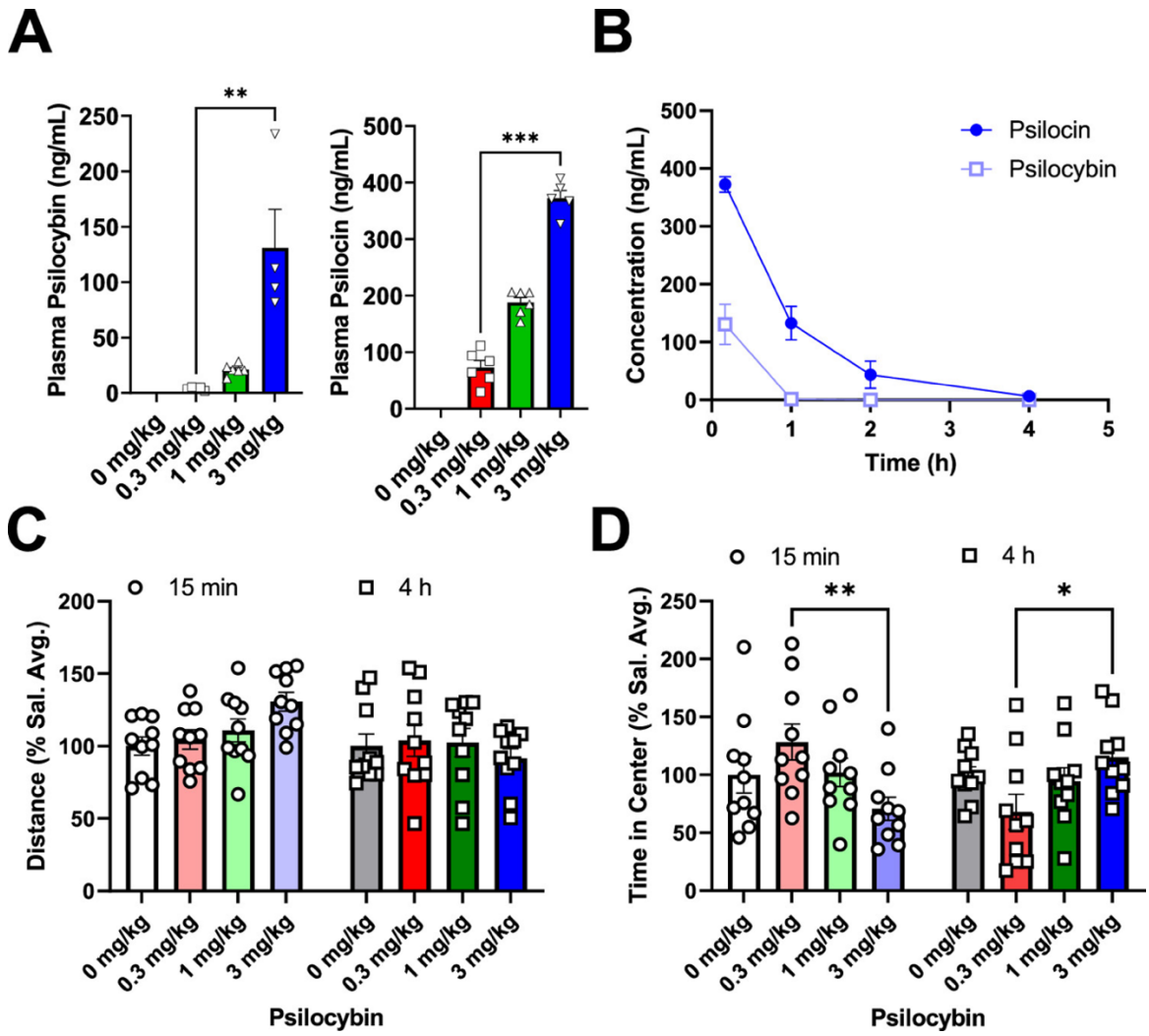
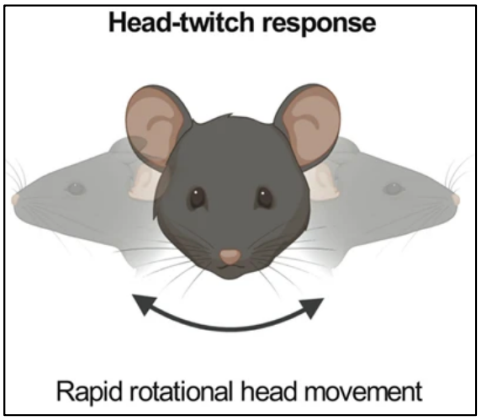
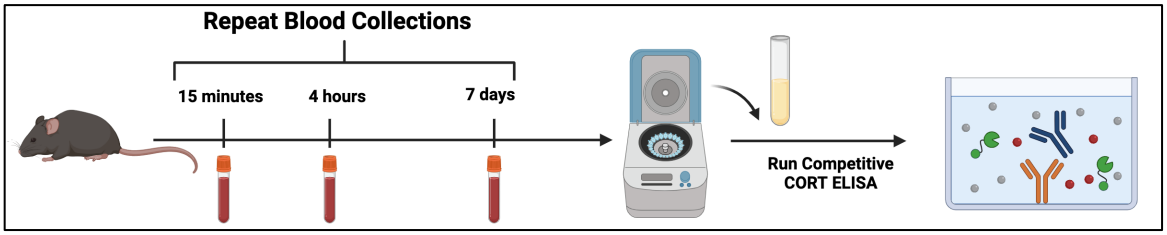


Figure 1: Psilocybin's effects on anxiety-like behavior are inversely related during acute exposure and post-acute clearance. (A) Plasma psilocybin and psilocin concentrations at 15 min after IP administration of psilocybin. Kruskal–Wallis (n = 5; psilocybin, $p < 0.0001$; psilocin, $p < 0.0001$) with Dunn's multiple comparisons test (**p = 0.0014, ***p = 0.0005). **(B)** Time course of psilocybin and psilocin concentrations following administration of 3 mg/kg IP psilocybin (n = 4–5). **(C)** Dose–response relationship of the distance traveled relative to the saline average during a 10 min exposure to an open-field arena at 15 min and 4 h after IP administration of psilocybin. Two-way RM ANOVA (dose × time interaction, p = 0.034; dose main effect, p = 0.65; time main effect, p = 0.025). **(D)** Dose–response relationship of time in the center during a 10 min exposure to an open-field arena at 15 min and 4 h after IP administration of psilocybin. Two-way RM ANOVA (dose × time interaction, p = 0.0019; dose main effect, p = 0.95, time main effect, p = 0.50) with Sidak's multiple comparisons (*p = 0.047, **p = 0.001). Data shown as mean ± SEM for all panels.

The 3 mg/kg dose of psilocybin was selected for further observations, given its observed significant effects on acute anxiogenesis and post-acute anxiolysis in this initial experiment. Animals given this dose were assessed for HTRs, which are a classical proxy of hallucinogenic activity in mice. Psilocybin-treated animals demonstrated a clear elevation in the number of HTRs occurring in the first 10 min after IP administration, which rapidly returned to baseline (2A). Next, animals given 3 mg/kg psilocybin IP were assessed for acute elevations in plasma corticosterone at 15 min; this was observed to occur at significantly higher levels compared to saline administration at 15 min postinjection, confirming that this dose would be adequate to test our hypothesis about drug-induced corticosterone release (Figure 2B). This increase in plasma corticosterone returned to baseline by 4 h following administration and remained there 7 days later.



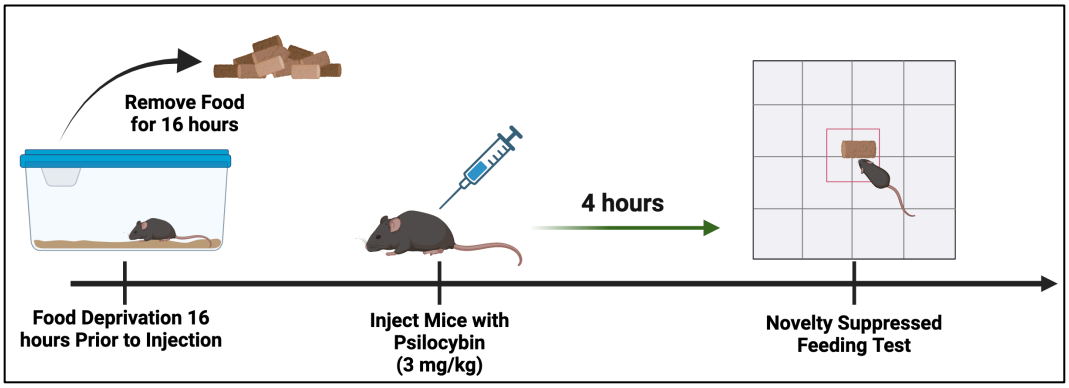
Scheme 5: Sensitivity to Serotonergic Activity. Reliably indicates psilocybin's effects on 5HT-2A due to its sensitivity to serotonergic activity.



Scheme 6: Assess drug-induced stress response and CORT concentrations following IP psilocybin.

With this evidence of meaningful *in vivo* pharmacologic activity and induction of plasma corticosterone elevations occurring acutely after being treated with 3 mg/kg IP psilocybin, we next looked at the NSF to confirm anxiolytic-like effects of this intervention at the 4 h post-acute period, which follows drug clearance from the plasma. At this post-acute period, psilocybin was found to reduce the latency to first consumption in the NSF assay (Figure 2C), while there was no significant effect on the probability of feeding overall (Fisher's Exact Test, $p = 0.24$).

Animals treated with 3 mg/kg IP psilocybin also exhibited increased time in the center of this assay at 4 h posttreatment (Figure 2D). No effects of this dose were seen post-acutely in either the sucrose preference test or the FST (Figure S2), indicating that anhedonic and behavioral despair-associated behaviors are not altered at 4 h after administering this psilocybin dose.



Scheme 7: Assess post-acute anxiolytic effects following IP psilocybin in the NSF. The time point of 4 hours was selected due to the absence of the drug and ongoing drug activity.

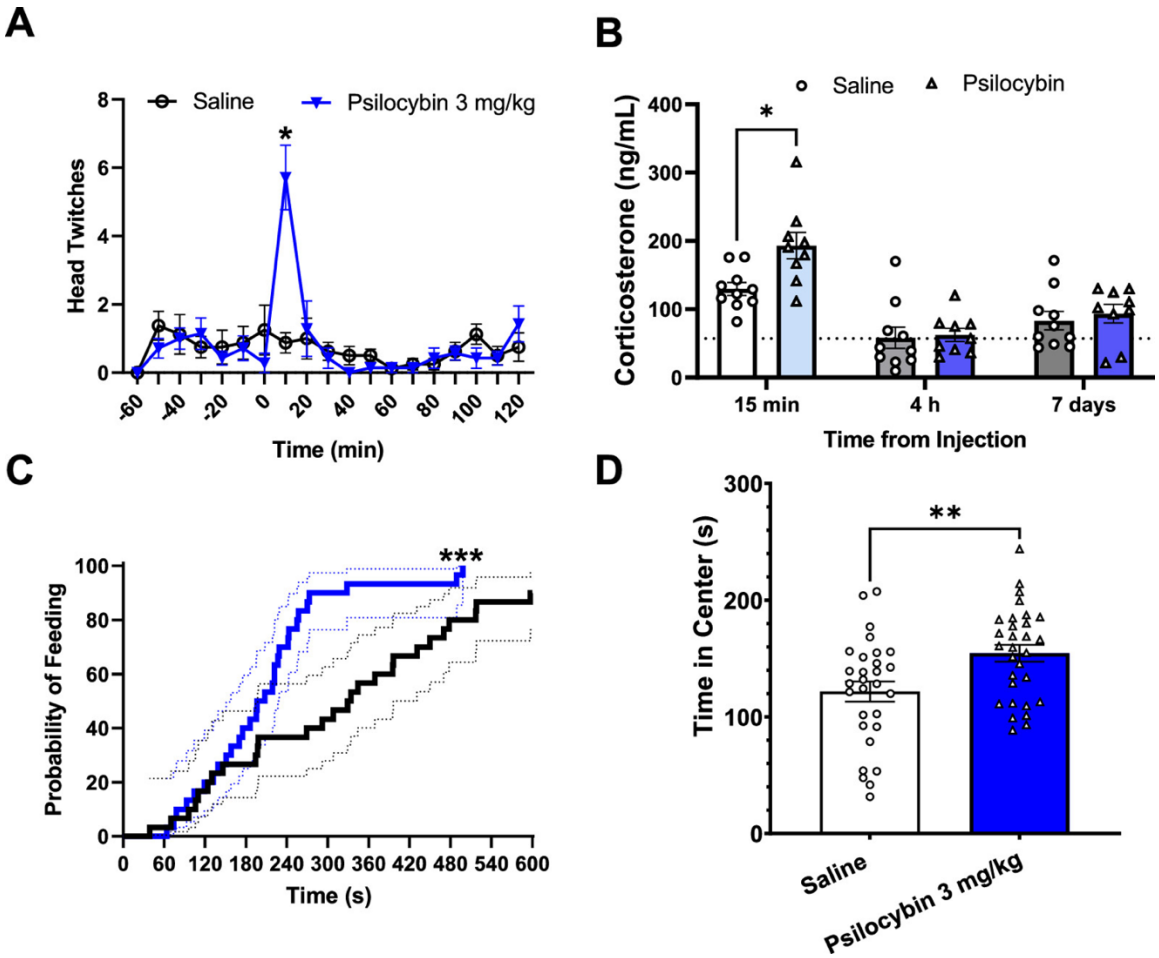
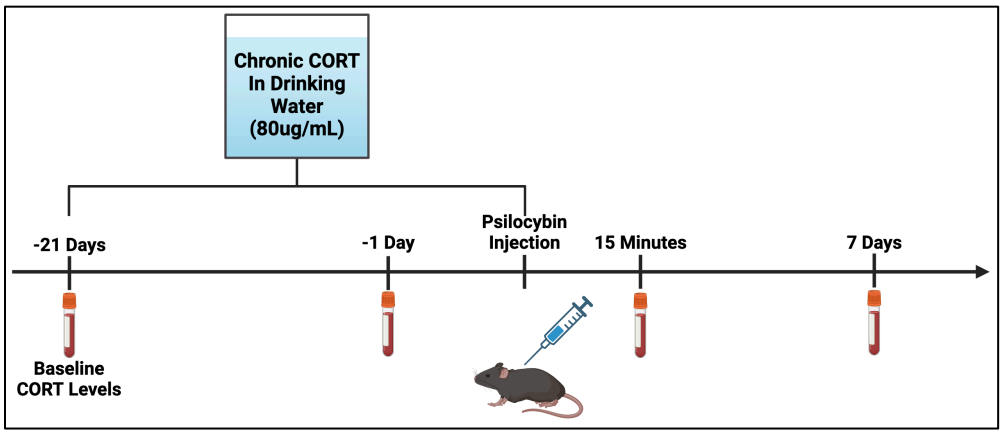
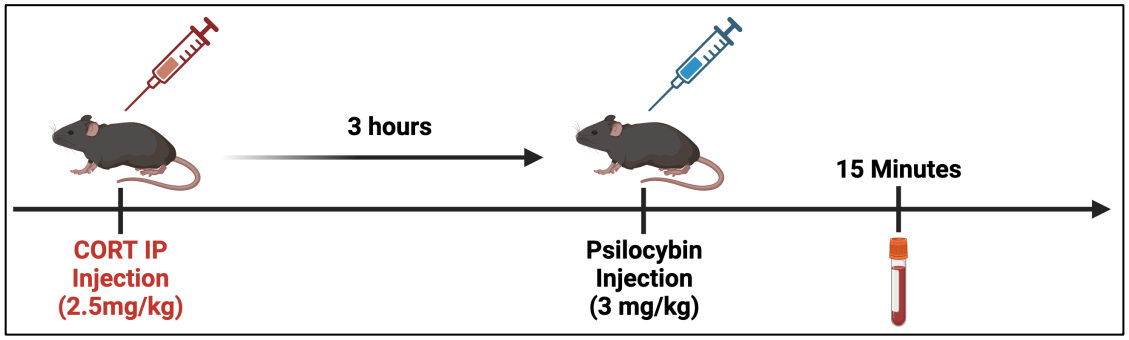


Figure 2: IP administration of 3 mg/kg psilocybin induces acute head twitches and elevations in plasma corticosterone and post-acute reductions in anxiety-like behavior. **(A)** Head twitch counts across 10 min bins following psilocybin administration, mean ± SEM. Two-way RM ANOVA (n = 7–8; drug × time interaction, $p < 0.0001$; drug main effect, $p < 0.0001$; time main effect, $p = 0.48$) with Sidak's multiple comparisons (*p = 0.031). **(B)** Plasma corticosterone concentrations at 15 min, 4 h, and 7 days after IP injection, mean ± SEM. Two-way RM ANOVA (drug × time interaction, $p = 0.0441$, time main effect, $p < 0.0001$, drug main effect, $p = 0.066$) with Sidak's multiple comparisons (*p = 0.036). **(C)** Survival curves for feeding behavior in a 10 min exposure to a novelty-suppressed feeding test at 4 h following IP injection. Mantel–Cox, values ±95% CI (n = 30; ***p = 0.001). **(D)** Time in the center of the novelty-suppressed feeding chamber under the same conditions, mean ± SEM. t-test (n = 30; **p = 0.0047).

After observing both psilocybin-induced acute glucocorticoid release and psilocybin-induced post-acute anxiolysis occurring after treatment with 3 mg/kg IP, we next tested whether blockade of either glucocorticoid release or glucocorticoid action could suppress the post-acute anxiolytic effects of psilocybin. Adapting an established protocol previously used in rats, corticosterone pretreatment at 2.5 mg/kg IP, 3 h before drug administration, was first conducted. (31) This acute intervention was able to significantly blunt all administration-associated glucocorticoid release at 15 min, with corticosterone concentrations from the psilocybin-treated animals reduced such that they were now like those from untreated animals (Figure 3A). Passive exposure to 80 µg/mL of chronic oral (PO) corticosterone in drinking water for 28-days (about 4 weeks) was next explored to establish suppression of the HPA axis response to drug treatment over longer periods of time. Induction of negative feedback and HPA axis suppression were observed following the time-course of plasma corticosterone without drug treatment. This passive corticosterone exposure successfully led to significant elevations in plasma corticosterone concentrations, as measured on the 20th day of exposure, one day prior to drug administration. Importantly, it also resulted in a complete blockade of injection-induced corticosterone elevations in response to either saline or 3 mg/kg psilocybin injected IP at 15 min after administration these values were not significantly different from those measured on the previous day (Figure 3B). Furthermore, evidence of chronic HPA axis suppression was seen with this paradigm, as corticosterone concentrations fell significantly below the baseline on day 7 after drug treatment, following an 8 h period of withdrawal from passive exposure (Figure 3B).



Scheme 8: Chronic CORT or vehicle exposure in drinking water for 21 days prior to psilocybin injection.



Scheme 9: Pretreatment with a CORT or vehicle injection 3 hours prior to psilocybin administration.

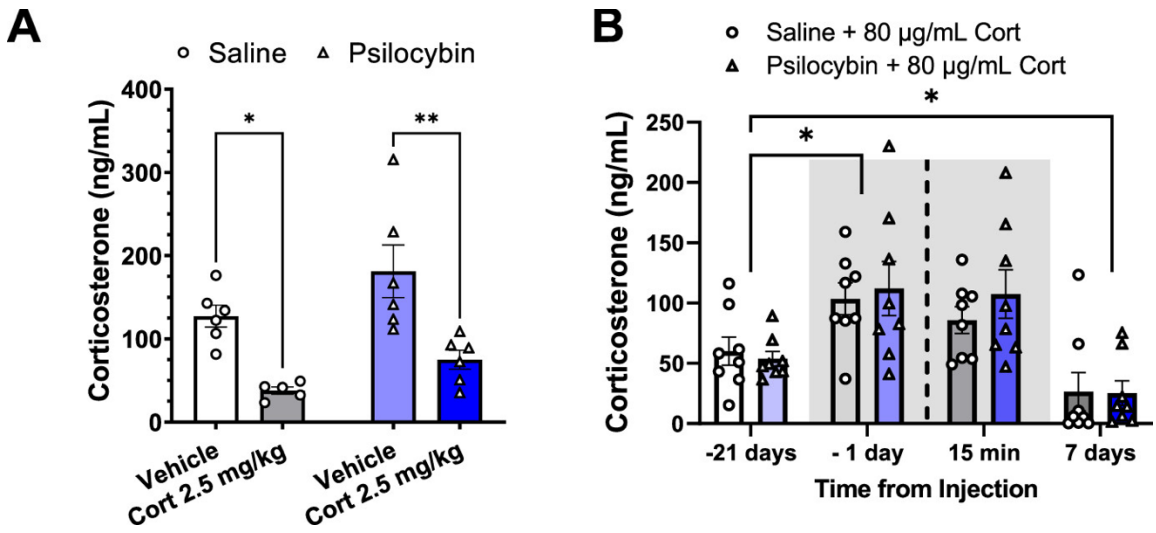
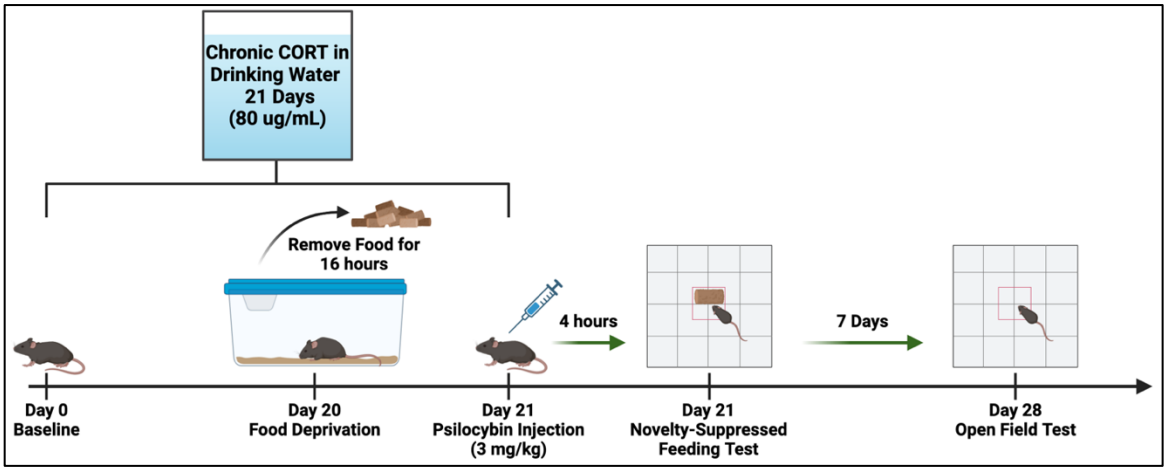


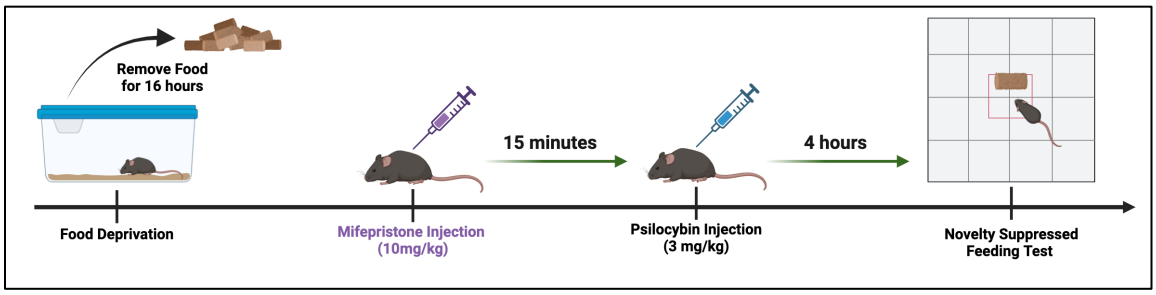
Figure 3: Acute or chronic pretreatment with corticosterone suppresses HPA axis function and blocks psilocybin-induced corticosterone elevations. (A) Plasma corticosterone concentrations at 3 h after pretreatment with IP corticosterone and 15 min after IP injection of saline or psilocybin. Two-way ANOVA (drug × pretreatment interaction, $p = 0.66$, drug main effect, $p = 0.028$, pretreatment main effect, $p < 0.0001$) with Sidak's multiple comparisons (*$p = 0.026$, **$p = 0.004$). **(B)** Plasma corticosterone concentrations at baseline (–21 days), following 20-days of treatment with oral corticosterone (–1 day), at 15 min after drug administration, or 7 days after treatment, following withdrawal of oral corticosterone. Two-way RM ANOVA (drug × time interaction, $p = 0.79$, drug main effect, $p = 0.58$, time main effect, $p < 0.0001$) with Sidak's multiple comparisons for all groups between time points (*$p \leq 0.05$). Data is shown as mean ± SEM for all panels.

To assess whether such suppression of drug-induced corticosterone release would block the post-acute reductions in latency to feed previously seen with psilocybin, behavior in the NSF was measured in animals either during an ongoing period of chronic glucocorticoid exposure (80 µg/mL × 21 days) or following withdrawal after a prior period of chronic glucocorticoid exposure (50 µg/mL × 28 days). Crucially, the ability of psilocybin to induce post-acute anxiolysis was blocked in both cases (Figure 4A, B). This pretreatment had no significant effects on the overall probability of feeding (Fisher's Exact Test; 4A, $p = 0.30$; 4B, $p = 0.21$).

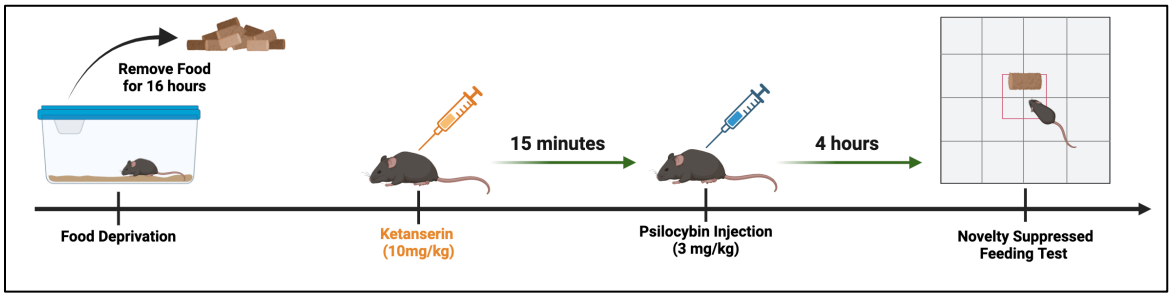
Next, the post-acute effects of psilocybin in the NSF were measured following pretreatment with mifepristone, a glucocorticoid, mineralocorticoid, and progesterone receptor antagonist. While mifepristone treatment alone had no significant effects on feeding latency as compared to saline at the 10 mg/kg dose used, mifepristone-induced steroid receptor blockade was found to significantly blunt the effect of subsequent psilocybin administration, as compared to psilocybin alone (Figure 4C), without modifying the overall probability of feeding (Fisher's exact test; Psil. vs Psil. + Mifep., $p > 0.999$). In contrast, pretreatment with 1 mg/kg of ketanserin, a 5-HT_{2A} receptor antagonist, had no effect on the post-acute NSF feeding profile of animals treated with psilocybin (Figure 4D). This pretreatment also did not modify the overall probability of feeding (Fisher's exact test; Psil. vs Psil. + Ketans., $p > 0.999$).



Scheme 10: Chronic CORT or vehicle exposure in drinking water prior to psilocybin injection. Injected on day 21, following an 16 hour food deprivation. Tested in the NSF 4 hours post-injection. Then tested in the OFT 7 days following injection to assess the long-term anxiolytic effects.



Scheme 11: Mifepristone – Glucocorticoid receptor antagonist. Blocks CORT from binding to its receptors. Pretreatment with Mifepristone prior to administration of psilocybin.



Scheme 12: Ketanserin – Serotonin 5HT-2A antagonist. Pretreatment with ketanserin prior to administration of psilocybin.

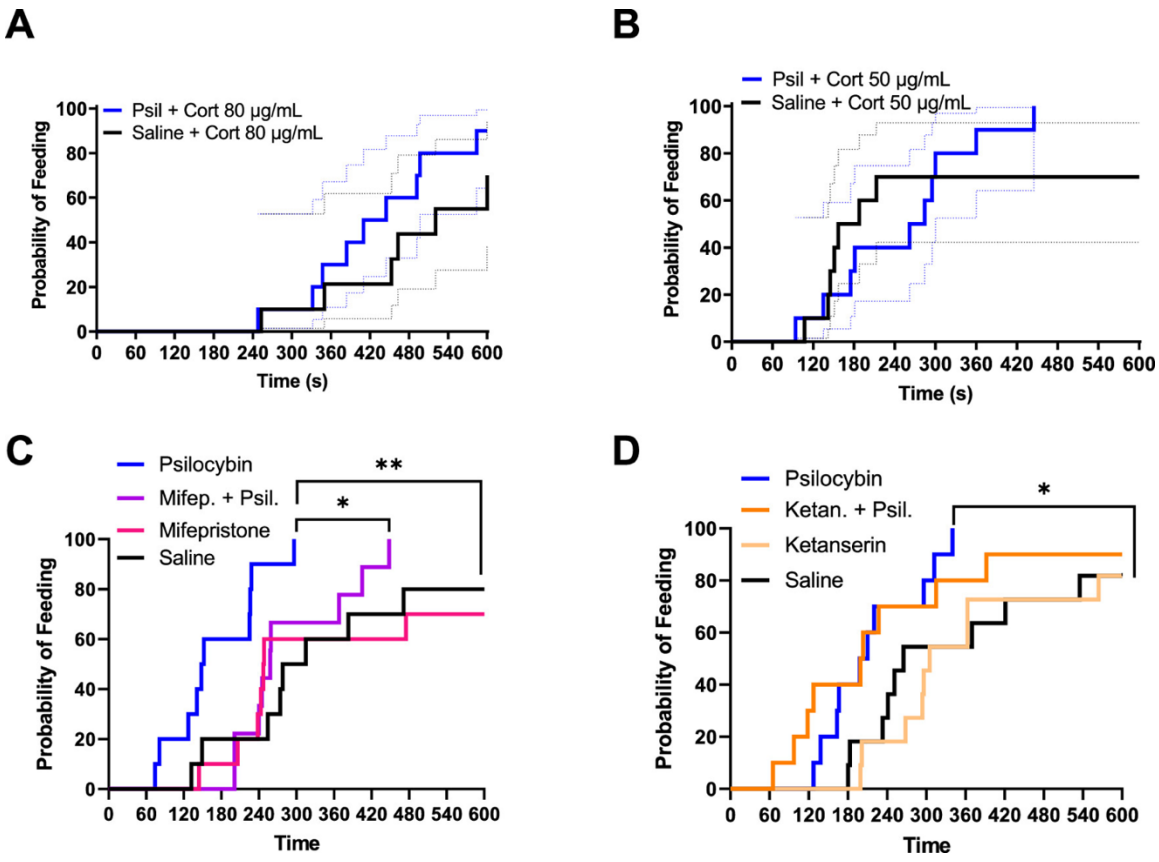
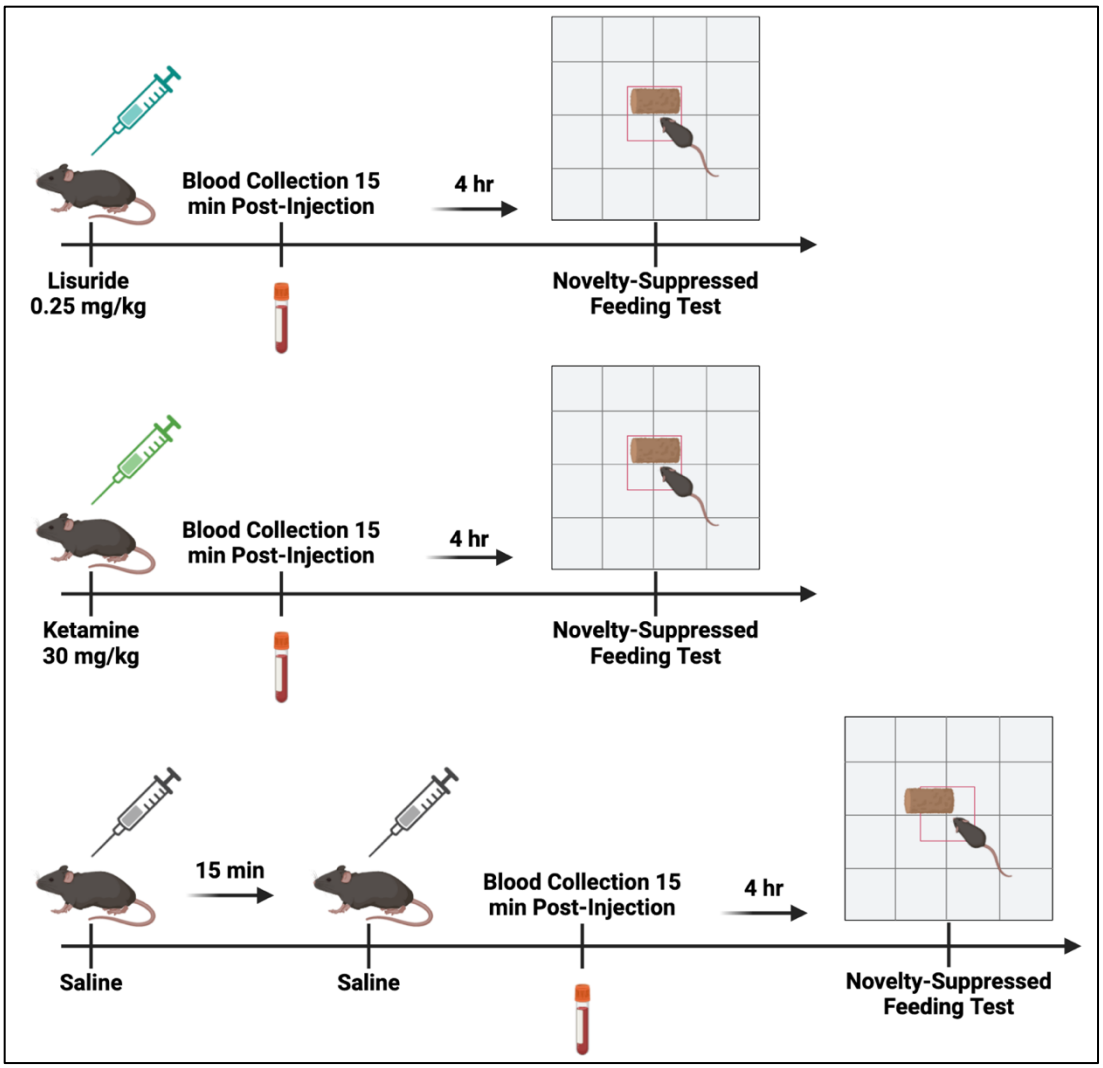


Figure 4: Suppression of psilocybin-induced CORT elevations or blockade of CORT binding to glucocorticoid receptors blocks psilocybin's post-acute reductions in anxiety-like behavior. Survival curves for feeding behavior in a 10 min exposure to a novelty-suppressed feeding test at 4 h following IP injection of 3 mg/kg psilocybin and (A) 21-day pretreatment with 80 µg/mL oral corticosterone. Mantel-Cox ($p = 0.16$), values ± 95% CI. (B) 4 days after withdrawal of a 28-day pretreatment with 50 µg/mL of oral corticosterone. Mantel-Cox ($p = 0.59$), values ± 95% CI. (C) 15 min pretreatment with 10 mg/kg of mifepristone administered IP. Mantel-Cox (all curves, $p = 0.0003$) and multiple comparisons (Psil. vs. saline, $^{**}p = 0.0075$; Psil. vs Psil. + Mifep., $^{*}p = 0.0174$; Mifep. vs saline, $p = 0.95$). (D) 15 min pretreatment with 1 mg/kg of ketanserin administered IP. Mantel-Cox (all curves, $p = 0.041$) and multiple comparisons (Psil. vs. saline, $^{*}p = 0.05$; Psil. vs Psil. + Ketans., $p = 0.45$; Ketans. vs saline, $p = 0.78$).

With these indications that blockade of glucocorticoid release or binding could mitigate the post-acute effects of psilocybin in the NSF assay, we next assessed whether alternative interventions that induce glucocorticoid release but do not share either the perception-altering

effects or the receptor-binding profile of psilocybin would still be sufficient to demonstrate similar post-acute anxiolytic effects to those of psilocybin. To this end, a group of animals were given 0.25 mg/kg of lisuride, a compound with robust 5-HT_{2A} agonist activity but no overt alterations in conscious perception that has recently been reported to be a RAAD. (32) Another group of animals was given 30 mg/kg of ketamine, a RAAD compound that acts independently of 5-HT_{2A} activation but does induce alterations in conscious perception. (33)

At these doses, both compounds were found to induce elevations in plasma corticosterone at 15 min after administration, with lisuride having a significantly greater effect than ketamine. Notably, two injections of saline separated by 15 min showed similar elevations in plasma corticosterone to the ketamine injection, though not as much as lisuride (Figure 5A). Plasma corticosterone concentrations returned to baseline by 4 h for both the lisuride and ketamine conditions (Figure S3). Injection of lisuride or double injections of saline also generated significant reductions in latency to feed in the NSF assay at 4 h post-administration, as compared to a single saline injection, mimicking the profile seen with psilocybin (Figure 5B). Furthermore, when repeated saline injection stress-was preceded by treatment with mifepristone, there was a significant reversal of the dual-injection anxiolytic-like effects (Figure S4). Overall, these results indicated that repeated glucocorticoid release, downstream of either drug-induced effects or injection-associated stress, was sufficient to induce post-acute anxiolysis.



Scheme 13: Lisuride – Non-hallucinogenic 5HT-2A agonist. Ketamine – NMDA antagonist rapidly acting antidepressant (RAAD) with marked psychoactive effects**.** Double – saline injections 15 min apart. With blood collected at 15 min post-injection and then tested in the NSF at 4 hours.

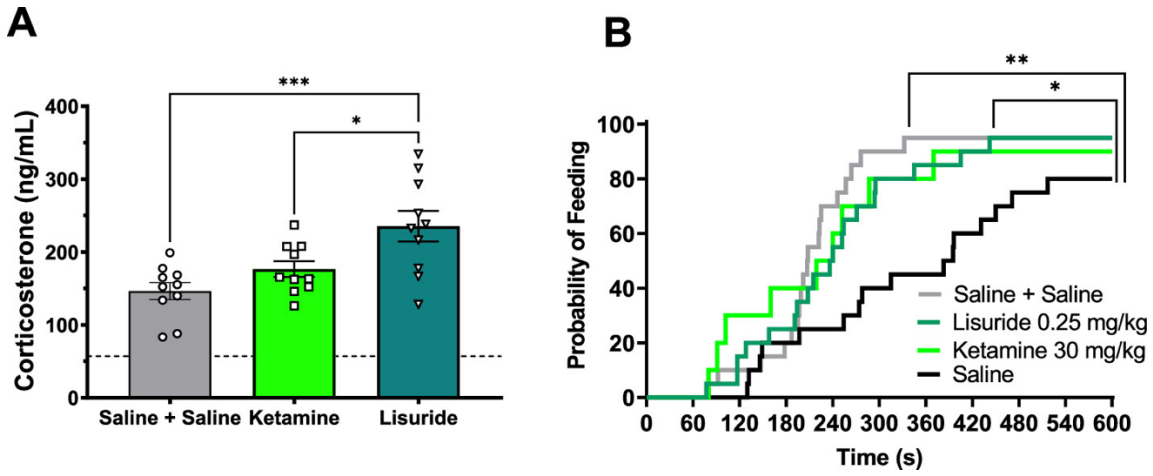


Figure 5. Repeat injection and drug conditions that increase plasma corticosterone also reduce latency to feed in novelty-suppressed feeding. (A) Plasma corticosterone concentrations at 15 min after IP injection with saline + saline, 0.25 mg/kg lisuride, or 30 mg/kg ketamine. Dotted line reference is the mean concentration of a no-injection baseline. One-way ANOVA ($p = 0.0011$) with Tukey's multiple comparisons (*$p = 0.278$, ***$p = 0.0009$), mean ± SEM. Survival curves for feeding behavior in a 10 min exposure to a novelty-suppressed feeding test at 4 h following IP injection of **(B)** saline, saline + saline, lisuride, or ketamine. Mantel–Cox (All curves, $\chi2 = 9.858$, $p = 0.0198$) and multiple comparisons (saline + saline vs saline, $\chi2 = 8.741$, **$p = 0.009$; lisuride vs saline, $\chi2 = 5.776$, *$p = 0.049$; ketamine vs saline, $\chi2 = 3.634$, $p = 0.17$).

Finally, to see if the anxiolytic-like effects of psilocybin were persistent beyond this post-acute period, we assessed behavior in an open field 7 days following drug administration. Although no significant difference was seen for drug treatment within the first 10 min of the assay, significant psilocybin-induced increases in time spent in the center of the chamber were apparent when mice were allowed to explore the apparatus for a longer (2.5 h) period Additionally, in mice that were exposed to chronic oral corticosterone in their drinking water (80 µg/mL × 28 days), psilocybin did not demonstrate an anxiolytic-like effect at 7 days later. Instead, there was a trend toward reducing the time in the center for psilocybin-treated animals compared to animals treated with saline. This behavioral pattern yielded a significant psilocybin–corticosterone treatment interaction for time in the center of the OFT at 7 days (Figure 6A). In contrast, there was no significant interaction effect for psilocybin and corticosterone treatment regarding overall distance

traveled (Figure 6B), indicating that alterations in time spent in the center were not due to long-term modification of locomotor activity.

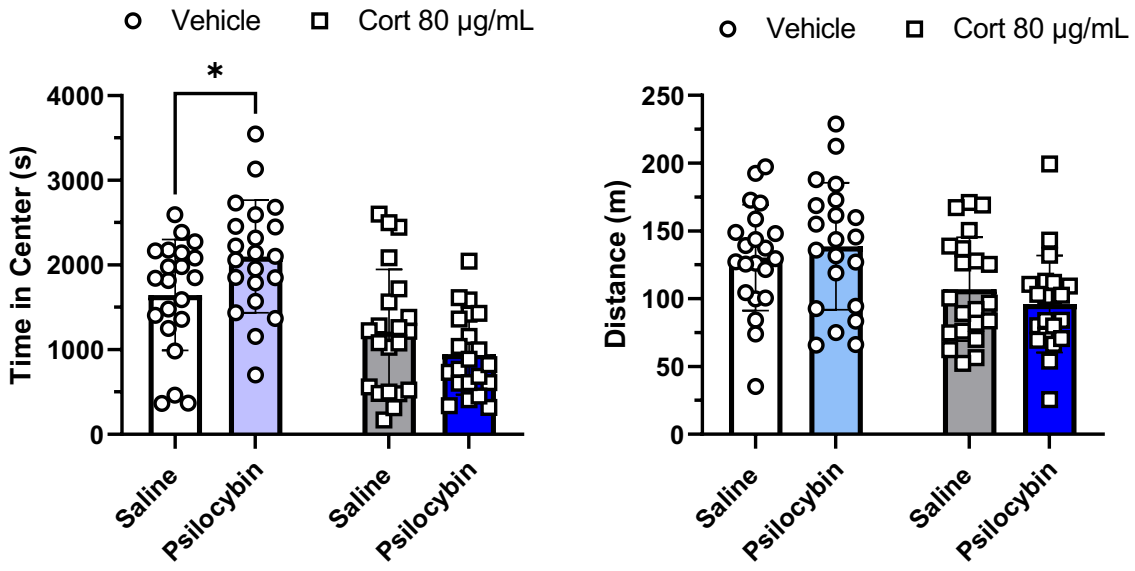


Figure 6. Psilocybin's long-term anxiolytic effects are sensitive to chronic elevations in plasma corticosterone concentration. Behavioral effects in an open-field apparatus during a 150 min exposure at 7 days following IP administration of saline or psilocybin (3 mg/kg) ± 28 days (about 4 weeks) of 80 µg/mL oral corticosterone. **(A)** Time in the center. Two-way ANOVA (psilocybin × corticosterone interaction, $p = 0.0115$; drug main effect, $p < 0.0001$; chronic corticosterone main effect, $p = 0.50$) with Sidak's multiple comparisons vs saline (*$p = 0.046$). **(B)** Distance traveled. Two-way ANOVA (psilocybin × corticosterone interaction, $p = 0.27$; drug main effect, $p = 0.87$; chronic corticosterone main effect, $p = 0.0003$). All data shown as mean ± SEM.

2.4 Discussion:

The results of these experiments suggest that psilocybin-induced glucocorticoid release is a key mechanism for inducing post-acute anxiolytic-like effects in mice and that transient psilocybin-induced plasma glucocorticoid elevations are a necessary factor for generating long-term anxiolytic-like effects in mice as well.

Notably, the psilocybin-induced post-acute anxiolysis observed here was also induced by alternative interventions that also cause plasma corticosterone increases through mechanisms independent from either overt perceptual alterations or direct 5-HT_{2A} receptor activation. Indeed,

even repeated handling and injection stress separated by 15 min could induce a similar outcome. However, this effect of repeated handling and injection was blocked when glucocorticoid receptors were antagonized with mifepristone. To help contextualize these findings, it is notable that repeated corticosterone administration alone has recently demonstrated bidirectional effects on stress-associated behavioral adaptation in rodents; a single corticosterone exposure can enhance future anxiety-like responses, but rapid stress or second corticosterone increase postexposure is sufficient to prevent this behavioral change. (27,28)

In this instance, IP psilocybin administration may be acting similarly to generate a corticosterone response profile that provides resilience to subsequent exposure to post-acute stress. Given our findings here, we suggest that the interpretation of both acute and post-acute effects of psilocybin or other psychedelics on anxiety-like behaviors in mice should routinely account for multiple injection-associated glucocorticoid release as a potential confounding factor, particularly when performing antagonist blockade experiments. As the anxiety-modifying effects of repeated corticosterone injections have been hypothesized to be dependent on delayed glutamate-sensitive neuroplastic effects occurring in the basolateral amygdala, future investigations into the mechanistic basis for the observed interaction between psilocybin (as well as other RAADs, like ketamine and other classical psychedelics) and corticosterone will benefit from monitoring both immediate and long-term changes in cortical and amygdalar glutamate concentrations following 5-HT_{2A} agonist administration. (28,34)

Furthermore, these results indicate that the chronic glucocorticoid plasma concentration profile is a crucial factor in modifying long-term anxiety-associated outcomes. Specifically, when glucocorticoid plasma concentrations were chronically experimentally elevated before, during, and after psilocybin administration, the long-term anxiolytic profile of psilocybin was lost This observation of psilocybin's sensitivity to exogenous glucocorticoid manipulation will likely be useful for future experiments measuring the functional impact of stress paradigms applied at

different times in relation to psychedelic drug administration, including paradigms that manipulate stress via changes to the dosing environment itself. Intriguingly, such environmentally dependent drug effects have already been observed in mice regarding the effects of other antidepressants, such as selective serotonin reuptake inhibitors. (35)

Finally, these findings demonstrate that chronic oral corticosterone suppression of drug-induced acute glucocorticoid release is a beneficial model for the study of neuroendocrine modulation of psychedelic effects in rodents. Future directions include an assessment of whether the interaction of chronic corticosterone-induced HPA axis suppression and psilocybin effects on long-term anxiety-like behaviors generalizes to other means of manipulating HPA axis function and to other psychedelic drugs. Measurement of functional and structural neuroplastic outcomes following manipulation of acute and long-term glucocorticoid release in the presence of 5-HT_{2A} activation will also be important.

One notable limitation of these studies is the use of male mice only; direct assessment of sex differences in this response is being pursued, given well-established knowledge regarding differential stress responses (26,36) and emerging evidence of differential psychedelic responses in male and female rodents. (37,38) Another limitation is that the roles of IP injection-related stress versus drug-related stress in the treated animals were not fully isolated from one another; the use of minimally stressful administration techniques for psilocybin (such as infusion through a pre-implanted IV catheter) could lend further clarity to this dimension in future studies. A final consideration is that a longer period was required to observe the long-term effects of drug administration in the OFT, potentially making measurements at this time point more reflective of perseverative exploratory behaviors than of behavioral response to a novel, anxiety-provoking environment.

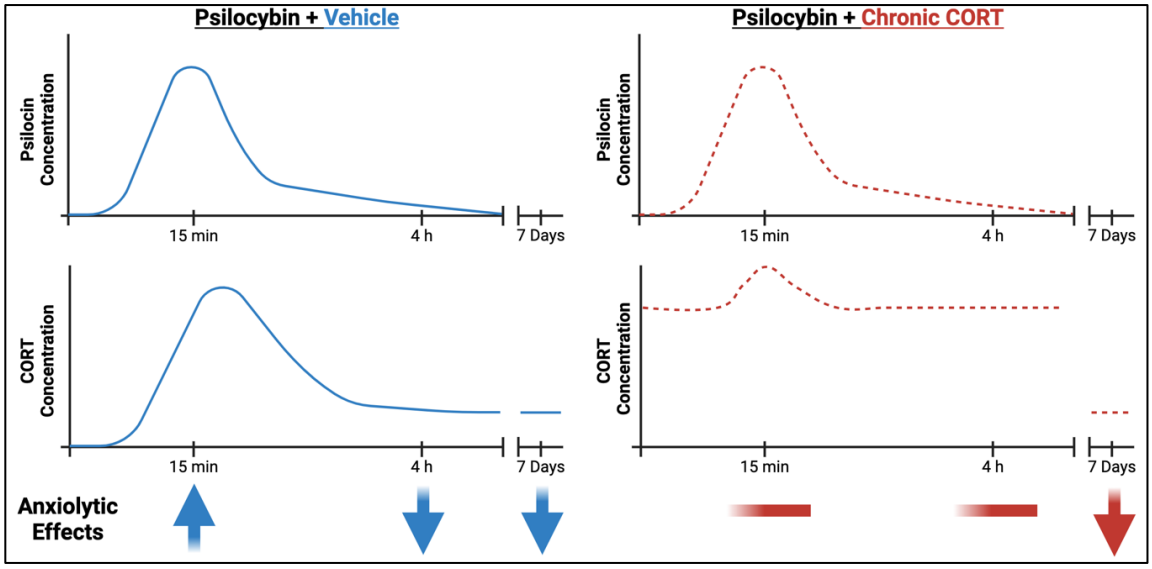
Notably, the enduring effects seen here occurred in the absence of psychedelic messaging and a species with more limited evidence for self-awareness and narrative

metacognition. (39,40) This indicates that psilocybin's long-term behavioral effects are at least partially independent of the expectation biases surrounding psychedelics in humans. Further studies undertaking explicit measurement of neuroplasticity would be valuable in addressing this translationally relevant issue. (20,41–44)

Additionally, the observations made here demonstrate that the progression from acute anxiogenesis, through post-acute anxiolysis, to the long-term effects of psilocybin on anxiety-like behavior in mice was supported by and sensitive to the temporal profile of plasma glucocorticoids. These findings indicate that repeated measurement of cortisol concentrations in the blood before, during, and after psychedelic exposure could be employed as a human biomarker. This would help improve our understanding of how environmental conditions, including set and setting, preparation, and integration, can modify the progression and resolution of both biological stress-associated hormonal responses and anxious behavior following psilocybin-assisted therapy.

Such biomarker measurement could also help resolve existing tensions and controversies in best practices for how to approach acute drug-induced stress and anxiety occurring during psychedelic administration. On one hand, both self-reported anxiety and plasma cortisol concentrations have been reported to be transiently elevated by high-dose psilocybin treatment in healthy human subjects (45), and a particular form of acute anxiety, "dread of ego dissolution", has also been negatively associated with therapeutic efficacy in individuals with treatment-resistant depression. (46) On the other hand, observational studies have identified a positive correlation between acutely challenging psychedelic experiences and long-term well-being, and at least one model of psychedelic efficacy proposes that overcoming difficult experiences through acceptance is of therapeutic benefit overall. (47,48) The "pivotal mental states" model further proposes that stress-induced upregulations of the 5-HT_{2A} system, such as those generated with the application of psychedelics, are essential for the generation of time-bound opportunities for transformative change. (49)

Whether, as seen here in rodents, acute psychedelic-induced glucocorticoid release similarly supports post-acute enhancement of stress resilience in clinical trials for anxiety-associated psychiatric indications remains an important question for translational consideration. Likewise, examination of whether the return to baseline from transiently elevated cortisol concentrations directly interacts with long-term anxiety outcomes in humans, as was seen here with rodents, is another critical area for clinical and translational study. Such studies would be particularly informative if undertaken in concert with an assessment of basal HPA axis function across different treatment populations to address the identification of psychiatric diagnostic subpopulations that may be most likely to respond to psychedelic-assisted therapy approaches. (50–53).



Scheme 14: Psilocybin's anxiolytic-like effects persisted at 7 days following administration. The long-term anxiolytic effects of psilocybin were lost when psilocybin was administered to animals with ongoing chronic elevations in plasma corticosterone concentrations. Overall, these experiments indicate that acute, resolvable psilocybin-induced glucocorticoid release drives the post-acute anxiolytic-like effects of psilocybin in mice and that its long-term anxiolytic-like effects can be abolished in the presence of chronically elevated plasma glucocorticoid elevations.

2.5 Impact Statement:

This study demonstrates that psilocybin-induced corticosterone release is a critical factor driving reductions in anxiety-like behavior in mice in the hours following drug clearance and that ongoing, unresolved glucocorticoid elevations can invert the long-term outcome of psilocybin administration on anxiety-like behavior up to seven days later. These findings point to HPA axis activation and changes in glucocorticoid concentration profiles over time as important translational factors for mechanistic consideration when investigating classical serotonergic psychedelic-assisted therapy for the treatment of psychiatric disorders.

Author Contributions

Nathan T Jones: Investigation, methodology, formal analysis, visualization, validation, writing, review and editing. Conceptualization: Developed the initial ideas and framework for the research study. Investigation: Conducted the experimental procedures and gathered the data necessary for the study. Methodology: Developed and refined the techniques and approaches used in the research. Formal Analysis: Performed the statistical and analytical procedures to interpret the data. Execution of Experiment Ideas: Implemented and tested the experimental concepts and hypotheses. Visualization: Created graphical representations and visual aids to illustrate the findings. Validation: Ensured the accuracy and reliability of the results through repeated experiments and cross-verification. Writing: Drafted the manuscript, integrating research findings and theoretical discussions. Review and Editing: Critically reviewed the manuscript, providing substantial feedback and making necessary revisions for clarity and coherence.

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Jones, N. T., *Zahid, Z., Grady, S. M., Sultan, Z. W., Zheng, Z., Razidlo, J., Banks, M. I., & Wenthur, C. J. (2023). Transient Elevation of Plasma Glucocorticoids Supports Psilocybin-Induced Anxiolysis in Mice. ACS pharmacology & translational science, 6(8), 1221–1231.* [*https://doi.org/10.1021/acsptsci.3c00123*](https://doi.org/10.1021/acsptsci.3c00123)

2.6 Supplemental Figures:

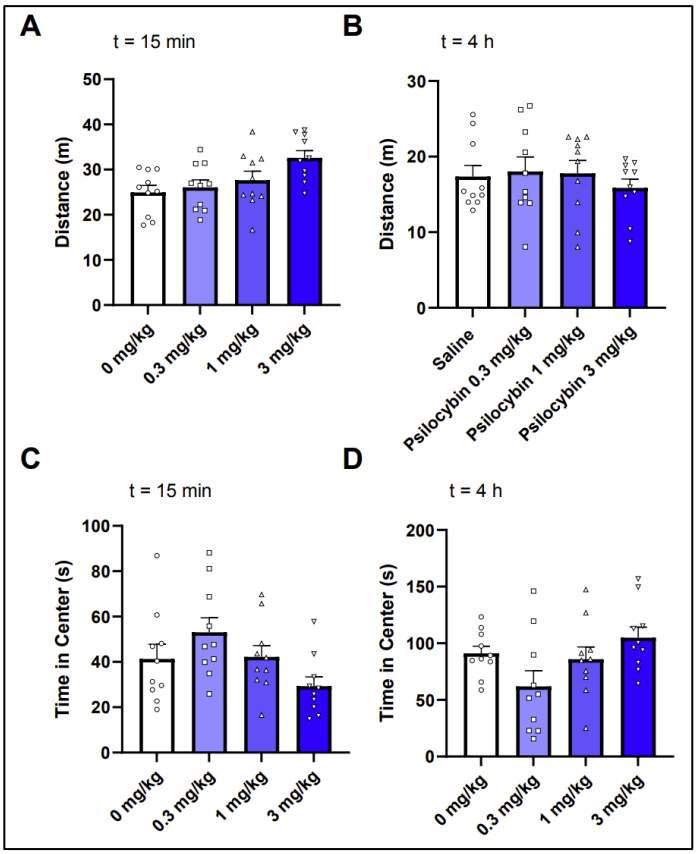


Figure S1. Dose-response relationship of distance traveled relative to saline average during a 10- minute exposure to an open field arena at **A)** 15 min and **B)** 4 h after IP administration of psilocybin. Dose-response relationship of time in center during a 10-minute exposure to an open field arena at **C)** 15 min and **D)** 4 h after IP administration of psilocybin.

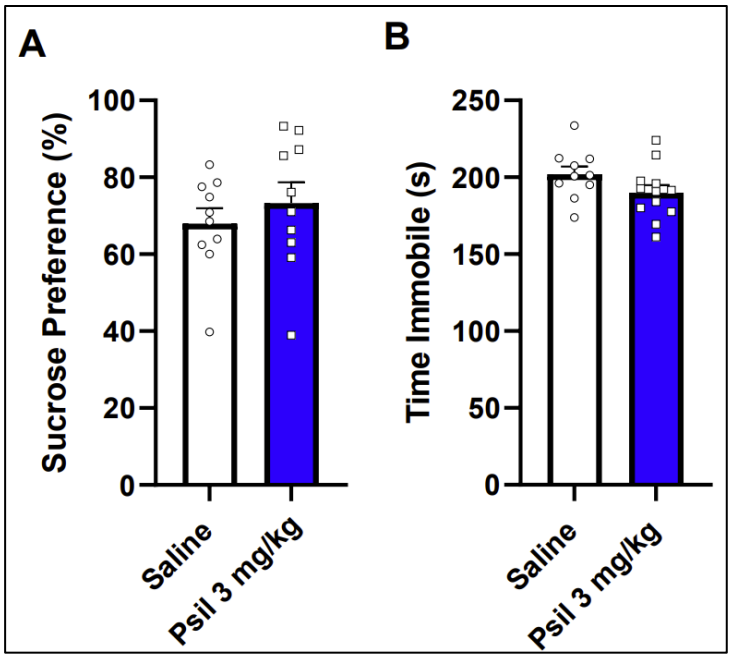


Figure S2. Effects at 4 h following administration of saline or 3mg/kg psilocybin IP on A) Sucrose Preference or B) Forced Swim Test.

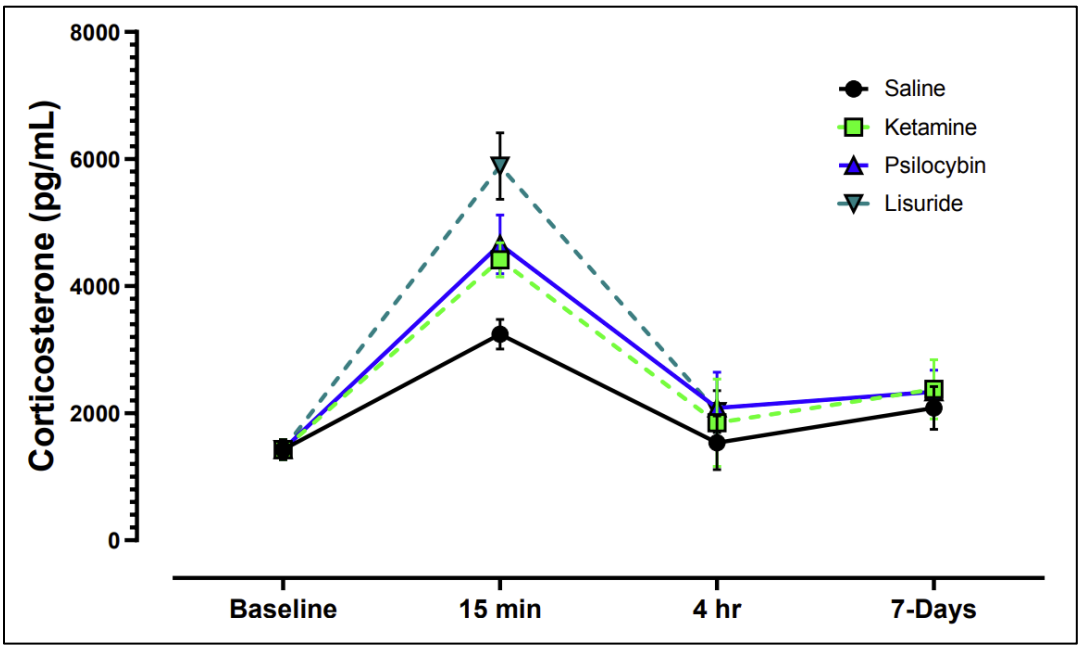


Figure S3. Time course of plasma corticosterone following administration of saline, 30 mg/kg ketamine IP, psilocybin 3 mg/kg IP, or lisuride 0.25 mg/kg IP. Two-Way ANOVA with Sidak's Multiple Comparisons: Baseline vs. 4 h, $p > 0$ .05 for all conditions.

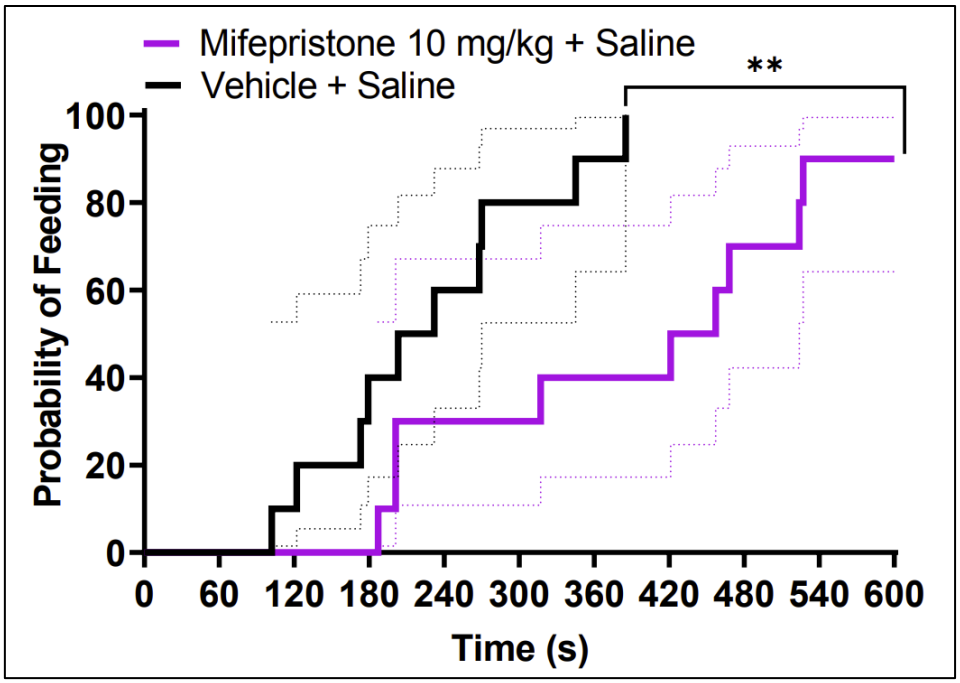


Figure S4. Survival curves for latency to feed in a novelty suppressed feeding assay assessing Mifepristone and Saline at 4 h prior to IP administration of saline, in the presence of 15 min pretreatment with either vehicle or mifepristone 10 mg/kg, values ± 95% CI. Mantel Cox ($p = 0.0059$).

REFERENCES

1. Madsen, M. K.; Fisher, P. M.; Burmester, D.; Dyssegaard, A.; Stenbæk, D. S.; Kristiansen, S.; Johansen, S. S.; Lehel, S.; Linnet, K.; Svarer, C.; et al. Psychedelic Effects of Psilocybin Correlate with Serotonin 2 A Receptor Occupancy and Plasma Psilocin Levels. Neuropsychopharmacology 2019, 44, 1328–1334.
2. Geiger, H. A.; Wurst, M. G.; Daniels, R. N. DARK Classics in Chemical Neuroscience: Psilocybin. ACS Chem. Neurosci. 2018, 9, 2438–2447.
3. Horita, A.; Weber, L. J. Dephosphorylation of Psilocybin to Psilocin by Alkaline Phosphatase. Proc. Soc. Exp. Biol. Med. 1961, 106, 32–34.
4. Johnson, M. W.; Richards, W. A.; Griffiths, R. R. Human Hallucinogen Research: Guidelines for Safety. J. Psychopharmacology. 2008, 22, 603–620.
5. Hartogsohn, I. The Meaning-Enhancing Properties of Psychedelics and Their Mediator Role in Psychedelic Therapy, Spirituality, and Creativity. Front. Neurosci. 2018, 12, 129.
6. Hartogsohn, I. Set and Setting, Psychedelics and the Placebo Response: An Extra Pharmacological Perspective on Psychopharmacology. J. Psychopharmacology. 2016, 30, 1259–1267.
7. Neitzke-Spruill, L.; Glasser, C. A. G. G. A Gratuitous Grace: The Influence of Religious Set and Intent on the Psychedelic Experience. J. Psychoact. Drugs 2018, 50, 314–321.
8. Hartogsohn, I. Constructing Drug Effects: A History of Set and Setting. Drug Sci. Policy Law 2017, 3, 205032451668332.
9. Johnson, M. W.; Richards, W.; Griffiths, R. Human Hallucinogen Research : Guidelines for Safety. J. Psychopharmacol. 2008, 22, 603–620.
10. Schenberg, E. E. Psychedelic-Assisted Psychotherapy: A Paradigm Shift in Psychiatric Research and Development. Front. Pharmacol. 2018, 9, 733.
11. Cameron, L. P.; Benson, C. J.; Dunlap, L. E.; Olson, D. E. Effects of N,N-Dimethyltryptamine on Rat Behaviors Relevant to Anxiety and Depression. ACS Chem. Neurosci. 2018, 9, 1582–1590.

12. Hibicke, M.; Landry, A. N.; Kramer, H. M.; Talman, Z. K.; Nichols, C. D. Psychedelics, but Not Ketamine, Produce Persistent Antidepressant-like Effects in a Rodent Experimental System for the Study of Depression. ACS Chem. Neurosci. 2020, 11, 864–871.

13. Matsushima, Y.; Shirota, O.; Kikura-Hanajiri, R.; Goda, Y.; Eguchi, F. Effects of Psilocybe Argentipes on Marble-Burying Behavior in Mice. Biosci., Biotechnol., Biochem. 2009, 73, 1866–1868.

14. Cameron, L. P.; Benson, C. J.; DeFelice, B. C.; Fiehn, O.; Olson, D. E. Chronic, Intermittent Microdoses of the Psychedelic N,N-Dimethyltryptamine (DMT) Produce Positive Effects on Mood and Anxiety in Rodents. ACS Chem. Neurosci. 2019, 10, 3261–3270.

15. Mahmoudi, E.; Faizi, M.; Hajiaghaee, R.; Razmi, A. Alteration of Depressive-like Behaviors by Psilocybe Cubensis Alkaloid Extract in Mice : The Role of Glutamate Pathway. Res. J. Pharmacogn. 2018, 5, 17–24.

16. Jefsen, O.; Højgaard, K.; Christiansen, S. L.; Elfving, B.; Nutt, D. J.; Wegener, G.; Müller, H. K. Psilocybin Lacks Antidepressant-like Effect in the Flinders Sensitive Line Rat. Acta Neuropsychiatr. 2019, 31, 213–219.

17. Catlow, B. J.; Song, S.; Paredes, D. A.; Kirstein, C. L.; Sanchez-Ramos, J. Effects of Psilocybin on Hippocampal Neurogenesis and Extinction of Trace Fear Conditioning. Exp. Brain Res. 2013, 228, 481–491.

18. Meinhardt, M. W.; Güngör, C.; Skorodumov, I.; Mertens, L. J.; Spanagel, R. Psilocybin and LSD Have No Long-Lasting Effects in an Animal Model of Alcohol Relapse. Neuropsychopharmacology 2020, 45, 1316–1322.

19. Hesselgrave, N.; Troppoli, T. A.; Wulff, A. B.; Cole, A. B.; Thompson, S. M. 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, 1–7.

20. Preller, K. H.; Vollenweider, F. X. Phenomenology, Structure, and Dynamic of Psychedelic States. Current Topics in Behavioral Neurosciences; Springer Verlag, 2018; Vol. 36, pp 221–256. DOI: 10.1007/7854_2016_459.

21. Schindler, E. A. D.; Wallace, R. M.; Sloshower, J. A.; D'Souza, D. C. Neuroendocrine Associations Underlying the Persistent Therapeutic Effects of Classic Serotonergic Psychedelics. Front. Pharmacol. 2018, 9, 1–16.

22. Rao, R. P.; Anilkumar, S.; McEwen, B. S.; Chattarji, S. Glucocorticoids Protect against the Delayed Behavioral and Cellular Effects of Acute Stress on the Amygdala. Biol. Psychiatr. 2012, 72, 466–475.

23. Ashokan, A.; Sivasubramanian, M.; Mitra, R. Seeding Stress Resilience through Inoculation. Neural Plast. 2016, 2016, 4928081.

24. Wegman-Points, L.; Pope, B.; Zobel-Mask, A.; Winter, L.; Wauson, E.; Duric, V.; Yuan, L. L. Corticosterone as a Potential Confounding Factor in Delineating Mechanisms Underlying Ketamine's Rapid Antidepressant Actions. Front. Pharmacol. 2020, 11, 1–9.

25. Georgiou, P.; Zanos, P.; Mou, T. C. M.; An, X.; Gerhard, D.M.; Dryanovski, D. I.; Potter, L. E.; Highland, J. N.; Jenne, C. E.;Stewart, B. W.; Pultorak, K. J.; Yuan, P.; Powels, C. F.; Lovett, J.;Pereira, E. F. R.; Clark, S. M.; Tonelli, L. H.; Moaddel, R.; Zarate, C.A.; Duman, R. S.; Thompson, S. M.; Gould, T. D. Experimenters' SexModulates Mouse Behaviors and Neural Responses to Ketamine viaCorticotropin Releasing Factor. Nat. Neurosci. 2022, 25, 1191–1200.

26. Fernández-Guasti, A.; Fiedler, J. L.; Herrera, L.; Handa, R. J. Sex, Stress, and Mood Disorders: At the Intersection of Adrenal and Gonadal Hormones. Horm. Metab. Res. 2012, 44, 607–618.

27. Chakraborty, P.; Chattarji, S. Interventions after Acute Stress Prevent Its Delayed Effects on the Amygdala. Neurobiol. Stress 2019, 10, 100168.

28. Chakraborty, P.; Datta, S.; McEwen, B. S.; Chattarji, S. Corticosterone after Acute Stress Prevents the Delayed Effects on the Amygdala. Neuropsychopharmacology 2020, 45, 2139–2146.

29. Halberstadt, A. L.; Geyer, M. A. Characterization of the Head-Twitch Response Induced by Hallucinogens in Mice: Detection of the Behavior Based on the Dynamics of Head Movement. Psychopharmacology 2013, 227, 727–739.

30. Calhoon, G. G.; Tye, K. M. Resolving the Neural Circuits of Anxiety. Nat. Neurosci. 2015, 18, 1394–1404.

31. Osterlund, C.; Spencer, R. L. Corticosterone Pretreatment Suppresses Stress-Induced Hypothalamic-Pituitary-Adrenal Axis Activity via Multiple Actions That Vary with Time, Site of Action, and de Novo Protein Synthesis. J. Endocrinol. 2011, 208, 311–322.

32. Qu, Y.; Chang, L.; Ma, L.; Wan, X.; Hashimoto, K. Rapid antidepressant-like effect of non-hallucinogenic psychedelic analog lisuride, but not hallucinogenic psychedelic DOI, in lipopolysaccharide-treated mice. Pharmacol., Biochem. Behav. 2023, 222, 173500.

33. Vesuna, S.; Kauvar, I. V.; Richman, E.; Gore, F.; Oskotsky, T.;Sava-Segal, C.; Luo, L.; Malenka, R. C.; Henderson, J. M.; Nuyujukian, P.; Parvizi, J.; Deisseroth, K. Deep Posteromedial Cortical Rhythm in Dissociation. Nature 2020, 586, 87–94.

34. Yasmin, F.; Patel, S. Corting" Stress: Post-Stress Corticosterone Administration Prevents Delayed-Onset Biobehavioral Consequences. Neuropsychopharmacology 2020, 45, 2135–2136.

35. Branchi, I.; Santarelli, S.; Capoccia, S.; Poggini, S.; D'Andrea, I.; Cirulli, F.; Alleva, E. Antidepressant Treatment Outcome Depends on the Quality of the Living Environment: A Pre-Clinical Investigation in Mice. PLoS One 2013, 8, No. e62226.

36. Tylš, F.; Páleníček, T.; Kadeřábek, L.; Lipski, M.; Kubešová, A.; Horáček, J. Sex Differences and Serotonergic Mechanisms in the Behavioural Effects of Psilocin. Behav. Pharmacol. 2016, 27, 309–320.

37. Effinger, D. P.; Quadir, S. G.; Ramage, M. C.; Cone, M. G.; Herman, M. A. Sex-Specific Effects of Psychedelic Drug Exposure on Central Amygdala Reactivity and Behavioral Responding. Transl. Psychiatry 2023, 13, 119.

38. Jaster, A. M.; Younkin, J.; Cuddy, T.; de la Fuente Revenga, M.; Poklis, J. L.; Dozmorov, M. G.; González-Maeso, J. Differences across Sexes on Head-Twitch Behavior and 5-HT2A Receptor Signaling in C57BL/6J Mice. Neurosci. Lett. 2022, 788, 136836.

39. Mogil, J. S. Mice Are People Too: Increasing Evidence for Cognitive, Emotional and Social Capabilities in Laboratory Rodents. Can. Psychol. 2019, 60, 14–20.

40. Ly, C.; Greb, A. C.; Vargas, M. V.; Duim, W. C.; Grodzki, A. C. G.; Lein, P. J.; Olson, D. E. Transient Stimulation with Psychoplastogens Is Sufficient to Initiate Neuronal Growth. ACS Pharmacol. Transl. Sci. 2020, 4, 452–460.

41. Studerus, E.; Gamma, A.; Kometer, M.; Vollenweider, F. X. Prediction of Psilocybin Response in Healthy Volunteers. PLoS One 2012, 7, No. e30800.

42. Vollmayr, B.; Henn, F. A. Stress Models of Depression. Clin. Neurosci. Res. 2003, 3, 245–251.

43. Yang, L.; Zhao, Y.; Wang, Y.; Liu, L.; Zhang, X.; Li, B.; Cui, R. The Effects of Psychological Stress on Depression. Curr. Neuropharmacol. 2015, 13, 494–504.

44. Bekhbat, M.; Merrill, L.; Kelly, S. D.; Lee, V. K.; Neigh, G. N. Brief Anesthesia by Isoflurane Alters Plasma Corticosterone Levels Distinctly in Male and Female Rats: Implications for Tissue Collection Methods. Behav. Brain Res. 2016, 305, 122–125.

45. Hasler, F.; Grimberg, U.; Benz, M. A.; Huber, T.; Vollenweider, F. X. Acute Psychological and Physiological Affects of Psilocybin in Healthy Humans: A Double-Blind, Placebo-Controlled Dose-Effect Study. Psychopharmacology 2004, 172, 145–156.

46. Roseman, L.; Nutt, D. J.; Carhart-Harris, R. L. Quality of Acute Psychedelic Experience Predicts Therapeutic Efficacy of Psilocybin for Treatment-Resistant Depression. Front. Pharmacol. 2018, 8, 974.

47. Carbonaro, T. M.; Bradstreet, M. P.; Barrett, F. S.; MacLean, K. A.; Jesse, R.; Johnson, M. W.; Griffiths, R. R. Survey Study of Challenging Experiences after Ingesting Psilocybin Mushrooms: Acute and Enduring Positive and Negative Consequences. J. Psychopharmacol. 2016, 30, 1268–1278.

48. Wolff, M.; Evens, R.; Mertens, L. J.; Koslowski, M.; Betzler, F.; Gründer, G.; Jungaberle, H. Learning to Let Go: A Cognitive- Behavioral Model of How Psychedelic Therapy Promotes Acceptance. Front. Psychiatr. 2020, 11, 5.

49. Brouwer, A.; Carhart-harris, R. L. Pivotal Mental States. J. Psychopharmacol. 2021, 35, 319–352.

50. Galvão, A. C. d. M.; de Almeida, R. N.; Silva, E. A. D. S.; Freire, F. A. M.; Palhano-Fontes, F.; Onias, H.; Arcoverde, E.; Maia-de-Oliveira, J. P.; de Araújo, D. B.; Lobão-Soares, B.; Galvão-Coelho, N.L. Cortisol Modulation by Ayahuasca in Patients With Treatment Resistant Depression and Healthy Controls. Front. Psychiatr. 2018, 9,185.

51. Sousa, G. M. de; de Oliveira Tavares, V. D.; de Menezes Galvão, A. C.; de Almeida, R. N.; Palhano-Fontes, F.; Lobão-Soares, B.; de Morais Freire, F. A.; Nunes, E. A.; Maia-de-

Oliveira, J. P.; Perkins, D.; Sarris, J.; de Araujo, D. B.; Galvão-Coelho, N. L. Moderators of Ayahuasca's Biological Antidepressant Action. Front. Psychiatr. 2022, 13, 1033816.

52. Lewis, C. R.; Tafur, J.; Spencer, S.; Green, J. M.; Harrison, C.; Kelmendi, B.; Rabin, D. M.; Yehuda, R.; Yazar-Klosinski, B.; Cahn, B. R. Pilot Study Suggests DNA Methylation of the Glucocorticoid Receptor Gene (NR3C1) Is Associated with MDMA-Assisted Therapy Treatment Response for Severe PTSD. Front. Psychiatr. 2023, 14, 959590.
53. Mayer, S. E.; Peckins, M.; Kuhlman, K. R.; Rajaram, N.; Lopez-Duran, N. L.; Young, E. A.; Abelson, J. L. The Roles of Comorbidityand Trauma Exposure and Its Timing in Shaping HPA Axis Patternsin Depression. Psychoneuroendocrinology 2020, 120, 104776.

CHAPTER 3: *IN VIVO,* VALIDATION OF PSILACETIN AS A PRODRUG YIELDING MODESTLY LOWER PERIPHERAL PSILOCIN EXPOSURE THAN PSILOCYBIN

The intricate mechanisms underlying psilocybin's effects on anxiety-like behavior have significant implications for the broader field of psychedelic-assisted therapy. Specifically, the first part of my thesis work, highlights the pivotal role of psilocybin-induced corticosterone release in mitigating anxiety-like behaviors in mice shortly after drug clearance. Conversely, it also underscores that sustained, unresolved glucocorticoid elevations can negatively impact the long-term efficacy of psilocybin in reducing anxiety-like behaviors up to a week post-administration. These observations suggest that the HPA axis activation and the dynamic changes in glucocorticoid concentration over time are crucial translational factors that need to be considered when investigating the fundamental pharmacology and underlying mechanism of action for classical serotonergic psychedelic-assisted therapy in treating psychiatric disorders (200). To continue exploring novel mechanisms of action for psilocybin and other serotonergic psychedelic compounds, it is crucial to provide more scientists with access to these substances, fostering a multidisciplinary approach.

Nevertheless, one of the major barriers in working with psilocybin is its classification as a Schedule 1 drug, as discussed in Chapter 1. This designation, under the Controlled Substances Act in the United States, categorizes psilocybin alongside other substances deemed to have a high potential for abuse, no currently accepted medical use, and a lack of accepted safety for use under medical supervision (201–205). As a result, obtaining the necessary approvals and licenses for research involving psilocybin is a complex and time-consuming process. Researchers must navigate through a series of stringent regulatory hurdles, including obtaining a Schedule 1 license from the Drug Enforcement Administration (DEA). This process requires extensive documentation to demonstrate the legitimacy and safety of the proposed research. Institutions must also have

secure storage facilities that meet DEA specifications, that not all institutions have, to handle Schedule 1 substances. Additionally, the approval process involves multiple layers of review, including institutional review boards (IRBs) and possibly the Food and Drug Administration (FDA) (201–203). These regulatory barriers can significantly delay the initiation of research projects and increase the costs associated with conducting studies. Furthermore, the stigma attached to Schedule 1 substances can deter funding from public and private sources, limiting the resources available for psychedelic research. This challenging regulatory landscape not only hinders scientific progress but also restricts the opportunities for new investigators to enter the field and contribute to the growing body of knowledge on psilocybin and its potential therapeutic benefits.

Enabling more researchers to investigate the effects of psilocybin's active metabolite, Psilocin, through alternative prodrugs holds significant promise for advancing our understanding of psilocin's pharmacology and therapeutic effects (206,207). By providing a more accessible and efficient means of studying psilocin, we can incorporate a multidisciplinary approach and gain a more comprehensive understanding of its pharmacology and therapeutic potential. One promising approach to facilitating this research is the use of synthetic prodrug for Psilocin (208–211). Synthetic prodrugs are compounds designed to metabolize into the active substance, in this case, psilocin, after administration (212,213). This method not only offers an alternative route to study Psilocin but also provides several significant advantages in research settings.

One key advantage of using synthetic prodrugs for psilocin is the enhanced control over their production and dosing. Synthetic prodrugs are manufactured under strict quality control protocols, ensuring a high degree of consistency in their chemical composition and purity. This consistency allows researchers to conduct studies with highly standardized compounds, thereby reducing variability and increasing the reliability of their findings (208–213). Such control is particularly important in pharmacological research, where precise dosing and compound purity are essential for obtaining accurate and reproducible results. When working with natural

compounds like psilocybin, variability in the source material can lead to fluctuations in the concentration of the active ingredient, potentially skewing results and making it difficult to replicate studies. In addition, the synthesis for psilocybin is rather complex, difficult, and more expensive (214). Synthetic prodrugs eliminate these issues by providing a simpler synthesis, consistent purity, and controlled means of delivering psilocin (212–214). Furthermore, synthetic prodrugs can be designed to have specific pharmacokinetic properties, such as improved bioavailability or extended-release profiles, which can enhance their therapeutic potential and make them more suitable for clinical use, a concept that will be explored further in Chapter 4. This ability to fine-tune the properties of the prodrug also allows researchers to explore different dosing regimens and treatment protocols, contributing to a more comprehensive understanding of psilocin's effects and mechanisms of action.

Furthermore, by making these advanced tools and techniques more accessible, we democratize research, enabling a greater diversity of scientific inquiry and innovation. Therefore, expanding the number of researchers able to investigate psilocin accelerates the exploration of its pharmacodynamics and pharmacokinetics. A better understanding of how Psilocin interacts with serotonin receptors, the time course of its effects, and its metabolic pathways can provide deeper insights into its therapeutic mechanisms. This knowledge is crucial for optimizing dosing regimens, minimizing potential side effects, and enhancing the overall efficacy of psilocin-based treatments. This could lead to more personalized treatment approaches tailored to the specific needs of patients with conditions such as major depressive disorder, anxiety, PTSD, and other mental health issues.

3.1 Introduction:

Comparison of Psilocybin and Psilacetin

Psilocybin is a Schedule 1 compound being investigated for the treatment of major depressive disorder and other psychiatric conditions (129,215–223). Psilocybin is rapidly dephosphorylated in the body and is thought to predominantly act as a prodrug to deliver the active metabolite psilocin, another Schedule 1 substance (206,207). In human studies, the pharmacokinetics and exposure of Psilocin have been well validated *in vivo* after administering psilocybin in accordance with both fixed-dose and weight-based protocols (137,224–226). While psilocybin has the longest history of human consumption of any known psilocin prodrug, as this natural product found in Psilocybe mushrooms has been available since antiquity, it is not the only psilocin prodrug known (Figure 1) (51,201,202,227,228)

Another notable example is the synthetic tryptamine psilacetin, also known as O-acetylpsilocin and 4-acetoxy-N,N-dimethyltryptamine (4-AcO-DMT). Psilacetin was first disclosed in a patent by Hofmann and Troxler in 1961, with an improved synthesis disclosed by Nichols and Frescas in 1999 (210,229). Recently, both fumarate and hemifumarate crystalline forms of psilacetin have been isolated (230,231). Although psilacetin induces psychedelic effects in humans, it is not presently included in any international drug schedules, including the UN 1971 Convention on Psychotropic Substances, which established a system for classifying controlled psychoactive drugs into four schedules based on their potential for abuse and therapeutic value (51,203,232,233)

In recent years, there has been a growing interest in studying psilacetin as an alternative to psilocybin, for several reasons. First, due to its entirely synthetic nature, psilacetin offers researchers greater control over its production, distribution, and dosing compared to the variability inherent in extractions of psilocybin from naturally occurring psilocybin-containing mushrooms

(208,210,234–237). Even when comparing the synthetic production of psilacetin versus psilocybin, the production of psilacetin is notably simpler, with superior atom economy and fewer steps. This is primarily due to the difficulty encountered in the installation of the phosphate group of psilocybin (208,235,238). Additionally, substituting psilacetin for Psilocin may reduce regulatory access barriers for researchers who do not hold a Schedule I DEA research license.

Psilacetin Usage, Effects, and Pharmacology

Investigation of psilacetin is also of significant public health relevance due to its recreational use. Synthetic tryptamines have been available on the designer drug market since at least the late 1990s (239), with psilacetin being a notable contributor to this marketplace. In Spain, the drug testing organization Energy Control has identified psilacetin as the most prevalent non-regulated tryptamine in samples submitted between 2006 and 2015 (240). Furthermore, the prevalence of psilacetin use has even been suggested to surpass that of psychedelic mushroom use in recent years (241). Individuals taking psilacetin describe its effects as comparable to those of psilocybin, yet without the adverse side effects associated with the use of whole Psilocybe mushrooms, such as nausea (241,242). Nevertheless, there remains a paucity of academic studies concentrating on psilacetin (243) in comparison to the numerous research efforts on the pharmacological impacts and metabolism of psilocybin and Psilocin (244–247).

While metabolism to Psilocin has been suggested as the likely source of psilacetin's psychoactivity, there remains ambiguity as to whether the parent drug itself exerts additional behaviorally meaningful pharmacologic effects of its own across species (51,243). Some 4-acetoxy-N,N-dialkyltryptamines have been reported in humans to exhibit effects reminiscent of lysergic acid diethylamide (LSD) and have been suggested to have enhanced passive access to the brain due to the acetoxy group enhancing lipid solubility, thereby aiding in crossing the blood–brain barrier (238). The 5-HT_{2A} receptor is viewed as a significant target for psychoactive

tryptamines such as psilacetin, as it is with other classical psychedelics such as psilocybin. *In vitro,* psilacetin's receptor potency is approximately 10- to 20-fold lower than that of Psilocin, though this difference has little apparent influence on HTR potency *in vivo* (235,238). Psilacetin induced equivalent head twitch responses to psilocin on an equimolar basis: psilocin at 0.81 µmol/kg and psilacetin at 1.12 µmol/kg (238). Psilacetin has also demonstrated overlapping 95% confidence intervals with psilocybin regarding potency for inducing head twitch, hypolocomotion, and hypothermia in rodents, despite psilacetin having substantially higher affinity and potency at 5-HT_{2A} than psilocybin (235). Together, these results are consistent with psilacetin's behavioral effects in animal models being predominantly driven by psilocin liberation *in vivo*.

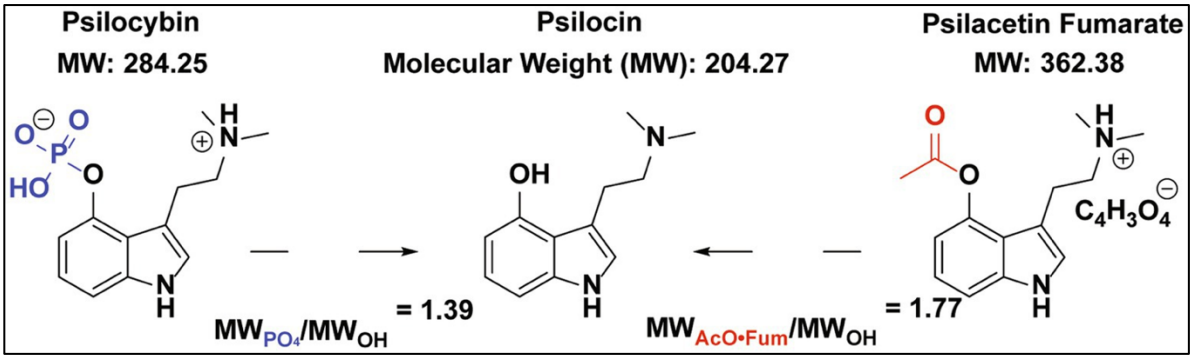


Figure 1. Psilocybin and psilacetin (in fumarate salt form) as prodrugs for Psilocin. The relevant protecting groups for Psilocin's hydroxyl group are shown in blue for psilocybin and red for psilacetin fumarate. Based on relative molecular weights, doses of 1.39 mg/kg of Psilocin or 1.77 mg/kg of psilacetin fumarate by weight are needed to yield a 1 mg/kg equivalent dose of Psilocin.

The Present Study

There is a standing call in the field for direct evidence of psilacetin's *in vivo* conversion to Psilocin to confirm its long-assumed prodrug status; evidence for this transformation has come only from *in vitro* studies to date (235,238). In this study, we directly respond to this request. A liquid chromatography–tandem mass spectrometry method suitable for the quantitative analysis

of psilocin concentrations is assessed for accuracy and applied to determine the plasma concentrations of liberated Psilocin following the administration of psilacetin fumarate and psilocybin to mice.

3.2 Materials and Methods:

Animals and Husbandry

All experimental procedures were approved by the University of Wisconsin – Madison Animal Care and Use Committee (IACUC) and completed in full accordance with Research Animal Resources and Compliance (RARC) guidelines. All 115 mice used in this study were acclimated to the University of Wisconsin vivarium conditions for at least 7 days prior to handling or experimentation. Food pellets (LabDiet) and water (Inno-Vive) were available ad libitum, unless otherwise noted. All C57Bl6/J mice used (male and female; 6–8 weeks old; The Jackson Laboratory, ME, USA) were housed in groups of three or four while under a 12 h artificial, reversed light/dark cycle. The room temperature remained constant between 22 and 24°C.

Drugs

All controlled substances were handled by authorized users on Schedule I and Schedules II – V DEA research licenses and WI Special Use Authorizations held by Dr. Cody Wenthur. For *in vivo* injections, psilocybin powder (Usona Institute; Madison, WI; > 99% purity) 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. Psilacetin fumarate (1:1) (Usona Institute; Madison, WI; >99% purity) was diluted in 0.9% sterile saline at pH 6–7. These materials were passed through a 0.2 µm filter and administered intraperitoneally (IP). All IP injections were given at a volume of 10 mL/kg. Chemical purity was assessed for all compounds using high-resolution LC–MS, and the fumarate

anion 1:1 molar ratio for psilacetin fumarate was verified using 1H NMR. No mouse was given more than one injection of psilocybin or psilacetin fumarate or re-used following a washout period.

Blood Sample Collection

Animals were briefly anesthetized with isoflurane (to prevent loss of righting reflex) prior to decapitation and trunk blood collection. Collections occurred at time points between 15 and 240 min after drug administration using EDTA-coated microcentrifuge tubes. Following collection, the samples were then centrifuged at 10,000 rpm (11,292 g) for 10 min at 4°C. The plasma fraction was separated and stored in the dark at –80°C until LC–MS/MS analysis.

Liquid Chromatography/Tandem Mass Spectrometry (LC–MS/MS)

LC–MS/MS analysis and quantitation occurred in the Analytical Instrumentation Center (AIC) at the University of Wisconsin-Madison School of Pharmacy. For the preparation of analytical standards, Psilocin (Usona Institute, Madison, WI; > 99% purity) was prepared in Optima LC–MS-grade methanol (Fisher Scientific, Hampton, NH). d10-Psilocin solution was purchased from Cerilliant (Round Rock, TX) for use as an internal standard (ITSD). Blank mouse plasma for preparing calibration curves and quality control samples (QCs) was purchased from Innovative Research (Novi, MI). All solvents for liquid chromatography were Optima LC/MS grade (Fisher Scientific, Hampton, NH). Additives for LC/MS analysis were purchased from Sigma Aldrich (St. Louis, MO).

Calibrators and QCs were prepared from stock methanol solutions of active pharmaceutical ingredients diluted to between 0.5 and 400 ng/mL in blank plasma. Samples, calibrators, and QCs were prepared for LC–MS/MS by protein precipitation and filtration using Waters Sirocco plates (Milford, MA) according to the manufacturer's instructions. A precipitation mix containing the ISTD was prepared and aliquoted to a Sirocco plate mounted on a 96-well receiver. Samples, QCs, and calibrators were added to the plate, incubated for 2 min, and pushed

through the plate using a positive pressure manifold (Waters, Milford, MA). Processed samples were then dried under nitrogen and resuspended in 100 µL of 98% A/2% B solvent prior to LC/MS/MS analysis.

Quantitative LC–MS/MS was performed using a Waters Acquity I-Class binary pump (Waters Corp., Milford MA) coupled to a Sciex QTRAP 5500 mass spectrometer (Sciex Corp., Framingham MA). Samples were separated on a Kinetex Core-Shell phenyl-hexyl 2.1 × 100 mm column (Phenomenex, Torrence, CA) using a 3-min gradient with a flow rate of 0.4 mL/min and a column temperature of 28°C. The initial conditions consisted of 95% solvent A (2.5 mM ammonium formate in water with 0.1% formic acid) and 5% solvent B (acetonitrile with 0.1% formic acid). The elution gradient began at 5% B, increased to 8.6% B over 1.8 min, and then quickly increased to 95% B in 0.15 min. This was held for 0.6 min, then decreased to 5% B in another 0.15 min, followed by a 0.25-min re-equilibration period. The column temperature was maintained at 28°C with a flow rate of 0.4 mL/min. All samples were injected in triplicate in randomized order, and the average of these injections was used for analysis. Blanks were injected between each calibrant, QC, or sample injection. For quantitation, the area under the curve of analyte peaks relative to ISTD peaks was modeled for each identified transition using a quadratic curve fit with 1/x2 weighting. Data was processed using MultiQuant software (Sciex, Framingham, MA). Any calibrator points differing by more than 15% from theoretical values were eliminated from the model.

Pharmacokinetic Calculations

The elimination rate (Ke) for Psilocin was determined by fitting a linear regression to the plots of the natural log of psilocin concentration over time for each tested condition, then averaging the values of the reported slopes. The elimination rate was reported as the inverse of this average slope. Half-life was calculated from this elimination rate using T1/2 = Ln (2)/Ke. Relative exposure of Psilocin was calculated for each pairwise comparison between psilacetin (A) and psilocybin (B)

doses using F = (AUCA/Dose A)/(AUCB/Dose B). Relative exposure was then reported from the average of the four pairwise dose comparisons.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism, version 10 (San Diego, CA). All tests were run as two-tailed analyses, with a value of p of <0.05 as the threshold for significance. Post-hoc tests for ANOVA are reported with p-values corrected for multiple comparisons when follow-up tests were run to assess differences between specific conditions. Tests for outliers in biological data were run using ROUT at a 1% threshold, and one outlier was removed based on this threshold overall.

3.3 Results:

Analytical Methodology Assessment

The LC–MS/MS method used for the identification of Psilocin in mouse plasma samples yielded three distinct transitions for Psilocin at 205/160, 205/115, and 205/89 m/z. Curve fits for each independent transition, as well as a total fit weighted across the three transitions, yielded suitable standard curves for analytical application with R2 values >0.995 and coefficients of variation $<10\%$ across all QC samples, injections, and transitions (Figure 2). The total weighted curve fit was selected for use in the assessment of biological samples, as it had the highest R2 value overall at 0.9968.

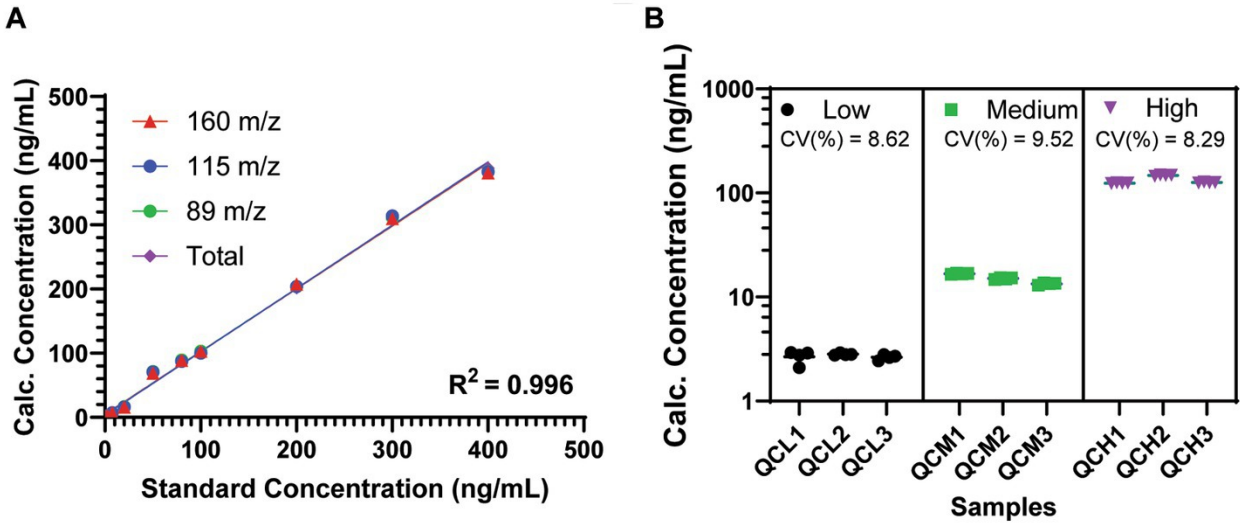


Figure 2. Performance of the tandem LC–MS/MS method for psilocin detection in mouse plasma. (A) Standard curves for observed psilocin transitions. R2 is shown as the average of all four curves (range, 0.9951–0.9968). **(B)** Quality control samples show variation for multiple injections and across multiple samples at low, medium, and high concentrations. Coefficients of variation (CV%) are shown across all transitions, injections, and samples at each concentration. Data are shown as mean ± SEM.

Verification of *in vivo* Production of Psilocin from Psilacetin and Psilocybin

This LC–MS/MS approach was used to assess plasma samples collected from animals treated with psilocybin and psilacetin fumarate at 15 min after administration (Figure 3). To look at relative concentrations of Psilocin liberated into the plasma, the doses were administered on an equimolar basis and were selected to be equivalent to the administration of either 1 mg/kg of Psilocin or 3 mg/kg of Psilocin. Notably, micromolar concentrations of Psilocin were found in the plasma of animals treated with psilacetin at both doses, indicating robust metabolic transformation *in vivo*. The psilocin concentration resulting from the 1 mg/kg equivalent dose of psilacetin was 225 ng/mL, which was 90% of that from psilocybin (250 ng/mL). For the 3 mg/kg equivalent dose, the psilacetin concentration was 860 ng/mL, or 75% of that from psilocybin (1,145 ng/mL). These

psilocin concentrations were not found to be significantly different between prodrugs at these sample sizes (Student's t-test: 1 mg/kg, $p = 0.37$; 3 mg/kg, $p = 0.08$).

The trend showing a lower fraction of psilocin exposure following psilacetin exposure than following psilocybin exposure was supported in a second experiment using doses of psilacetin fumarate and psilocybin that were interleaved across an escalating dose scale. As expected, these doses resulted in significantly different concentrations of plasma psilocin overall (ANOVA, $F = 45.74$, $p < 0.0001$). However, there was not a smoothly increasing concentration of Psilocin across escalating doses, as would be expected if psilocin exposure from both prodrugs was equal. Instead, the 5.63 µmol/kg dose of psilacetin fumarate had a lower psilocin concentration than that from the 4.90 µmol/kg dose of psilocybin.

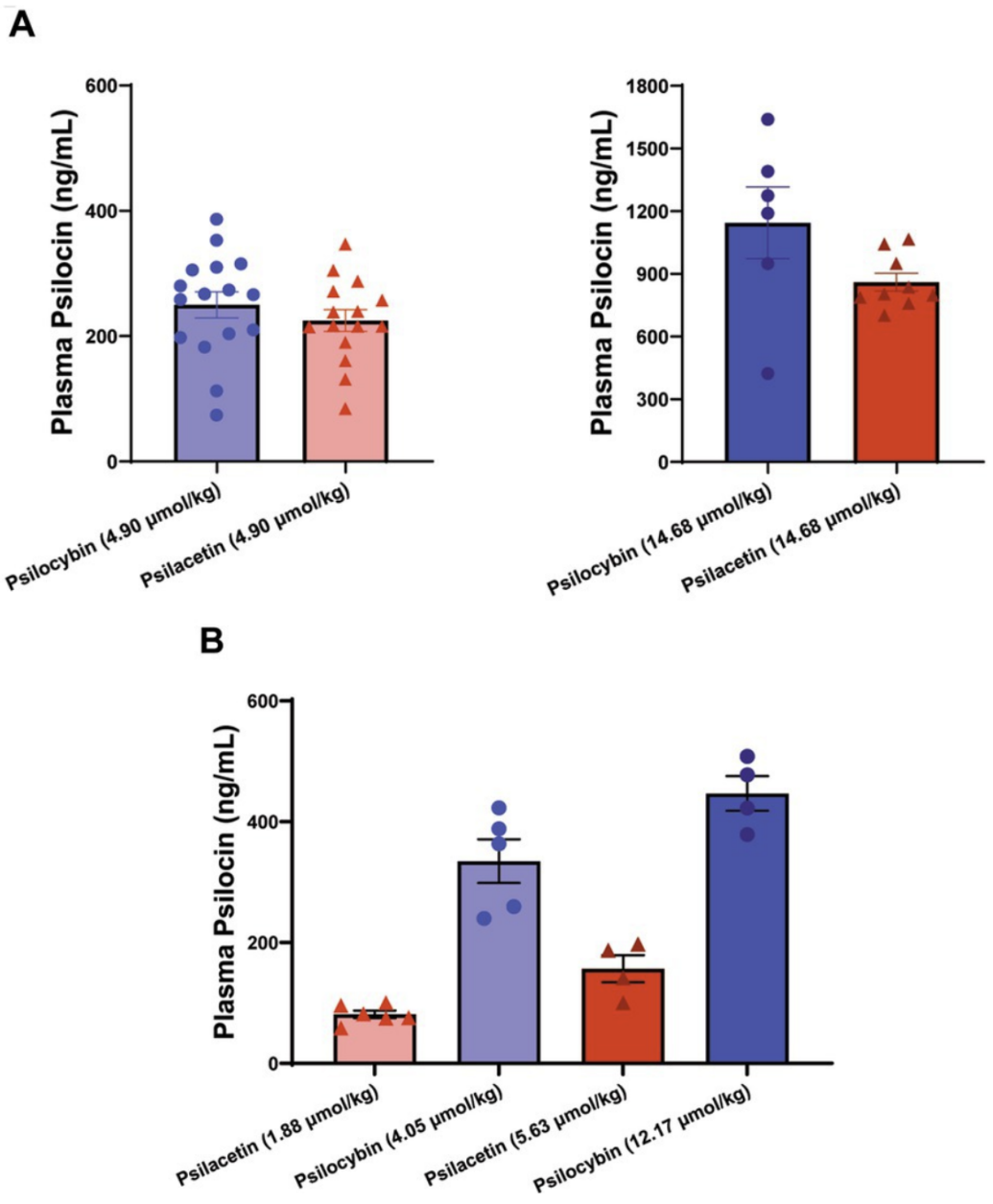


Figure 3. Metabolically derived Psilocin is rapidly detectable following psilocybin and psilacetin fumarate injections in mice. Psilocin concentrations were detected in plasma 15 min after intraperitoneal injection. **(A)** Matched equimolar doses equivalent to either 1 mg/kg psilocin (4.90 µmol/kg) or 3 mg/kg psilocin (14.68 µmol/kg). One outlier was removed for psilacetin at 14.68 µmol/kg (ROUT, 1%). **(B)** Interleaved doses of psilacetin fumarate (1.88 µmol/kg; 5.63 µmol/kg) and psilocybin (4.05 µmol/kg; 12.17 µmol/kg) across an escalating range. Data are shown as mean ± SEM.

Time Course and Magnitude of Psilocin Exposure from Psilacetin and Psilocybin

Given the relatively smaller sample size used and higher variability observed for the single time point dose escalation experiment, a follow-up assessment of psilocin concentrations over time was also undertaken to further illuminate the full profiles of psilocybin and psilacetin fumarate as prodrugs. This analysis was undertaken using a separate cohort of animals from the single time point experiments (Figure 4). The relative plasma profiles of Psilocin liberated from either psilacetin fumarate or psilocybin demonstrated dose-dependency between 15 and 240 min that was consistent with the observations at 15 min. The elimination of Psilocin liberated from either source was observed to adhere to first-order kinetics across doses. The terminal elimination rate was found to be 0.026 min−1 (range, 0.020–0.038 min1). This corresponds to a psilocin half-life of approximately one-half hour, aligning with previously reported results for the clearance of this molecule (200). Plasma psilocin concentrations fell to near or below the lower limit of quantitation for the LC/MS/MS assay (0.1 ng/mL) by 4 h after administration, or approximately 6 half-lives.

Notably, in an observation consistent with the single time point experiments, psilacetin fumarate generated lower levels of plasma exposure to Psilocin than psilocybin on an equimolar basis. When relative psilocin bioavailability was calculated using dose-corrected areas under the curves (AUCs) between 15 and 60 min, there were significant differences in AUCs across all groups (ANOVA, $F = 107.6$, $p < 0.0001$), and both drugs also independently demonstrated their own dose-dependent exposure trends (Sidak's, psilacetin, $p = 0.0002$; psilocybin, $p < 0.0001$). However, psilacetin was found to yield only between 67 and 89% as much psilocin exposure in comparison to psilocybin. On average, across all possible pairwise dose comparisons, psilacetin resulted in approximately 30% less psilocin exposure than psilocybin on an equimolar basis. This is effectively demonstrated by comparing the profiles from the 5.63 µmol/kg dose of psilacetin fumarate and the 4.05 µmol/kg dose of psilocybin—these two conditions generated nearly

identical psilocin exposure (Sidak's, p = 0.95) despite the psilocybin dose being approximately 30% smaller than the psilacetin dose in terms of molar equivalents.

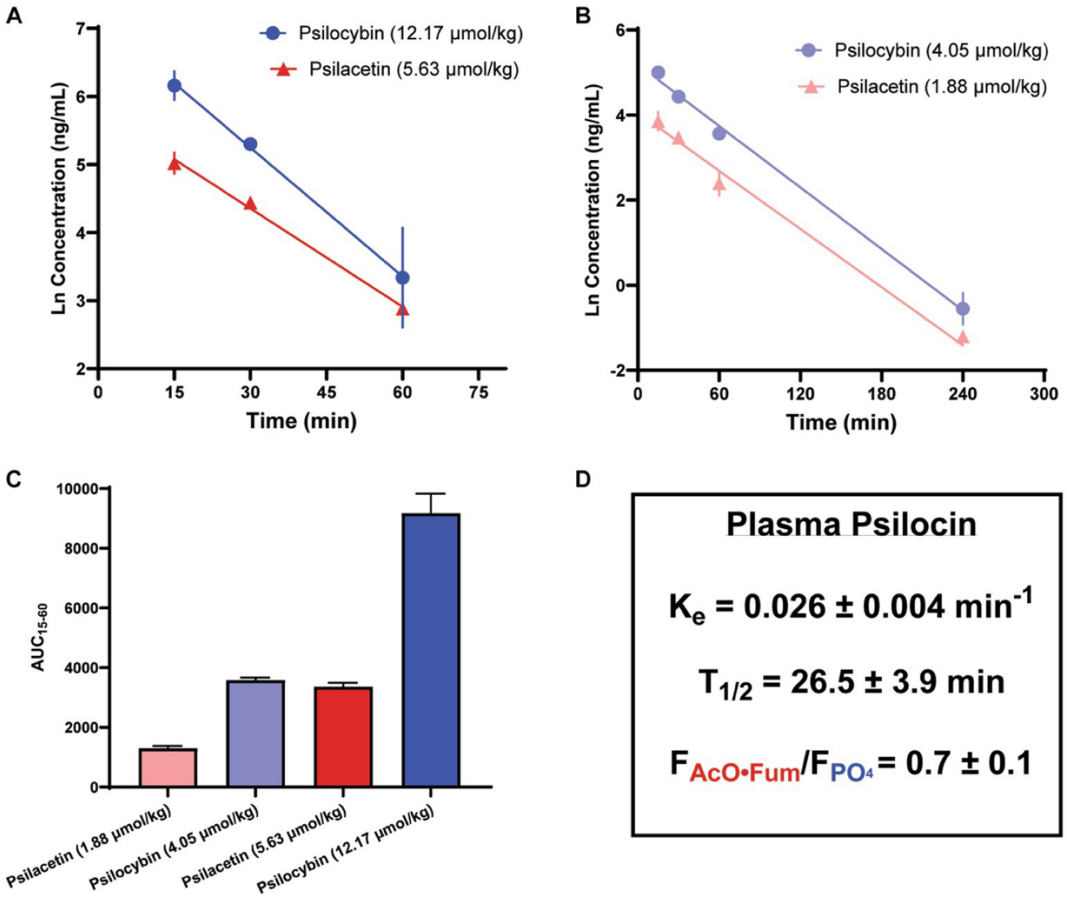


Figure 4. Metabolism of psilacetin fumarate in mice yields a lower psilocin plasma exposure as compared to the metabolism of psilocybin. Psilocin plasma concentration time courses from **(A)** doses of psilocybin (12.17 µmol/kg; n = 12, 4/timepoint) and psilacetin fumarate (5.68 µmol/kg; n = 9, 3/timepoint) between 15 and 60 min after intraperitoneal injection and **(B)** doses of psilocybin (4.05 µmol/kg; n = 9, 3/timepoint) and psilacetin fumarate (1.88 µmol/kg n = 9, 3/timepoint) between 15 and 240 min after intraperitoneal injection. Lines show best-fit linear regressions for a first-order elimination model. **(C)** Comparison of areas under the curves from 15 to 60 min following intraperitoneal injection for all doses in panels (A,B). **(D)** Elimination rate and half-life of liberated Psilocin, as well as relative bioavailability of Psilocin from psilacetin fumarate in comparison to psilocybin. Data are shown as mean ± SEM.

3.4 Discussion:

Summary of the Findings

The results of these experiments demonstrate that psilacetin fumarate acts as a prodrug for Psilocin in both male and female C57Bl6/J mice. There was no sexual dimorphism in the production of Psilocin from either psilacetin or psilocybin. While the *in vivo* action of psilacetin as a psilocin prodrug has long been hypothesized, this is the first formal, publicly available pharmacokinetic report validating this status *in vivo* of which we are aware.

Theoretical and Practical Implications

This validation has important implications for pre-clinical psychedelic research programs. Most notably, regular substitution of psilacetin fumarate as an unscheduled (240) alternative to psilocybin in pre-clinical studies may enable broader access and more rapid progress on mechanistic questions surrounding Psilocin's effects. For clinical studies using human participants, there is less likelihood of accelerating progress through substitution alone, given the additional regulatory considerations involved. However, there may be other compensatory benefits to the pursuit of psilacetin or other novel psilocin prodrug strategies as alternatives to psilocybin for human research studies, such as the possibility of reduced ethical, legal, and sustainability concerns by avoiding the commercialization of a natural product with a long-documented history of sacramental use by indigenous peoples (201,204,248).

Limitations and Future Directions

There are several limitations to this study worth noting when considering psilacetin fumarate substitution for psilocybin in C57Bl6/J mice. First, while these data support the liberation of Psilocin as an active metabolite that contributes to the actions of psilacetin, they do not address the relevant intrinsic pharmacologic activity of psilacetin, which may occur alongside Psilocin.

Second, these studies were limited to plasma, and central nervous system exposure may be different. Together, these factors mean that pharmacodynamically equivalent doses are not likely to be the same as the peripherally pharmacokinetically equivalent doses noted here. Furthermore, this effort used the fumarate, rather than hemifumarate, crystalline form—if using the hemifumarate form, equimolar dosage adjustments will be required to account for the half-weight of fumarate complexed with each psilacetin molecule. Finally, while this study aimed to explore dose ranges (0–5 mg/kg) for psilocybin and psilacetin that are commonly employed in pre-clinical studies, the resulting plasma concentrations observed (100–1,200 ng/mL) are significantly larger than those seen in human pharmacokinetic studies of psilocybin (5–50 ng/mL). This notable difference means that attempts to translate relative exposure outcomes across species are likely premature.

3.5 Conclusion:

In summary, the results of the experiments reported here provide direct evidence to validate the long-standing assumption that psilacetin acts as a psilocin prodrug *in vivo*. They also provide initial evidence suggesting that psilacetin fumarate leads to a quantifiably lower psilocin peripheral exposure as compared to psilocybin on an equimolar basis. Together, these findings provide an empirical basis for pre-clinical investigators to thoughtfully substitute the unscheduled compound psilacetin for the Schedule 1 compound psilocybin as a pharmacokinetically reasonable means to address a significant regulatory barrier to entry for new scientists interested in contributing to the growing field of psychedelic studies.

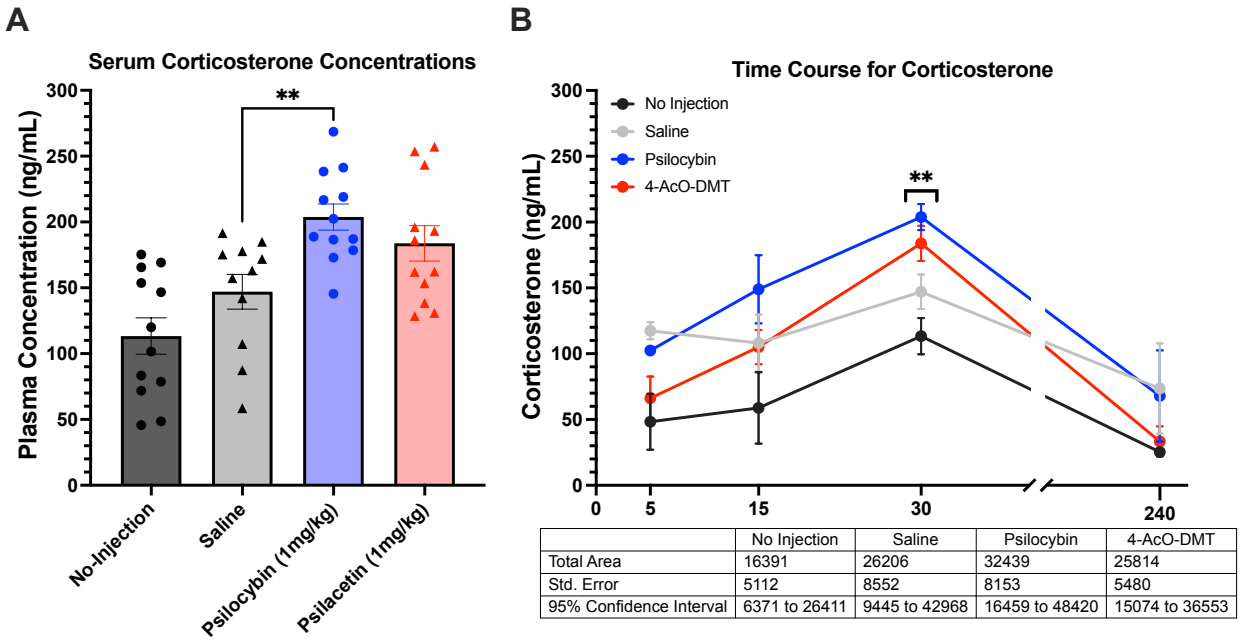
3.6 Temporal Dynamics of Corticosterone Release Following Psilocybin and Psilacetin Administration in Mice

Given the structural and functional similarities between psilocybin and psilacetin, it is important to understand how psilacetin influences corticosterone levels. To investigate the effects

of psilocybin and psilacetin on corticosterone levels in C57BL/6 mice, plasma corticosterone concentrations were measured at 5-, 15-, 30-, and 240- minutes in both male and female mice. The number of mice in each treatment group was as follows: 5 minutes (n=2), 15 minutes (n=4), 30 minutes (n=12), and 240 minutes (n=4). Repeated retro-orbital bleeds were collected from each mouse at all timepoints except for the terminal 240-minute timepoint, where blood was collected via decapitation. The mice were anesthetized with isoflurane prior to blood collection, and the samples were centrifuged to separate the serum, which was then stored at -80°C. Plasma corticosterone concentrations were assessed using a colorimetric ELISA analysis, and concentrations were corrected for assay dilution (200,209).

The results indicated that psilocybin treatment led to a significant increase in corticosterone levels compared to controls ($p = 0.0086$), while psilacetin did not produce a statistically significant change ($p = 0.1198$) at the 30-minute timepoint post-injection. At the 5-minute mark, both treatments showed a slight increase in corticosterone levels. By 15 minutes, a substantial rise in corticosterone concentration was observed in the psilocybin-treated group, peaking at 30 minutes, and returning to baseline by 240 minutes post-injection. These findings suggest that psilocybin induces a robust stress response in C57BL/6 mice, as evidenced by the significant elevation in corticosterone levels, indicating activation of the hypothalamic-pituitary-adrenal (HPA) axis. These findings reaffirm our previous observations that psilocybin induces a transient increase in corticosterone levels at a dose of 3 mg/kg, as described in Chapter 2 (200). Psilacetin, did not elicit a statistically significant increase in corticosterone concentrations. Further research is needed to understand the differential effects of these compounds on stress-related hormonal pathways and their underlying mechanisms. Furthermore, the finding that a 1 mg/kg dose of psilacetin did not significantly increase corticosterone levels suggests that this dosage may be below the threshold required to elicit a notable stress response in C57BL/6 mice. It is

possible that a higher dose, such as 3 mg/kg, might be necessary to observe significant changes in corticosterone concentration.



	No Injection	Saline	Psilocybin	4-AcO-DMT
Total Area	16391	26206	32439	25814
Std. Error	5112	8552	8153	5480
95% Confidence Interval	6371 to 26411	9445 to 42968	16459 to 48420	15074 to 36553

Figure 5: Effects of Psilocybin and Psilacetin on Plasma Corticosterone Concentrations in C57BL/6 Mice: (A) Plasma corticosterone concentrations measured at 30-minutes post-administration. Bars represent the mean corticosterone levels for each treatment group, with error bars indicating the standard error of the mean (SEM). Individual data points are shown as symbols: circles for control no-injection (n=12), squares for saline (n=11), triangles for psilocybin (n=12), and diamonds for psilacetin (n=12). Psilocybin (0.0086) treatment resulted in a significant increase in corticosterone levels compared to control, while psilacetin (0.1198) did not show a significant effect. (B) Time-course of plasma corticosterone concentrations measured at 5-, 15-, 30-, and 240-minutes post-administration. Each line represents the mean corticosterone levels for a different treatment group: black (control), gray (vehicle), blue (psilocybin), and red (psilacetin). Error bars indicate SEM. Psilocybin-treated mice exhibited a significant increase in corticosterone levels, peaking at 30-minutes, while psilacetin-treated mice did not show significant changes at any time points compared to controls. For the time course study, the number of mice in each treatment group was as follows: 5 minutes (n=2), 15 minutes (n=4), 30 minutes (n=12), and 240 minutes (n=4).

Author contributions

Nathan T. Jones: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Conceptualization:** Developed the initial research ideas and framework, laying the foundation for the study**. Formal Analysis:** Performed the statistical and analytical procedures to interpret the data accurately and ensure robust results. **Investigation:** Conducted the experimental procedures, gathered the data, and ensured the integrity of the research process. **Methodology:** Developed and refined the techniques, protocols, and approaches used throughout the research to ensure methodological rigor. **Visualization:** Created graphical representations, charts, and visual aids to effectively communicate the research findings and insights. **Writing – Original Draft:** Composed the initial version of the manuscript, integrating research findings, theoretical discussions, and key conclusions. **Writing – Review & Editing:** Critically reviewed and revised the manuscript, providing substantial feedback, refining the content for clarity, coherence, and academic rigor.

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***Jones, N. T.,** Wagner, L., Hahn, M. C. P., Scarlett, C. O., & Wenthur, C. J. (2024).* In vivo *validation of psilacetin as a prodrug yielding modestly lower peripheral psilocin exposure than psilocybin. Frontiers in psychiatry, 14, 1303365. https://doi.org/10.3389/fpsyt.2023.1303365*

REFERENCES

1. Jones NT, Zahid Z, Grady SM, Sultan ZW, Zheng Z, Razidlo J, Banks MI, Wenthur CJ. Transient elevation of plasma glucocorticoids supports psilocybin-induced anxiolysis in mice. *ACS Pharmacol Transl Sci* (2023) 6:1221–1231.
2. Sharma P, Nguyen QA, Matthews SJ, Carpenter E, Mathews DB, Patten CA, Hammond CJ. Psilocybin history, action and reaction: A narrative clinical review. *Journal of Psychopharmacology* (2023) 37:849–865.
3. Hartogsohn I. Constructing drug effects: a history of set and setting. Drug science, Policy and Law 3. *Link: https://bit ly/2Op2JGI* (2017)
4. Khan I. Convention on psychotropic substances, 1971: the role and responsibilities of the World Health Organization. *Prog Neuropsychopharmacol* (1979) 3:11–14.
5. Penedos S, Ramos C, Miguel M, Alves M, Paulino L, Azevedo A, Magalhães M, Moreno L, Ribeiro N, Fonseca I, et al. P.0729 Highlights of psychedelic history and current research on psilocybin application for treatment of depression – a comprehensive literature review. *European Neuropsychopharmacology* (2021) 53:S532–S533. doi: https://doi.org/10.1016/j.euroneuro.2021.10.797
6. Sharma P, Nguyen QA, Matthews SJ, Carpenter E, Mathews DB, Patten CA, Hammond CJ. Psilocybin history, action and reaction: A narrative clinical review. *Journal of Psychopharmacology* (2023) 37:849–865. https://api.semanticscholar.org/CorpusID:261395845
7. Hasler F, Bourquin D, Brenneisen R, Bär T, Vollenweider FX. Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. *Pharm Acta Helv* (1997) 72:175–184.
8. Horita A, Weber LJ. The enzymic dephosphorylation and oxidation of psilocybin and pscilocin by mammalian tissue homogenates. *Biochem Pharmacol* (1961) 7:47–54.
9. Pham DNK, Chadeayne AR, Golen JA, Manke DR. Psilacetin derivatives: fumarate salts of the methyl–ethyl, methyl–allyl and diallyl variants of the psilocin prodrug. *Acta Crystallogr E Crystallogr Commun* (2021) 77:101–106.
10. Jones, Nathan T. Wagner, Laura Scarlett, Cameron 0. Hanh, Molly C. Wenthur CJ. In Vivo Validation of Psilacetin as a Prodrug Yielding Modestly Lower Peripheral Psilocin Exposure than Psilocybin. *Front Psychiatry* (2023) 14: doi: doi: 10.3389/fpsyt.2023.1303365

11. Nichols DE, Frescas S. Improvements to the synthesis of psilocybin and a facile method for preparing the O-acetyl prodrug of psilocin. *Synthesis (Stuttg)* (1999) 1999:935–938.
12. Chadeayne AR, Golen JA, Manke DR. Bis(4-acetoxy-N,N-dimethyltryptammonium) fumarate: a new crystalline form of psilacetin, an alternative to psilocybin as a psilocin prodrug. *Acta Crystallogr E Crystallogr Commun* (2019) 75:900–902. https://api.semanticscholar.org/CorpusID:191146517
13. Elliott SP, Holdbrook T, Brandt SD. Prodrugs of New Psychoactive Substances (NPS): A New Challenge. *J Forensic Sci* (2020) 65: https://api.semanticscholar.org/CorpusID:210335207
14. Rautio J, Laine K, Gynther M, Savolainen J. Prodrug Approaches for CNS Delivery. *AAPS J* (2008) 10:92–102. doi: 10.1208/s12248-008-9009-8
15. Sherwood AM, Meisenheimer P, Tarpley G, Kargbo RB. An improved, practical, and scalable five-step synthesis of psilocybin. *Synthesis (Stuttg)* (2020) 52:688–694.
16. Geiger HA, Wurst MG, Daniels RN. DARK classics in chemical neuroscience: psilocybin. *ACS Chem Neurosci* (2018) 9:2438–2447.
17. Bogenschutz MP, Forcehimes AA, Pommy JA, Wilcox CE, Barbosa PCR, Strassman RJ. Psilocybin-assisted treatment for alcohol dependence: a proof-of-concept study. *Journal of psychopharmacology* (2015) 29:289–299.
18. Davis AK, Barrett FS, May DG, Cosimano MP, Sepeda ND, Johnson MW, Finan PH, Griffiths RR. Effects of psilocybin-assisted therapy on major depressive disorder: a randomized clinical trial. *JAMA Psychiatry* (2021) 78:481–489.
19. Mertens LJ, Wall MB, Roseman L, Demetriou L, Nutt DJ, Carhart-Harris RL. Therapeutic mechanisms of psilocybin: changes in amygdala and prefrontal functional connectivity during emotional processing after psilocybin for treatment-resistant depression. *Journal of Psychopharmacology* (2020) 34:167–180.
20. Roseman L, Demetriou L, Wall MB, Nutt DJ, Carhart-Harris RL. Increased amygdala responses to emotional faces after psilocybin for treatment-resistant depression. *Neuropharmacology* (2018) 142:263–269.
21. Doss MK, Považan M, Rosenberg MD, Sepeda ND, Davis AK, Finan PH, Smith GS, Pekar JJ, Barker PB, Griffiths RR. Psilocybin therapy increases cognitive and neural flexibility in patients with major depressive disorder. Transl Psychiatry 11 (1): 574. (2021)

22. Goldberg SB, Pace BT, Nicholas CR, Raison CL, Hutson PR. The experimental effects of psilocybin on symptoms of anxiety and depression: A meta-analysis. *Psychiatry Res* (2020) 284:112749.
23. Carhart-Harris RL, Bolstridge M, Rucker J, Day CMJ, Erritzoe D, Kaelen M, Bloomfield M, Rickard JA, Forbes B, Feilding A. Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. *Lancet Psychiatry* (2016) 3:619–627.
24. Carhart-Harris RL, Bolstridge M, Day CMJ, Rucker J, Watts R, Erritzoe DE, Kaelen M, Giribaldi B, Bloomfield M, Pilling S. Psilocybin with psychological support for treatment-resistant depression: six-month follow-up. *Psychopharmacology (Berl)* (2018) 235:399–408.
25. Tylš F, Páleníček T, Horáček J. Psilocybin–summary of knowledge and new perspectives. *European Neuropsychopharmacology* (2014) 24:342–356.
26. Brown RT, Nicholas CR, Cozzi N V, Gassman MC, Cooper KM, Muller D, Thomas CD, Hetzel SJ, Henriquez KM, Ribaudo AS. Pharmacokinetics of escalating doses of oral psilocybin in healthy adults. *Clin Pharmacokinet* (2017) 56:1543–1554.
27. Kolaczynska KE, Liechti ME, Duthaler U. Development and validation of an LC-MS/MS method for the bioanalysis of psilocybin's main metabolites, psilocin and 4-hydroxyindole-3-acetic acid, in human plasma. *Journal of Chromatography B* (2021) 1164:122486.
28. Holze F, Ley L, Müller F, Becker AM, Straumann I, Vizeli P, Kuehne SS, Roder MA, Duthaler U, Kolaczynska KE. Direct comparison of the acute effects of lysergic acid diethylamide and psilocybin in a double-blind placebo-controlled study in healthy subjects. *Neuropsychopharmacology* (2022) 47:1180–1187.
29. Holze F, Becker AM, Kolaczynska KE, Duthaler U, Liechti ME. Pharmacokinetics and Pharmacodynamics of Oral Psilocybin Administration in Healthy Participants. *Clin Pharmacol Ther* (2023) 113:822–831. doi: 10.1002/cpt.2821
30. Geiger H, Wurst M, Daniels RN. DARK Classics in Chemical Neuroscience: Psilocybin. *ACS Chem Neurosci* (2018) 9 10:2438–2447. https://api.semanticscholar.org/CorpusID:49591766
31. Nichols DE, Nichols CD, Mckenna DJ, Mangini M, Grigsby J, Lsd C 8., Panik K, Presti DE, Lancelotta R, Davis AK, et al. Handbook of MEDICAL HALLUCINOGENS. (2021) https://api.semanticscholar.org/CorpusID:261210049

32. Pearson C, Siegel J, Gold JA. Psilocybin-assisted psychotherapy for depression: Emerging research on a psychedelic compound with a rich history. *J Neurol Sci* (2022) 434:120096.
33. Hofmann Albert TF. C07F9/5728 Five-membered rings condensed with carbocyclic rings or carbocyclic ring systems. (1963) https://patents.google.com/patent/US3075992A/en?oq=US3075992#patentCitations
34. Chadeayne AR, Golen JA, Manke DR. Bis (4-acetoxy-N, N-dimethyltryptammonium) fumarate: a new crystalline form of psilacetin, an alternative to psilocybin as a psilocin prodrug. *Acta Crystallogr E Crystallogr Commun* (2019) 75:900–902.
35. Chadeayne AR, Golen JA, Manke DR. The crystal structure of 4-AcO-DMT fumarate. *Psychedelic Sci Rev* (2019)
36. Sessa B. Can psychedelics have a role in psychiatry once again? *The British Journal of Psychiatry* (2005) 186:457–458.
37. United Nations AddressNew York, NY 10017 US. Convention on Psychotropic Substances, 1971. (1977). https://www.ojp.gov/ncjrs/virtual-library/abstracts/convention-psychotropic-substances-1971
38. Glatfelter GC, Manke DR, Chadayne AR, Baumann MH. Receptor binding profiles and behavioral effects of psilocybin analogs. *The FASEB Journal* (2022) 36:
39. Sessa B, Glatfelter GC, Pottie E, Partilla JS, Sherwood AM, Kaylo K, Pham DNK, Naeem M, Sammeta VR, DeBoer S, et al. Structure–Activity Relationships for Psilocybin, Baeocystin, Aeruginascin, and Related Analogues to Produce Pharmacological Effects in Mice. *ACS Pharmacol Transl Sci* (2022) 5:1181–1196. doi: 10.1021/acsptsci.2c00177
40. Sanacora G, Yan Z, Popoli M, Carhart-Harris RL, Nutt DJ, Cao D, Yu J, Wang H, Luo Z, Liu X, et al. Structure-based discovery of nonhallucinogenic psychedelic analogs. *Journal of Psychopharmacology* (2022) 38:370–384. doi: 10.1016/j.jad.2022.01.104
41. Kargbo RB, Sherwood AM, Walker A, Cozzi N V, Dagger RE, Sable JH, O'Hern K, Kaylo KW, Patterson T, Tarpley G, et al. Direct Phosphorylation of Psilocin Enables Optimized cGMP Kilogram-Scale Manufacture of Psilocybin. *ACS Omega* (2020) 5:16959–16966. https://api.semanticscholar.org/CorpusID:220599227
42. Klein AK, Chatha M, Laskowski LJ, Anderson EI, Brandt SD, Chapman SJ, McCorvy JD, Halberstadt AL. Investigation of the structure–activity relationships of psilocybin analogues. *ACS Pharmacol Transl Sci* (2020) 4:533–542.

43. Meyer MR, Caspar A, Brandt SD, Maurer HH. A qualitative/quantitative approach for the detection of 37 tryptamine-derived designer drugs, 5 β-carbolines, ibogaine, and yohimbine in human urine and plasma using standard urine screening and multi-analyte approaches. *Anal Bioanal Chem* (2014) 406:225–237.
44. Palma-Conesa ÁJ, Ventura M, Galindo L, Fonseca F, Grifell M, Quintana P, Fornís I, Gil C, Farré M, Torrens M. Something new about something old: a 10-year follow-up on classical and new psychoactive tryptamines and results of analysis. *J Psychoactive Drugs* (2017) 49:297–305.
45. Palamar JJ, Acosta P. A qualitative descriptive analysis of effects of psychedelic phenethylamines and tryptamines. *Human Psychopharmacology: Clinical and Experimental* (2020) 35:e2719.
46. Palamar JJ, Barratt MJ, Ferris JA, Winstock AR. Correlates of new psychoactive substance use among a self-selected sample of nightclub attendees in the United States. *Am J Addict* (2016) 25:400–407.
47. Kargbo RB, Sherwood A, Walker A, Cozzi N V, Dagger RE, Sable J, O'Hern K, Kaylo K, Patterson T, Tarpley G. Direct phosphorylation of psilocin enables optimized cGMP kilogram-scale manufacture of psilocybin. *ACS Omega* (2020) 5:16959–16966.
48. Dinis-Oliveira RJ. Metabolism of psilocybin and psilocin: clinical and forensic toxicological relevance. *Drug Metab Rev* (2017) 49:84–91.
49. Higgins GA, Carroll NK, Brown M, MacMillan C, Silenieks LB, Thevarkunnel S, Izhakova J, Magomedova L, DeLannoy I, Sellers EM. Low Doses of Psilocybin and Ketamine Enhance Motivation and Attention in Poor Performing Rats: Evidence for an Antidepressant Property. *Front Pharmacol* (2021) 12: doi: 10.3389/fphar.2021.640241
50. Kamata T, Katagi M, Tsuchihashi H. Metabolism and toxicological analyses of hallucinogenic tryptamine analogues being abused in Japan. *Forensic Toxicol* (2010) 28:1–8. doi: 10.1007/s11419-009-0087-9
51. Manevski N, Kurkela M, Höglund C, Mauriala T, Court MH, Yli-Kauhaluoma J, Finel M. Glucuronidation of Psilocin and 4-Hydroxyindole by the Human UDP-Glucuronosyltransferases. *Drug Metabolism and Disposition* (2010) 38:386. doi: 10.1124/dmd.109.031138
52. Anderson BT, Danforth A, Grob CS. Psychedelic medicine: safety and ethical concerns. *Lancet Psychiatry* (2020) 7 10:829–830. https://api.semanticscholar.org/CorpusID:221807015

CHAPTER 4: NOVEL EXTENDED-RELEASE TRANSDERMAL FORMULATIONS OF THE PSYCHEDELIC N,N-DIMETHYLTRYPTAMINE (DMT)

In clinical settings, classical serotonergic psychedelics are administered under strict medical supervision to ensure patient safety and optimize therapeutic outcomes. The method of administration and dosage varies depending on the specific psychedelic compounds, the purpose of the treatment, and the individual characteristics of the patient (34,248–253). Psilocybin and LSD are typically administered orally, with psilocybin given in the form of capsules (34,168,198,222,254–256) and LSD often delivered via blotter paper or liquid form (250–252). Whereas DMT, due to its short duration of action, is commonly administered intravenously, allowing for precise control over the onset and duration of the psychedelic experience (251,253,257–262).

Once administered, the time to reach peak plasma concentrations and the duration of the psychedelic-induced experience vary quite a lot. For psilocybin, peak concentrations are typically achieved approximately two hours after oral ingestion, with the effects generally lasting for about 4 to 6 hours (226,254,263). LSD reaches its peak plasma concentration at around 1.5 to 2 hours post-administration, with the experience extending between 8 to 12 hours (226,250,264). In contrast, when administered intravenously, DMT reaches peak concentrations within minutes, leading to a rapid onset of effects that typically last for only 15 to 30 minutes (265,266). Despite these differences in duration, all three of these compounds induce profound and intense psychedelic, hallucinogenic, experiences. Regardless of the varying durations of their effects, the psychedelic experience induced by these substances is often intense and deeply impactful, characterized by a variety of perceptual, emotional, and cognitive changes (256,259,267–274).

One of the most notable aspects of the psychedelic experience is the alteration of sensory perceptions. Among these, patients commonly report visual hallucinations, which can range from

simple geometric patterns to complex, vivid images. These visuals often feature bright, shifting colors and intricate designs that seem to move and evolve, creating dynamic and captivating patterns (95,249). In addition, patients often report auditory hallucinations, such as hearing music or sounds that are not present in the environment. These sensory alterations can be profoundly engaging, frequently leading to a sense of awe and wonder. Beyond these hallucinations, patients often experience changes in their perception of time and space. Time may seem to slow down, speed up, or even stand still, creating a sense of timelessness. Spatial perceptions can also be distorted, with objects appearing larger or smaller than they are or distances between objects seeming to expand or contract (256,259,267–274). Moreover, these changes in perception can create a sense of detachment from reality, contributing to the overall intensity of the experience.

To manage these intense experiences safely, administering these psychedelics requires a controlled clinical environment with specific logistical considerations to ensure safety and efficacy (30,147). Consequently, patients undergo thorough screening for contraindications, which are specific conditions or factors that can increase the risks associated with the procedure or make it unsuitable for certain individuals. They are also prepared for the experience through pre-session counseling, which helps to set expectations and reduce anxiety (22,32,275,276). During the session, one or two trained psychiatrists must be present to monitor the patient and provide support. This supervision is crucial for managing any challenging experiences that may arise, such as intense emotional reactions or distressing hallucinations. After the session, post-session integration therapy is provided to help patients process and make sense of their experiences, facilitating long-term therapeutic benefits (251,276–280).

However, this comprehensive therapeutic process is not without financial implications. The need for extensive pre-session counseling, continuous monitoring by trained professionals, and post-session integration therapy can significantly increase the cost of treatment. As a result,

the economic burden of such interventions may limit accessibility for some patients, underscoring the importance of exploring cost-effective solutions and insurance coverage to make these potentially life-changing treatments more widely available (251,280–283). Despite these high costs, the potential therapeutic benefits make these treatments a promising area for research and clinical practice.

Nevertheless, while these serotonergic psychedelics offer profound therapeutic potential, this may not be the best treatment approach for all patients. The intense nature of the psychedelic experience can be overwhelming for some individuals, and the hallucinogenic effects may not be suitable for those seeking non-hallucinogenic alternatives (248,284,285). For example, elderly patients (285–287) or veterans with PTSD (43,280,284,288,289) may find the intensity of the psychedelic experience distressing rather than therapeutic. Elderly patients often have multiple comorbidities and may be more vulnerable to the side effects of hallucinogenic substances (285–287,290). They might benefit more from treatments that offer therapeutic benefits without altering their state of consciousness.

Similarly, veterans with PTSD may have heightened sensitivity to altered perceptions, which can exacerbate their symptoms rather than alleviate them. For these individuals, a novel approach is required, such as using transdermal patches to deliver psychedelic compounds offers a promising alternative.

Transdermal patches allow for the slow and controlled release of medication through the skin and into the bloodstream, ensuring steady and consistent therapeutic levels of the drug over time. These patches deliver medication through the skin and into the bloodstream and have been used to treat neurological conditions such as schizophrenia (291–294), Parkinson's disease (295–297), Alzheimer's disease (298–301), and nicotine addiction (302–306). Similarly, transdermal patches could be used to administer classical serotonergic psychedelics in a controlled manner. This method has several advantages. Firstly, it can minimize the peak concentrations of the drug

in the bloodstream that are often responsible for the intense hallucinogenic effects. By maintaining stable plasma levels, transdermal patches can potentially deliver the therapeutic benefits of psychedelics without inducing a psychedelic experience. By identifying the optimal dose, this method could achieve antidepressant efficacy without eliciting an experience (307–313). This is particularly beneficial for patients who require the therapeutic properties of psychedelics, such as their antidepressant or anxiolytic effects, without the associated alterations in consciousness.

Additionally, transdermal patches bypass the gastrointestinal system, reducing the risk of nausea and other digestive side effects commonly associated with oral administration (314–316). This mode of delivery can enhance patient comfort and adherence to the treatment regimen. Furthermore, the non-invasive nature of patches makes them a convenient and discreet option, especially for patients who may be reluctant to take oral medications or undergo comprehensive treatment regimens. By offering a controlled and gradual release of the medication, transdermal patches can provide a more manageable and predictable therapeutic experience (317–319). Moreover, the use of transdermal patches can enhance the safety, tolerability, and therapeutic potential of these compounds, allowing them to benefit a broader range of patients. This approach helps mitigate the risk of intense psychedelic experiences that may be distressing for some individuals (251,284,285,312). Consequently, studies have shown that low-dose psychedelic treatments can exert antidepressant effects through mechanisms that are not necessarily dependent on the hallucinogenic properties of these drugs (309–313). By administering the drug at non-hallucinogenic levels, patients can receive the therapeutic benefits associated with serotonergic psychedelics, such as mood enhancement (313,320) and neuroplasticity (321), without undergoing significant alterations in perception, cognition, or emotion (309–313). These findings open the possibility of using transdermal patch applications for psychedelics as a treatment for depression and other mental health disorders, broadening their accessibility to a wider range of patients.

4.1 Introduction:

Psychedelic compounds, largely overlooked as therapeutic approaches by industrialized nations since the 1960s, have emerged again as promising drug classes for psychiatric (322) and addiction disorders (275). The tryptamine or “classic psychedelic” compound class is being evaluated in several late-stage clinical trials (252,253,323–325) showing sustained efficacy in patients after only one or two doses. The doses selected in these trials are typically designed for maximum psychedelic subjective effects which require the use of therapists to observe patients within a clinical setting. This is a similar model to the now approved Spravato (esketamine) for treatment-resistant depression; (326) however, the methods of drug delivery to patients have not been optimized for non-orally active psychedelic drugs or those with low bioavailability.

One of these drugs, *N,N*-dimethyltryptamine (DMT), can produce rapid, intense, and short-lived psychedelic effects via serotonin 2A receptor (5-HT_{2A}R) agonism (260) Despite its potent nature, DMT is rapidly degraded by monoamine oxidase (MAO) enzymes limiting any potential of oral dosing (327–329). The use of ayahuasca, a plant-based aqueous extract consisting of naturally derived DMT and MAO inhibitors, has been used for ritualistic, spiritual (330), and therapeutic (331) purposes. Pure DMT formulations must be administered via intravenous, intramuscular, inhalation, and insufflation methods to reduce gastrointestinal first-pass effects. Definitive biological and ecological roles of DMT are unknown, but research shows it is ubiquitously produced throughout nature (262) and has been found in the brains of rats (332) and in human biological fluids (333). Given its structural similarities to serotonin and the amino acid tryptamine, its biosynthetic precursor, DMT is also hypothesized to be the endogenous ligand to 5-HT_{2A} (334). Despite these intriguing developments, DMT remains a schedule 1 drug in the United States and a highly controlled substance in most countries worldwide.

The therapeutic promise of DMT is currently being assessed in several FDA clinical trials including a phase I study (NCT05559931) with a focus on stroke and traumatic brain injury (TBI) and a phase I/IIa study for major depressive disorder (NCT04673383) (335). To date, most clinical DMT studies have incorporated individualized therapy alongside higher doses of 0.2–0.4 mg/kg, which are sufficient to induce psychedelic and hallucinogenic effects (258,336). Studies incorporating this approach include the use of ayahuasca for addiction (337). It is presumed that drug-induced neuroplasticity, increasing dendritic spines and formation of new neural pathways contribute to the therapeutic effects of these drugs and there has also been increasing use of preclinical models to study the impact of DMT on these potential mechanisms, both together with, and apart from, hallucinogenic effects.

Given the challenges in assessing altered states of consciousness in non-human species, the head-twitch response assay (HTR) has been used as a predictive pre-clinical method to define hallucinogenic and sub-hallucinogenic doses, as the plasma levels of drug required to induce head twitch in rodents has been demonstrated to be strongly correlated to the amount of drug required to induce subjective effects of psychedelic compounds in humans (338). In this assay, mice will repeatedly shake or twitch their head under the influence of strong 5-HT_{2A} agonists, like DMT, which can be accurately measured in real-time using an automated magnetometer approach to count head twitches. Sub-hallucinogenic doses (i.e., 10 % of hallucinogenic dose) of DMT produced similar synaptic signatures measured in rat brains to that of a larger, psychedelic dose (339). Furthermore, preclinical data suggests sub-hallucinogenic doses can be effective for ischemic stroke (340,341), mood disorders such as anxiety (342), and Alzheimer's disease (343).

A functional study showed that using an antagonist to block DMT's effects at the sigma-1 receptor, a transmembrane protein located on the endoplasmic reticulum, abolished neuroplasticity, but 5-HT_{2A}R antagonism still achieved DMT-induced neuroplasticity (344). These results indicate there are multiple cellular pathways affected by DMT and that higher doses of

DMT and 5-HT_{2A}R mediated hallucinations are not solely responsible for therapeutic effects (345). Therefore, non-invasive low-dose DMT dosage forms could offer a new paradigm to achieve therapeutic effects with fewer side effects such as hallucinations.

Transdermal drug delivery systems (TDDS) of psychedelics have only been postulated with no peer-reviewed literature supporting viable technology in this field despite the positive application of TDDS in psychiatry with Emsam (selegiline) and Secuado (asenapine) (294,315,346,347) as well as Adlarity (donepezil) for Alzheimer's disease (299). The physical properties of DMT (small molecule, low molecular weight, low boiling point, and suitable lipophilicity) make it an attractive candidate for transdermal delivery. Given that transdermal delivery is a direct route to the bloodstream without exposure to first-pass metabolism, it could offer a non-invasive alternative to intravenous administration. The major challenge to the druggability of DMT is its short half-life of about six minutes (348). Therefore, a drug delivery system must be designed to overcome the kinetics of metabolism to drive steady-state drug plasma concentrations with fewer fluctuations and blunting of maximum concentration (C_{max}) to avoid hallucinations.

This study outlines the design and development of single layer drug-in-adhesive (DIA) patches containing DMT freebase. Various formulations were trialed modifying adhesives, permeation enhancers, and concentrations of DMT to maximize drug loading and release characteristics. Franz cell diffusion was used as an initial tool to assess DMT release and flux. An optimized patch formulation was reproduced under good laboratory practices (GLP) and tested in both male and female mice for pharmacokinetic (PK) measurement of blood and brain concentrations of DMT, while the head-twitch response assay (HTR) was utilized to measure hallucinogenic potential. The goal of the study was to maintain plasma concentrations below that of 60 ng/mL, a concentration that has been clinically defined to cause subjective hallucinogenic and psychedelic effects in humans (258,336).

4.2 Materials and Methods:

Materials

DMT freebase (non-GLP) was synthesized for formulation screening and *in vitro* Franz cell release testing using the Speeter-Anthony method (349). DMT freebase (GLP) utilized in patches for dose-response in mice was purchased from Organix Inc., Boston, MA, USA with a purity of 98.5 %. Tryptamine, methanol, ethanol, ethyl acetate, water, acetonitrile (ACN), and formic acid (FA) were purchased from Fisher Scientific, Hampton, NH, USA. Duro-Tak 4098 and 6098 were provided by Henkel Corporation, Dusseldorf, Germany. Bio PSA 7-4302 and 4202 were provided by DuPont Inc., Missuagua, ON, Canada. Isopropyl myristate was provided by Croda International Plc, Edison, NJ, USA. Scotchpak 9709 and 9733 were provided by 3M Corporation, Saint Paul, MN, USA. Mylar packaging was purchased from Impak Corporation, Sebastian, FL, USA.

Formulation of DMT patches

A total of six formulations were trialed to optimize drug loading in the DIA matrix. To make the DIA formulations, non-GLP DMT was dissolved in either ethanol or ethyl acetate before the addition of adhesive and/or permeation enhancers. Once combined, an overhead stirrer (IKA RW20, Wilmington, NC, USA) was used to blend the formulation at 500 rpm and allowed to degas. The formulations were placed on the siliconized side of a Scotchpak 9709 liner and coated using a thin film applicator (150 mm, MTI Corporation, Richmond, CA, USA) at a thickness of 150-250 µm. Formulations were dried in an oven (Quincy Lab 10-180, Burr Ridge, IL, USA) for 2 h at 70 °C before lamination onto a Scotchpak 9733 backing. Patches were cut to size based on desired dose, heat sealed within Mylar packaging, and stored at room temperature until use.

The patches used for the *in vivo* studies were manufactured under Phase 1 GLP conditions at the Zeeh Pharmaceutical Experiment Station at the University of Wisconsin, Madison. The GLP DMT freebase from Organix was placed under stability and used to create two patch dosages at 0.4 mg/cm^2 using the same procedure for non-GLP DMT patches.

Quantification and stability sample preparation of All DMT patches

The release liner was removed, and patches folded in half onto itself with adhesive sides touching. The patch was cut into four pieces, placed into a vial, and 10 mL of ethyl acetate added to cover the material for thorough extraction. Vials were submerged in a sonicator (Branson 2510, Emerson Electric, Round Rock, TX, USA) to extract the DIA from the patch backing into the solvent for 15 min. Once complete, the DIA-less backing pieces were removed, and the resulting ethyl acetate extract was dried under an inert stream of nitrogen. The dried residue was reconstituted with 5 mL of methanol and sonicated for another 15 min. The resulting slurry was transferred into an Eppendorf tube and placed into a centrifuge (Mini Centrifuge, Fisher Scientific, Hampton, NH, USA) for 10 min at 6,000 rpm. The supernatant was pipetted into a 2 mL amber glass autosampler vial along with a tryptamine (50 µg/mL) internal standard.

In vitro release of non-GLP DMT patches

To determine the rate and quantity of non-GLP DMT released from patches, Franz cell apparatuses (10 mL unjacketed, PermeGear, Hellertown, PA, USA) were equipped with Strat-M membranes (25 mm, Millipore Sigma, Darmstadt, Germany). In our experience, these membranes provide a more consistent platform to analyze different formulations without changes in thickness and follicle density from excised skin. Inconsistencies in these factors can significantly alter drug flux parameters necessitating greater replicates along with extra

processing techniques (i.e. dermatome and stratum corneum removal). Strat-M membranes have excellent correlations to human skin across a number of different drugs further validating their usage (350).

Due to low aqueous solubility of freebase DMT, 10 % ethanol in water was used in the receiving well which was incubated in a 37 °C water bath to simulate human biological temperatures with continuous stirring at 500 rpm. Patches were stored at room temperature for at least one-week post-production to equilibrate before testing. Single replicates ($n = 1$) from each formulation were analyzed at multiple sampling times (0.5, 1, 2, 4, 8, 24, 30, 48, 72 hrs) whereby the entire contents of the receiving well were emptied into a vial and fresh 10 % ethanol solution was added to the receiving well. Each sample was homogenized with a vortexer (Standard Vortex Mixer, Fisher Scientific, Fisher Scientific, Hampton, NH, USA) before pipetted into a 2 mL amber glass autosampler vial along with a tryptamine (50 µg/mL) internal standard.

DMT patch quantitative analysis – *in vitro* and *in vivo*

An HPLC method was adopted (351) on an Agilent 1200 system to quantify DMT from patch formulations and Franz cell time points. A binary solvent system with water and ACN, each spiked with 0.1 % FA, was employed at a flow rate of 1.3 mL/min initially at 5 % ACN, ramping to 40 % ACN at 3.5 min, then up to 100 % ACN at 6 min and held for 1 additional minute. The stationary phase column, C18 InfinityLab Poroshell 120 (4.6 × 30 mm x 2.7 mm), was maintained at 35 °C. The sample injection volume was 5 μL and quantification was done at 280 nm. Samples were analyzed using Agilent ChemStation software whereby DMT peak areas were first normalized to a peak ratio of an external standard tryptamine vs. DMT at 50 µg/mL. The normalized DMT peak area was then divided by the peak area of tryptamine internal standard and multiplied by its concentration of 50 µg/mL.

Studies to determine recovery of DMT from the patch DIA were assessed by spiking 50 µg/mL DMT into the patch extraction protocol of Section 2.3. The DIA patch matrix did retain a portion of DMT with recovery determined to be 92 %. Reproducibility of DMT peak areas compared to the standard was 99.8 %. The lower limit of quantification for DMT was 20 ng/mL. Flux (µg/hr) was calculated from Franz cell (4.91 cm^2 active area) studies as the linear regression of the DMT concentration vs. time plot.

In vivo delivery of GLP DMT in mice

Animal experiments were approved by the University of Wisconsin, Madison Animal Care and Use Committee (IACUC). All procedures were followed in compliance with the Research Animal Resources and Compliance (RARC) guidelines. All mice (Swiss-Webster; male and female; 6-8 weeks; 30–40 g; Charles River Laboratories, Wilmington, MA) were handled for 7 days prior to experimentation to acclimate to both the experimenter and vivarium. Mice were housed in groups of two or three under a 12h reverse light/dark cycle; temperature was maintained between 22 and 24 °C. All food (LabDiet) and water (Inno-Vive) were available to the mice *ad libitum* unless stated otherwise.

For patch application, animals were initially anesthetized under a nose cone with 5 % isoflurane in oxygen flowing at 2 L/min, with maintenance anesthesia levels at 1.5-2 % isoflurane. Electric clippers (Fisher Scientific, Pittsburg, PA) were used to shave the dorsal neck area between the ears and shoulder blades. Veet hair removal cream (Andwin Scientific Industrial, Simi Valley, CA) was applied on the shaved portion and removed after 5 min using 70 % ethanol. Patches were cut to size prior to application. to deliver either 1 or 5 mg/kg of DMT. Patches containing either GLP DMT or vehicle (Psilera Inc., Tampa, FL) were placed on the hairless area and secured to the animals with jackets (Lomir Biomedical Inc, Malone, NY). All mice were placed

in a recovery chamber until they were fully awake before administering an IV tail vein injection of either DMT or vehicle and remained in their home cage for 60 min prior to experimentation. While all animals received both manipulations, an injection and patch, to control for differences in handling arising from these manipulations, active drug was only delivered through one of these routes for each group. Post-administration blood samples were collected at 5, 10, 15, 30, 60-, 240-, 480-, or 1440-min using microcentrifuge tubes. Following collection, the samples were then centrifuged at 10,000 rpm (11,292 g) for 10 min at 4 °C. The plasma fraction was separated and stored in the dark at –80 °C until LC–MS/MS analysis. Brain tissues were collected post-administration at 15 or 60 min and were flash frozen in liquid N_2 and stored in the dark at –80 °C until LC–MS/MS analysis. Tissue extracts for DMT quantitation were prepared by placing weighed brains in a 2-ml screw-cap tube with 1.4 mm ceramic beads (Fisherbrand cat. no. 15-340-153), adding 2 volumes of 150 mM ammonium bicarbonate per gram of tissue and homogenizing in an Omni Bead Mill Elite homogenizer.

DMT quantitative analysis – *in vivo*

For LC/MS/MS analysis plasma samples were prepared by precipitation and filtration. Briefly, 50 μl of plasma was precipitated with 150 μl ACN/1 % formic acid containing internal standard (6-methoxy DMT, Sigma Aldrich, St. Louis MO) in a 96-well format Sirocco plate (Waters Corp. Milford, MA) according to the manufacturer's protocol. Samples were pushed through the plate to a 2-ml receiver plate containing 800 μl of water. A similar protocol was used for preparation of brain extracts except that instead of a simple filtration plate, brain samples were processed through a Waters Ostro plate to reduce phospholipid content of the extracts. For both brain and plasma, calibration curves (1 ng/mL-2000 ng/mL) and QC samples for DMT were prepared in blank matrix and processed with unknown samples.

After processing samples were briefly vortexed then analyzed using a liquid chromatography tandem mass spectrometry (LC/MS/MS). Sample (2 μl) were injected onto a Phenomenex Kinetex Phenyl-Hexyl 2.1 × 100 mm column packed with 1.7 μm particles using a Waters Acquity UPLC system (Waters, Milford MA). The column was held at 35 °C and the flow-rate was 0.375 ml/min. Solvent A was water/0.1 % FA and solvent B was ACN/0.1 % FA. Analytes were eluted from the column with an increasing gradient of ACN from 5-40 % B in 1.75 min then to 95 % B in 0.35 min with a 0.4 minute hold at 95 % then a return to 5 % B in 0.25 min. Eluate from the column was analyzed in positive ion mode using a QTrap 5500 hybrid triple quadrupole mass spectrometer (SCIEX, Framingham MA) operating in multiple-reaction-mode (MRM) under conditions optimized for detection of the analyte and internal standard. For DMT the transitions were: parent ion 189.1/product ions 58, 144.1 and 91.1. The transitions for 6-methoxy DMT were: parent 219.1/product ions 77, 130.1 and 174.1. All transitions had a 50 msec dwell time. Triplicate injections of samples, calibrators and QCs were used for quantitative analysis allowing calculation of mean and standard deviation. The mean area under the curve (AUC) of the analyte relative to ISTD was used to construct a quadratic fit for the calibration curve in MultiQuant software (SCIEX, Framingham, MA). Calibrators were excluded from quantitation models if their calculated concentrations were >15 % different from theoretical. For all calibrators, samples, or QCs if the calculated %RSD of the triplicate injections was >15 % samples were not considered valid. All calibration curves had R-values ≥ 0.995. For all assays QC samples at each concentration fell within 15 % of theoretical concentrations. The lower limit of detection was based on a signal to noise value of three (determined in MultiQuant). The lower limit of quantitation for each assay was based on either a signal to noise value of ten, or set at a DMT concentration equal to the lowest concentration calibrator, whichever was higher. The upper limit of quantitation for each assay was set at the highest calibrator meeting the ±15 % of theoretical metric.

Head twitch response (HTR)

To assess the dose-dependent effects of DMT on HTR, male and female Swiss Webster mice, 6-8 weeks, were separated into two cohorts (cohort 1: DMT IV + vehicle patch; cohort 2: vehicle IV + DMT patch) and compared to animals receiving Saline IV + vehicle patch. All mice underwent a magnet implantation procedure and HTR analysis using procedures in previously reported methods (200).

Briefly, in this protocol, mice were anesthetized with isoflurane, as described above, and a nickel magnet (Eokoaiee multi-use fridge magnets; 4 × 2 mm) was cemented (DentalWorld, Bahadurgarh, India) to the skull attached to an anchor screw. All animals were allowed to recover for at least 5-7 days prior to any behavioral experimentation.

On the experimental day, mice were placed in a magnetometer cage (15.24 cm height × 15.24 cm diameter) containing ~300 rotations of 30-gauge copper wire (Essex, Fort Wayne, IN). All mice were initially analyzed for 60 min with jackets applied for baseline activity. After the initial habituation period, mice were administered the active treatment and placed immediately back into the magnetometer cage for another 60 min trial period.

All signals were transmitted through a Molecular Devices (Digidata 1440A) digitizer and analyzed with Clampex software. The sampling rate used in this study was set at 3003 Hz. All data analysis was performed with MATLAB software and Prism GraphPad. HTR was assessed at each timepoint as a percent of the corresponding saline response.

Pharmacokinetic calculations and statistical analysis

Elimination rate constants and half-lives were calculated from the slope of the best fit line for Ln (Concentration) over time. Y-intercepts were calculated for IV plots from this best fit. AUCs are reported for t = 0 – terminal collection, using the best fit Y-intercept value at t = 0 for IV plots,

and 0 at t = 0 for transdermal (TD) plots. Relative bioavailability (F) was calculated as: $F = (AUC_A / Dose_A) / (AUC_B / Dose_B)$. All statistical analyses were performed using GraphPad Prism, version 10 (San Diego, CA). In all cases, statistical significance threshold was set as $p < 0.05$. All data presented as Mean ± SEM.

4.3 Results:

Formulation of non-GLP DMT patches

Formulation screenings were undertaken with DMT freebase and the three most common types of transdermal adhesives (acrylate, polyisobutylene, and silicone) and isopropyl myristate, a known permeation enhancer (352). The formulations F1-F6 are listed in Table 1. The aim of screening was to maximize DMT concentrations in the adhesive-solvent system for *in vitro* drug release comparison. Ethanol was determined as the ideal solubilizer for DMT and was compatible across all adhesive systems. Ethyl acetate, used in F1, required the addition of ethanol to adequately solubilize DMT. Further formulations F2-F6 used ethanol as the sole solubilizer with greater DMT solvation capacity and increased drug loading. No crystallization was observed in any formulation (F1-F6). Solubility ranking of the adhesives (highest to lowest) are as follows: acrylate (Duro-Tak 4098) > silicone (Bio-PSA) > polyisobutylene (Duro-Tak 6098). The use of isopropyl myristate greatly increased solubility of DMT in the acrylate (Duro-Tak 4098) adhesive matrix. While DMT has a relatively low boiling point, care was taken to not evaporate DMT from the adhesive during the DIA drying process but did not pose problems for scale-up manufacturing.

Table 1. Wet weight concentration of DMT transdermal formulations (F1-F6).

Formulation	Ingredient (wet weight %)									
	DMT	Ethanol	Ethyl Acetate	Isopropyl Myristate	Duro-Tak 4098	Duro-Tak 6098	Bio-PSA 4302	Bio-PSA 4202	Coating Thickness (μm)	Dried Appearance
F1	1.8	7.2	7.2		83.8				150	Cloudy
F2	2.0	8.0			90.0				250	Clear
F3	2.0	8.0					45.0	45.0	250	Cloudy
F4	2.0	4.0				94.0			150	Cloudy
F5	3.8	15.9			80.3				200	Clear
F6	4.2	11.2		7.1	77.5				200	Clear

Table 1: Wet weight concertation of DMT transdermal formulations (F1-F6).

Characterization and quantification of non-GLP DMT patches

After drying the DIA laminate, each patch from formulations F1-F6 were weighed, thickness assessed, and average DMT content (dry w/w %) determined. The averages of these values are reported in Table 2. After removal of the outer edges of the laminate, which results in uneven coating due to edge effect, the intra-batch results for patches were consistent with coat weight variability rarely exceeding 10 %. In light of this, one patch from each formulation was quantitatively tested within the average thickness and coat weight.

Table 2. Average thickness, mass, and DMT concentration in dried drug-in adhesive (DIA) for each formulation (F1-F6).

Formulation	Average DIA Thickness (μm)	Average DIA Mass (mg)	DMT Dried Weight (%)
F1	50 ± 8	53 ± 3	2.4
F2	53 ± 8	58 ± 4	6.4
F3	53 ± 3	52 ± 5	3.9
F4	35 ± 7	25 ± 5	0.5
F5	40 ± 3	38 ± 4	10.5
F6	47 ± 6	44 ± 4	16.1

Table 2: Average thickness, mass, and DMT concertation in dried drug-in adhesive (DIA) for each formulation (F1-F6).

Polyisobutylene adhesive (F4) had the lowest DMT content, thickness, and DIA mass as its solubility hindered formulation. The characteristics of silicone adhesive formulation (F3) were similar to that of acrylate (F2), though higher DMT concentrations were achieved with F2. High drug loading of DMT is achievable with the acrylate adhesive, making Duro-Tak 4098 the ideal candidate for further formulation screening. The highest drug concentration without additives was F5, achieving 10.5 % w/w DMT. The addition of isopropyl myristate, a solubility and permeation enhancer, in F6 aided DMT dry weight concentrations up to 16.1 % w/w.

In vitro release of DMT from patches

Within two weeks of production and storage, each formulation was subjected to Franz cell diffusion studies to quantify *in vitro* release of DMT (Fig. 1). All formulations were able to achieve linear, zero-order DMT delivery over 72 h ($R^2 > 0.98$), except F4, under the tested conditions. The multiple timepoints for sampling ($n = 8$) add replicates for the flux calculations for a single Franz

cell diffusion analysis. This is congruent with prior experiments with the Strat-M membranes (unpublished data).

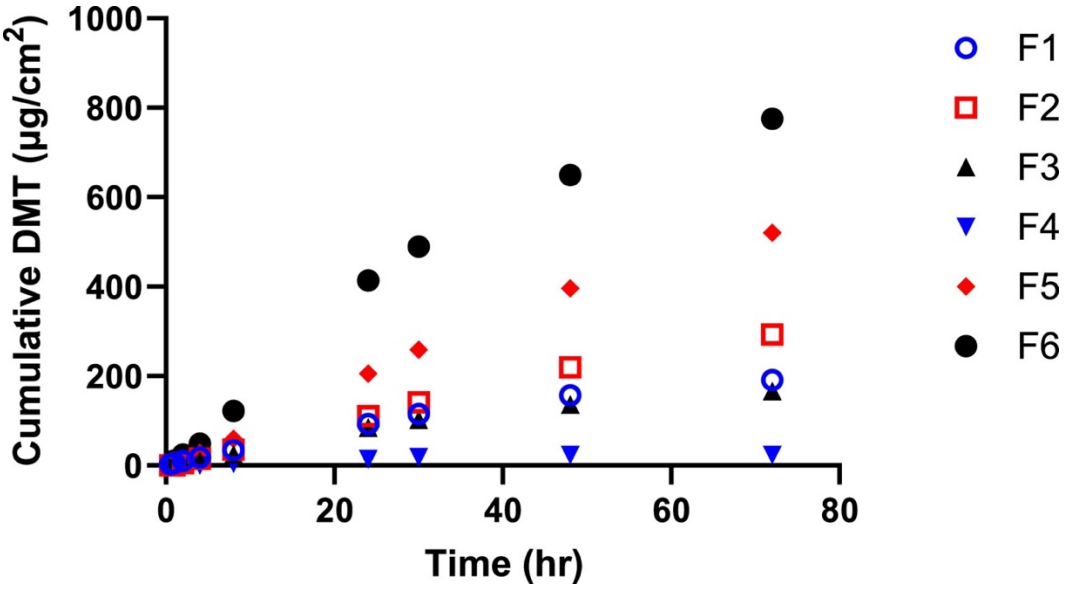

Figure 2: Time-based cumulative DMT delivered in Franz cell diffusion assay for formulations F1-F6.

Unsurprisingly, higher DMT concentrations tended to increase overall DMT permeation through the Strat-M membrane into the receiving well. Though there was one notable exception with F1 (2.4 % DMT w/w) having slightly greater diffusion than F3 (3.9 % w/w). This demonstrates that acrylate is a superior adhesive for drug delivery over silicone or polyisobutylene systems. Optimized formulation F5 (10.5 % DMT w/w) was able to deliver 520 µg/cm^2 over 72 h without any permeation enhancers. Only F6 (16.1 % w/w), with the addition of isopropyl myristate, bettered F5 with 775 µg/cm^2 cumulative DMT delivery. The flux values for all formulations F1-F6 are listed in Table 3.

Table 3. Flux values for each formulation F1-F6.

Formulation	Flux (μg/(cm^2*hr)$^{-1}$)
F1	4.30 ± 1.00
F2	3.22 ± 1.92
F3	2.19 ± 1.34
F4	0.29 ± 0.28
F5	6.54 ± 2.73
F6	13.3 ± 2.37

Table 3: Flux values for each formulation F1-F6.

This initial dataset confirms that transdermal delivery systems offer a plausible and novel administration route for DMT. Based on the *in vitro* diffusion data, it was determined that formulation F5 would be trialed *in vivo* to test the hypothesis that a transdermal delivery system, without permeation enhancers, could offer sustained DMT delivery. Patches from the F5 batch were subjected to Franz cell diffusion at one week, one month, and two months to determine stability. All of the resulting fluxes were within the range of F5 (6.54 ± 2.73 μg/(cm^2*hr)$^{-1}$) listed in Table 3, indicating suitable stability for *in vivo* testing.

In vivo delivery of DMT from intravenous or transdermal administration in mice

To quantify concentrations of DMT present in plasma and brain samples following delivery through both intravenous and transdermal routes, male and female mice were administered DMT doses of either 1 or 5 mg/kg. In these animals, samples were collected at 5-, 10-, 15-, 30-, 60-, and 240-min post-drug administration for IV DMT, and at 60-, 240-, 480-, and 1440- min post-drug administration for TD DMT. Precision and accuracy for the LC/MS/MS method was acceptable and reproducible (Table 4, Fig. 2) across runs.

Table 4. Analytical parameters for LC/MS/MS quantification of DMT concentration-time profiles.

Run	Tissue Type	Route	Doses (mg/kg)	LLOQ	LOD	ULOQ	Average %RSD Concentration	%RSD Range
1	Plasma	IV	5	1 ng/mL	0.3 ng/mL	2000 ng/mL	4.39 %	(0.04–12.04)
2	Plasma	IV	1	1 ng/mL	0.3 ng/mL	2000 ng/mL	3.16 %	(0.52–11.53)
3	Plasma	TD	5, 1	1 ng/mL	0.3 ng/mL	2000 ng/mL	2.50 %	(0.22–10.84)
4	Brain	IV	5, 1	150 ng/g	45 ng/g	6000 ng/g	1.08 %	(0.01–4.98)
5	Brain	TD	5, 1	150 ng/g	45 ng/g	6000 ng/g	0.93 %	(0.07–4.16)

Table 4: Analytical parameters for LC/MS/MS quantitation of DMT concertation-time profiles.

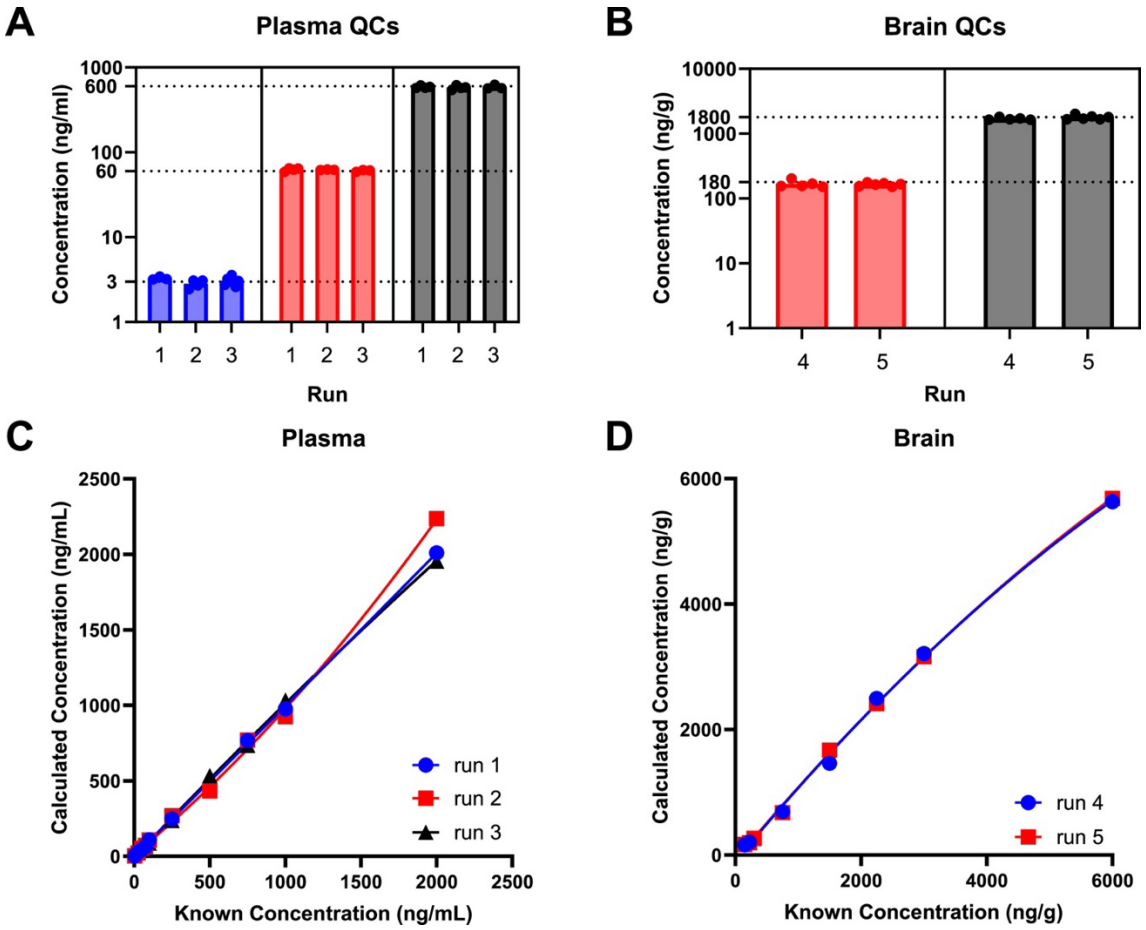

Figure 3: Validation of LC-MS/MS method development. (A, B) Quality controls (QCs) for the analysis of mouse plasma and brain samples. Each individual point represents an average of 3 technical replicates for each QC value on a plate. **(C, D)** Standard curves for plasma and brain concentration quantification with a second-order polynomial fit.

Regarding the pharmacokinetic profile for the IV infusion, this route exhibited rapid and robust maximum plasma concentrations (C_{max}) of 597 ng/mL for males and 1437 ng/mL for females with a 5 mg/kg dose (Fig. 3). All concentrations administered through IV infusions peaked at the initial 5-minute time point, irrespective of sex, and demonstrate a dose dependent plasma exposure profile. For the lower doses of DMT (1 mg/kg) administered to males, the average C_{max} reached 372 ng/mL, while the same dose in females achieved a concentration of 528 ng/mL.

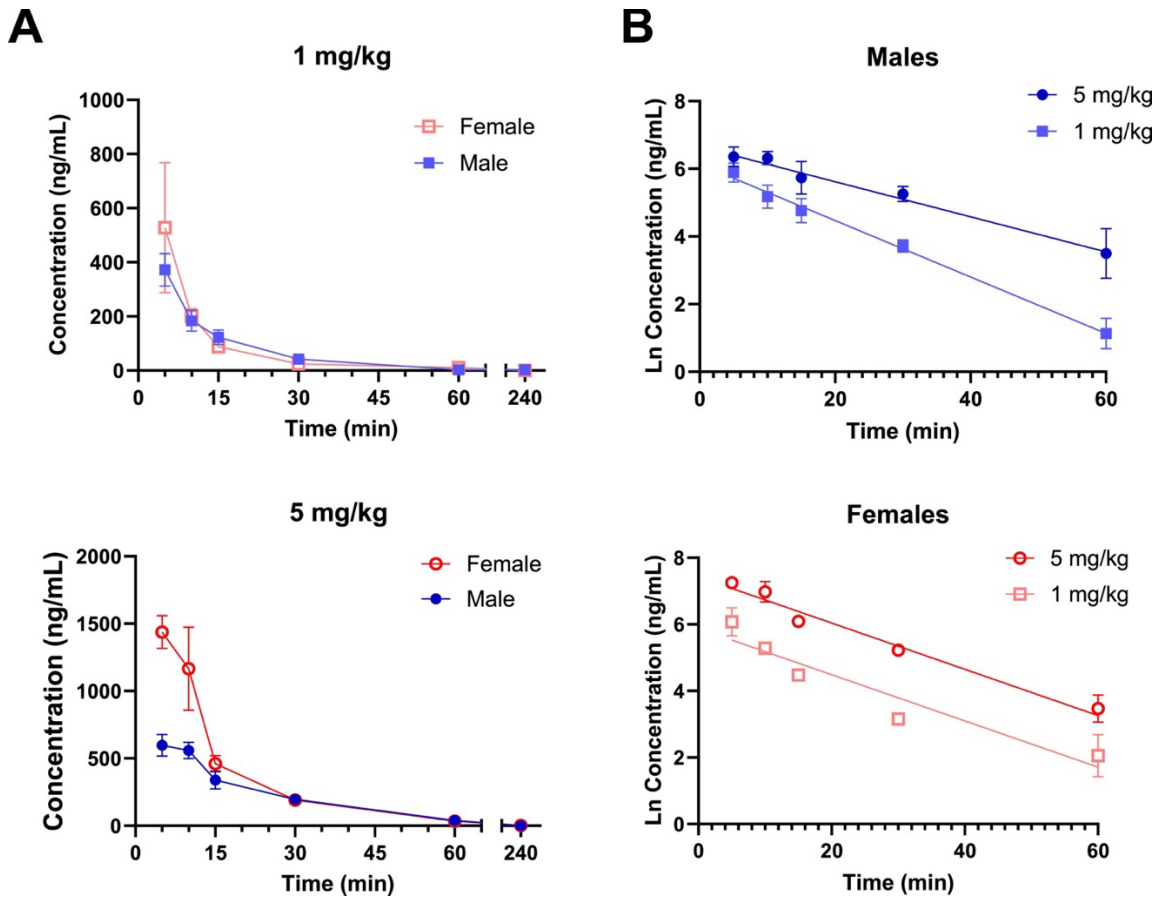

Figure 4: Plasma pharmacokinetics of intravenously administered DMT. (A) Plasma concentration time courses at 1 mg/kg (n = 18; 3/timepoint) and 5 mg/kg (n = 22; 3-5/timepoint). **(B)** Log-transformed concentration time-profiles by dose for males and females.

The transdermal DMT patch F5, on the other hand, demonstrated a sustained release profile, resulting in a prolonged time to peak concentration (Fig. 4), and much lower maximum concentrations overall. The average C_{max} for male animals reached 37.0 ng/mL at 5 mg/kg and 8.0 ng/mL at 1 mg/kg, both occurring at the 1-hour time point. Interestingly, female animals achieved a C_{max} of 54.8 ng/mL at the 4-hour time point with a 5 mg/kg dose. Average C_{max} for the 1 mg/kg dose was achieved at 1-hour post administration, peaking at 15.7 ng/mL.

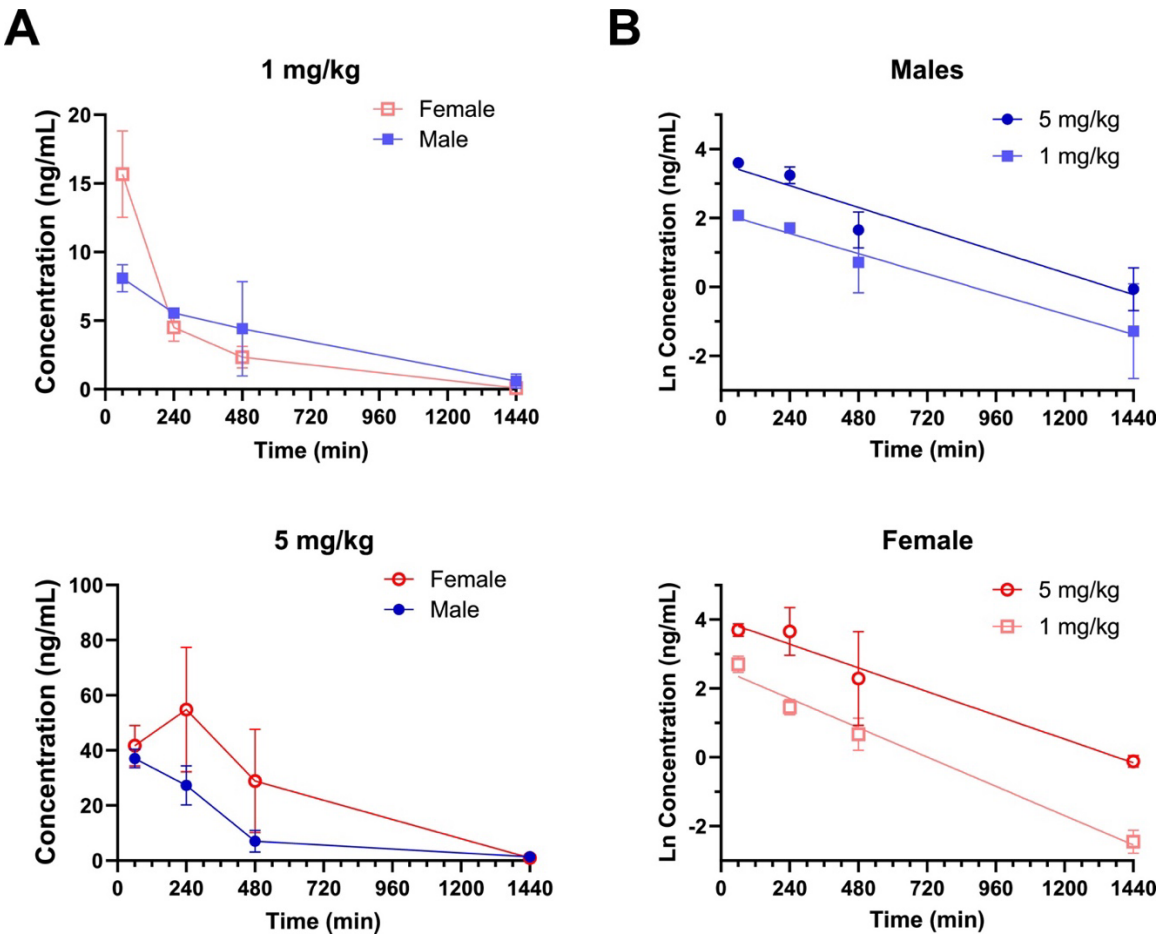

Figure 5: Plasma pharmacokinetics of transdermally administered DMT. (A) Plasma concentration time courses at 1 mg/kg (n = 12; 3/timepoint) and 5 mg/kg (n = 12; 3/timepoint). **(B)** Log-transformed concentration time-profiles by dose for males and females.

In addition to the DMT patch F5 blunting the peak concentrations of DMT achieved, these results reveal distinct prolongation of the apparent half-life ($t_{1/2}$) by the transdermal delivery system, as calculated using the transformed concentration-time profiles of plasma samples from the 1 mg/kg and 5 mg/kg doses. For the IV cohort, the average $t_{1/2}$ across was calculated to be 10.8 ± 2.5 min for males and 10.0 ± 0.1 min for females, whereas for the transdermal cohort, the $t_{1/2}$ was calculated to be 273 ± 10.5 min for males and 210 ± 14.6 min for females. Despite the quite different plasma concentration profiles demonstrated by the transdermal patch versus IV

administration, the relative plasma bioavailability of DMT following transdermal delivery remains 77 ± 10 % of that for IV administration across the 1 mg/kg and 5 mg/kg doses (Fig. 5).

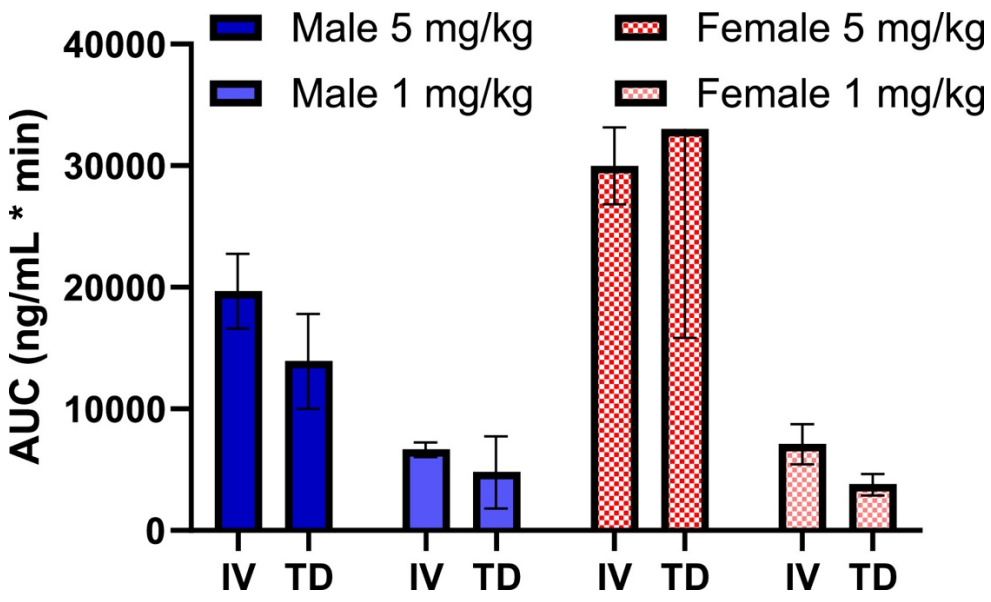

Figure 6: Relative Bioavailability of Transdermal versus Intravenous DMT. Areas under the concentration response curves are presented by dose, sex, and route of administration.

Concentrations of DMT were next assessed in brain samples taken from animals dosed with either IV or transdermal DMT (Fig. 6). In animals given IV DMT, brain concentrations of DMT were consistently found to be above the LLOQ only at the 15-minute time point. Interestingly, at 15 min, the 5 mg/kg IV dose induced the highest difference in brain DMT levels compared to plasma, with male and female brain samples reaching an average C_{max} of 4503 and 8080 ng/g, respectively. In contrast, at a dose of 1 mg/kg IV, brain DMT concentrations were lower than those found in plasma; males achieved an average C_{max} of 353 ng/g, and female animals reached a C_{max} of 250 ng/g. In animals given transdermal DMT, brain concentrations of DMT were consistently found to be above the LLOQ only at the 1-hour time point, reaching 156 ng/g in both male and female animals administered 5 mg/kg transdermally. The brain concentrations following 5 mg/kg transdermal DMT were significantly lower than those from 5 mg/kg IV DMT administration

(Two-Way ANOVA: F_{Route} [1,20] = 18.03, p = 0.0004). Brain concentrations of DMT were below the LLOQ at all timepoints for all animals given 1 mg/kg DMT transdermally. Overall, these data demonstrate that the transdermal preparation yielded a pharmacokinetic profile with dramatically reduced peak DMT concentrations in the periphery and CNS as compared to IV administration, while drug exposure is maintained at similar levels to IV due to a substantially prolonged half-life (Table 5).

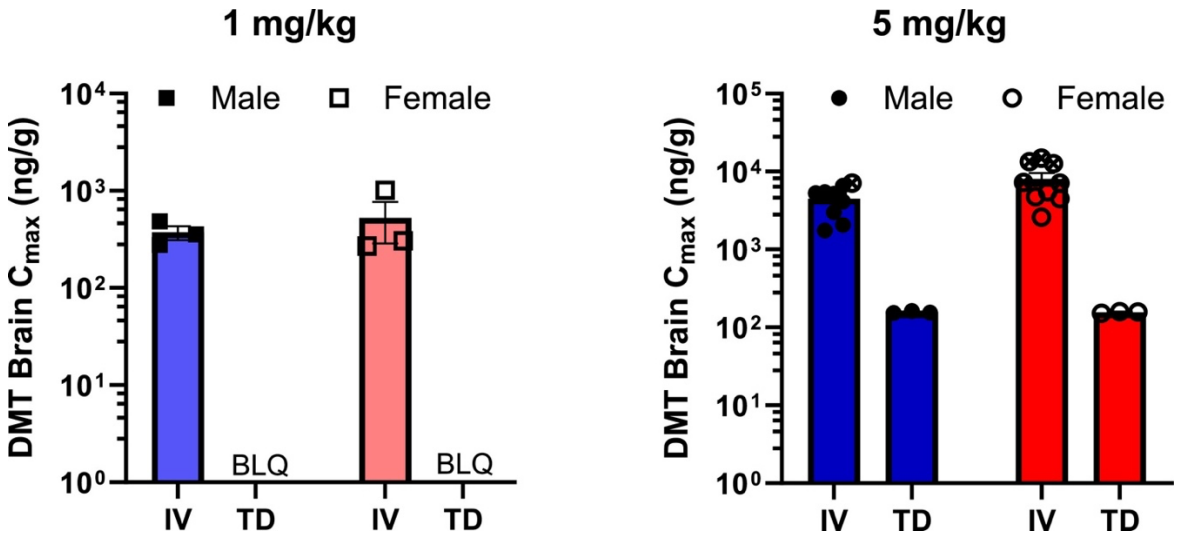

Figure 7: Maximum brain concentrations observed following IV (15 min) or transdermal (1 h) DMT administration at 1 or 5 mg/kg. BLQ = Below lower limit of quantitation. Symbols with X denote samples above top point of calibration curve.

Table 5. Summary of DMT pharmacokinetic parameters by route, dose, and sex.

	Transdermal				Intravenous			
	Male		Female		Male		Female	
	5 mg/kg	1 mg/kg	5 mg/kg	1 mg/kg	5 mg/kg	1 mg/kg	5 mg/kg	1 mg/kg
Plasma Cmax (ng/mL)[a]	37	8	55	16	597	372	1437	528
Brain Cmax (ng/g)	156	BLQ	156	BLQ	4503	353	>6000*	250
t1/2 (min)[b]	263 ± 36	284 ± 61	225 ± 29	195 ± 15	13	8	10	10
AUC (ng/mL * min)	13940 ± 3898	4817 ± 2975	33024 ± 17127	3805 ± 875	19686 ± 3048	6693 ± 604	29990 ± 3168	7130 ± 1639

a Values rounded to nearest whole unit b: SEM not shown where < 1 min.

* Above ULOQ; estimate – 8080 ng/g.

Table 5: Summary of DMT pharmacokinetic parameters by route, dose, and sex.

Assessing the hallucinogenic activity of DMT by administration route

To measure acute unconditioned responses to DMT mice were intravenously or transdermally dosed at 5 mg/kg (or given saline/vehicle) and assessed for HTR (338) (Fig. 7).

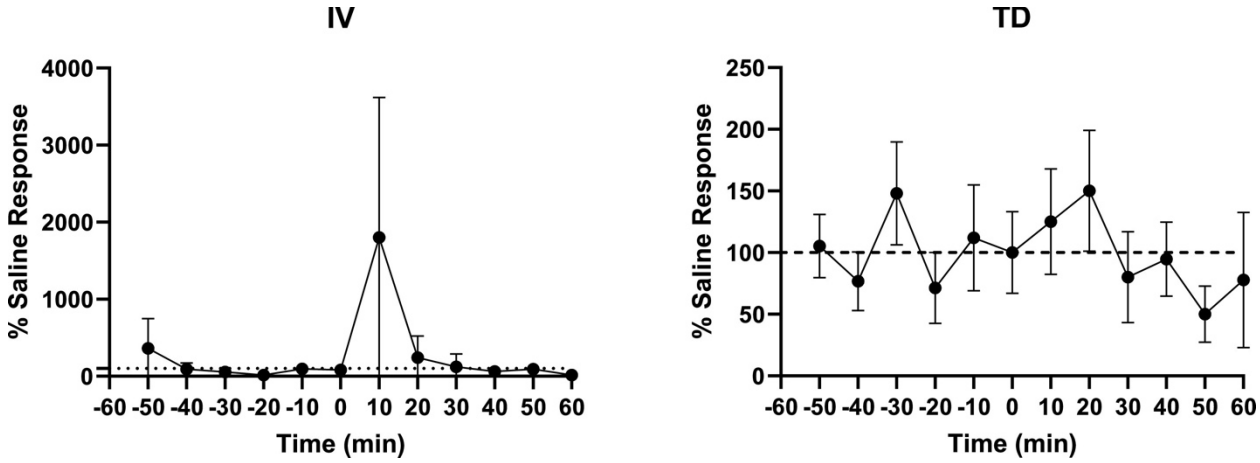

Figure 8: Head Twitch Responses due to intravenously (n = 5) or transdermally (n = 12) administered DMT at 5 mg/kg.

The IV cohort demonstrated significant variation in HTR responding by time (Two-Way RM ANOVA: $F_{RoutexTime}$ (11, 99) = 3.69, $p = 0.0002$), including a significant 18-fold increase in head twitches relative to animals receiving IV saline at 0-10 min post-administration (Sidak's Multiple Comparisons, $p = 0.0214$). This robust increase was observed transiently with only a 2.4 and 1.2-fold increase in HTR's relative to animals receiving IV saline within the next two chronological bins. In contrast, the transdermal cohort did not show any significant variation in HTR's, as compared to animals receiving a TD vehicle patch, within 1-hour post-drug administration at the same dose (Two-Way RM ANOVA: $F_{RoutexTime}$ (11, 242) = 0.66, $p = 0.77$).

4.4 Discussion:

This work herein demonstrates the first evidence of transdermal delivery of DMT, a notoriously low bioavailability and short half-life drug using oral administration. Despite increasing evidence of DMT's therapeutic potential, drug delivery for low or non-orally bioavailable psychedelic drugs have not been optimized. The development of a TDDS could offer a non-invasive and low-dose delivery option for DMT, inducing fewer hallucinations, and circumventing a side effect that requires therapists to continuously observe patients in clinical settings. A low-

dose DMT patch can also reduce abuse potential or product misuse relative to the current high, psychedelic dose administration of DMT including intravenous or inhalation.

The initial formulation strategy was to determine solvent systems which could maximize DMT drug loading into the DIA matrix. It was theorized that high drug loading of DMT would be necessary to increase flux and overcome the rapid metabolism of DMT. A solvent screening was employed to test DMT solubility in ethanol and ethyl acetate, two solvents which are both generally regarded as safe for topical use and are compatible with the adhesive systems. The first formulation (F1) was trialed using both solvents in a 1:1 ratio resulting in a cloudy formulation with a modest DMT concentration (2.4 %, dry w/w %). An optimized formulation (F2) was then tested using only ethanol in the same acrylate (Duro-Tak 4098) adhesive achieving a clear appearance with a much higher DMT concentration (6.4 %). Based on these initial results, ethanol was chosen for further screenings as the superior solvent with DMT solubility of ~400 mg/mL thereby reducing solvent evaporation and increasing DMT drug loading.

The next steps were to determine the optimal adhesive system to increase DMT drug loading. Nearly all commercial transdermal patches contain either acrylate, polyisobutylene, or silicone-based adhesives so a representative from each adhesive was trialed using ethanol as a drug solubilizer. Polyisobutylene (Duro-Tak 6098, F4) had significant solubility issues with the ethanolic DMT solution, leading to a cloudy formulation with the lowest DMT concentration (0.5 %). Compared to F4, silicone (Bio-PSA, F3) achieved a higher DMT concentration (3.9 %) with a cloudy appearance post-drying. Comparing the three adhesive types, acrylate-based F2 had the highest DMT concentrations with a clear formulation. Therefore, acrylate was chosen as the ideal adhesive for further analysis. DMT concentration was scalable from F2 into F5 achieving a 10.5 % DMT concentration with only DMT, ethanol, and the acrylate adhesive. Lastly, the addition of isopropyl myristate in F6 increased the DMT concentration to 16.1 %.

Franz cell diffusion assays, a common tool for transdermal formulation development, were used to test DMT drug flux for each formulation. A direct correlation was observed between DMT drug loading and flux and all formulations F1-F6 displayed the ability to permeate DMT through a skin-like membrane, with notable differences seen with adhesive types. When comparing adhesive types directly, acrylate (F2) displayed greater flux, though not significant, over silicone (F3) while both had significantly greater DMT fluxes over polyisobutylene (F4). This correlation could be driven by polarity with acrylate being the most polar, followed by silicone, then polyisobutylene being the most nonpolar. Increasing DMT concentration of F5 had a 2-fold greater impact on flux than F2 without the use of any permeation enhancers. Isopropyl myristate expectedly increased the flux in F6 over F5.

DMT patch F5 was chosen for *in vivo* screening to show the applicability of a simple DIA formulation, without the use of permeation enhancers, to deliver DMT transdermally. Furthermore, the drug loading (10.5 % DMT) and DMT flux of 6.54 ± 2.73 $\mu g/(cm^{2}*hr)^{-1}$ of F5 in Franz cell testing were theorized to show meaningful plasma concentrations in mice without hallucinogenic effects, measured by the HTR.

Compared to IV administration, DMT patch F5 elicited a dramatically lower C_{max} in both plasma and brain samples of Swiss-Webster mice. Additionally, while all DMT was cleared from the system within 1 h of IV administration, the DMT patch F5 resulted in measurable concentrations of peripheral DMT for at least 8 h, with a 20-fold increase in the apparent half-life of DMT at doses of 1 to 5 mg/kg. Interestingly, there were notable sex differences observed in drug exposure, with DMT reaching higher plasma concentrations at the 5 mg/kg and 1 mg/kg doses in female animals as compared to males, regardless of route of administration. Human clinical trials would be wise to monitor PK differences in male and female patients especially with high bolus dosages. Previous studies have shown variations in DMT effects in male versus female rats that correlate with weight (342) as well as efficacy differences in mice with the similar 4-

substituted tryptamine psilocybin (353). Future studies to address the source of this difference should be undertaken.

The rapid onset of DMT patch F5 is shown through detectable plasma concentrations *in vivo* at the first timepoint of 60 min. Peak plasma concentrations for DMT patch F5 were also detected at the first timepoint at both 1 mg/kg and 5 mg/kg. While initial drug onset and peak plasma concentration may be higher at timepoints prior to 1 h with TDDS, it is unlikely they approach levels seen in IV administration. This is especially true given the distinct lack of HTR response from the transdermal treatment group up through 1 h. There were notable differences between the *in vitro* Franz cell diffusion and the *in vivo* results for patch F5. The patches continued to show continuous delivery of DMT up to 72 h, however, the *in vivo* results showed no detectable DMT after 8 h. Presumably the lack of enzymes to metabolize DMT in the Franz cell led to these prolongated results *in vitro*. Rodents are known to be rapid metabolizers of drugs, often more than humans or pigs, the ideal species for transdermal drug development. In spite of this, Franz cell diffusion represents a good screening tool for transdermal formulation development, but durability of drug effects can only be accurately monitored *in vivo*.

Transdermal delivery is common to overcome the first-pass effect for drugs with low oral bioavailability as is the case with DMT. The plasma AUC for both IV and transdermal DMT were compared to show an apparent 77 ± 10 % bioavailability for DMT patch F5 across 1 and 5 mg/kg doses in males and females. The PK results obtained with DMT patch F5 are notably higher than expected given the lack of permeation enhancers or MAOIs to reduce drug metabolism. This is an incredibly efficient delivery method of DMT with high bioavailability for a drug which is commonly administered therapeutically in non-ideal delivery formats (intravenous, smoked or inhaled, or aqueous phytoid extracts of ayahuasca). It is yet to be seen if the level of drug exposure we achieve relates to DMT's efficacy or if the subjective psychedelic experience, a C_{max} driven effect, leads to enduring therapeutic effects. A 39-day study of non-hallucinogenic 1 mg/kg

subcutaneous DMT doses every third day did provide anxiolytic properties of fear extinction in both male and female Sprague Dawley rats; however, only females benefitted from antidepressant effects via the forced swim test (342). Our dataset also supports the key differences in rodent gender, with notably higher C_{max} and AUCs observed in females at 5 mg/kg.

During the HTR assay, intravenously administered DMT showed an increase in head twitches relative to saline within the first 10 min of drug administration. This is consistent with DMT IV administration leading to hallucinogenic-like activity in rodent models. In contrast, DMT patch F5 did not show significant increases in HTR relative to saline, suggesting that use of TDDS to avoid a rapid rise in plasma and brain DMT concentrations are also sufficient to avoid induction of hallucinogenic-like activity in mice. The average peak plasma concentrations of the 5 mg/kg DMT patch F5 did not exceed 60 ng/mL, a proposed threshold for the psychedelic or hallucinogenic effects of DMT (258). Taken together these characteristics address possible limitations of DMT administration including the opportunity for unsupervised or take-home dosing. Future studies could assess higher doses to see if the HTR could be elicited through TDDS or if permeation-enhanced formulations such as F6 can exceed DMT's psychedelic threshold.

Increased neural activity and dendritic densities are correlated with neuroplasticity, a proposed mechanism of action for DMT and other psychedelic 5-HT_{2A} agonists. Previous studies did not show increased dendritic spine densities with repeated 1 mg/kg doses (342) despite showing similar spontaneous excitatory postsynaptic currents for both 10 mg/kg and 1 mg/kg doses (339). However, a single 10 mg/kg dose did achieve psychedelic effects via HTR and increased spine density. It is still unclear whether lower repeated doses, often referred to as microdosing, can lead to therapeutic effects in animals and humans (354). The screening efforts landed on a novel TDDS formulation which was able to deliver a sustained dose of DMT through *in vitro* and *in vivo* models. This work provides evidence supporting the design and development of a single layer DIA system containing DMT freebase to achieve long-lasting peripheral exposure

to DMT without inducing hallucinogenic-like activity in rodent models, a condition that was achieved at a 5 mg/kg dose. While the results are quite surprising, the physical properties and lipophilicity of DMT make it an attractive drug for TDDS. These results demonstrate the adequacy of DMT as a transdermal drug candidate without using enhancers or techniques such as microneedles to bypass the stratum corneum, the outermost skin layer that can be impermeable for some drugs. Despite the promise of microneedles to aid the delivery of drugs, such as ketamine (316), challenges remain to manufacture these products reproducibly under strict regulatory guidelines, and to date, only a few microneedle transdermal patches have been approved by the FDA. The Franz cell and *in vivo* results of DMT patch F5 clearly validate the non-invasive approach of DMT TDDS.

Psychedelic compounds remain a promising class of drugs for a wide variety of neurological disorders. However, challenges remain to treat large numbers of patients with medical observation and limited options are available for patients unable or unwilling to undergo a psychedelic treatment. New drug delivery methods can reduce peak drug exposures to reduce or eliminate hallucinogenic side effects and improve overall patient compliance. Notably, many neuropsychiatric researchers still debate whether or not subjective effects like hallucinations are necessary for efficacy. For the first time, this research demonstrates a highly effective TDDS for DMT to extend half-life, reduce peak drug concentrations likely to limit hallucinogenic-like effects correlated with a rodent HTR. Further work dosing larger animals and humans will allow a better understanding of the translatability and therapeutic potential of low-dose DMT regimens. Non- or sub-hallucinogenic delivery systems capable of enabling neuroplasticity would expand patient populations beyond psychiatry to include neurodegenerative disorders. Convenient TDDS of DMT could significantly reduce medical burdens and provide new tools for physicians and patient treatments.

4.5 Conclusion:

A low-dose DMT product can offer new solutions for psychiatric and neurological disorders without inpatient dosing and supervision required for higher, psychedelic DMT doses. The primary goal was to demonstrate the viability of TDDS of DMT given general bioavailability concerns and rapid plasma clearance. The study was successful in producing optimized transdermal patch formulations of DMT with high bioavailability. The initial *in vitro* Franz cell diffusion screening provided examples of DMT in several commercially available adhesives and patch materials with increasing drug concentrations and drug fluxes with or without permeation enhancers. The optimized DMT patch F5 had suitable drug loading and flux to advance into *in vivo* studies in mice. Brain concentrations and pharmacokinetics of DMT along with the HTR were monitored to understand PK/PD drug effects with this novel TDDS. The IV half-life of DMT was extended by 20-fold with administration of the TDDS while plasma concentrations did not exceed 60 ng/mL, eliminating the HTR. Research to establish the therapeutic potential and neural effects of this delivery method along with PK profiling in further species will provide better value to this initial discovery of transdermally administered DMT.

Author Contributions

Nathan T. Jones: Investigation, Formal analysis, Data curation. **Investigation**: Conducted the experimental procedures for all plasma and brain pharmacokinetics of intravenously and transdermally administered DMT, ensuring meticulous data collection and adherence to experimental protocols. **Formal Analysis**: Performed the statistical and analytical procedures to interpret the pharmacokinetic data from both plasma and brain samples, applying rigorous methods to derive meaningful insights and conclusions. **Data Curation**: Organized, managed, and maintained the research data related to the pharmacokinetics studies, ensuring it was properly collected, cleaned, stored, and made accessible for analysis. This included handling data from LC-MS/MS protocol optimization and analysis to ensure accurate and reproducible results.

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REFERENCES

1. Anderson BT, Danforth A, Grob CS. Psychedelic medicine: safety and ethical concerns. *Lancet Psychiatry* (2020) 7 10:829–830. https://api.semanticscholar.org/CorpusID:221807015
2. Hill SL, Thomas SHL, Klein AK, Chatha MR, Laskowski LJ, Anderson EI, Brandt SD, Chapman SJ, McCorvy JD, Halberstadt AL, et al. Human hallucinogen research: guidelines for safety. *Drug Test Anal* (2020) 49:457–458. doi: 10.1021/acsptsci.2c00177
3. Family N, Maillet EL, Williams LTJ, Krediet E, Carhart-Harris RL, Williams TM, Nichols CD, Goble DJ, Raz S. Safety, tolerability, pharmacokinetics, and pharmacodynamics of low dose lysergic acid diethylamide (LSD) in healthy older volunteers. *Psychopharmacology (Berl)* (2020) 237:841–853.
4. Mocanu V, Mackay L, Christie D, Argento E. Safety considerations in the evolving legal landscape of psychedelic-assisted psychotherapy. *Subst Abuse Treat Prev Policy* (2022) 17:37.
5. Gasser P, Holstein D, Michel Y, Doblin R, Yazar-Klosinski B, Passie T, Brenneisen R. Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. *J Nerv Ment Dis* (2014) 202:513–520.
6. Gukasyan N, Davis AK, Barrett FS, Cosimano MP, Sepeda ND, Johnson MW, Griffiths RR. Efficacy and safety of psilocybin-assisted treatment for major depressive disorder: Prospective 12-month follow-up. *Journal of Psychopharmacology* (2022) 36:151–158. doi: 10.1177/02698811211073759
7. D'Souza DC, Syed SA, Flynn LT, Safi-Aghdam H, Cozzi N V, Ranganathan M. Exploratory study of the dose-related safety, tolerability, and efficacy of dimethyltryptamine (DMT) in healthy volunteers and major depressive disorder. *Neuropsychopharmacology* (2022) 47:1854–1862.
8. Brown RT, Nicholas CR, Cozzi N V, Gassman MC, Cooper KM, Muller D, Thomas CD, Hetzel SJ, Henriquez KM, Ribaudo AS. Pharmacokinetics of escalating doses of oral psilocybin in healthy adults. *Clin Pharmacokinet* (2017) 56:1543–1554.

9. Hasler F, Grimberg U, Benz MA, Huber T, Vollenweider FX. Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose–effect study. *Psychopharmacology (Berl)* (2004) 172:145–156.

10. Goodwin GM, Aaronson ST, Alvarez O, Arden PC, Baker A, Bennett JC, Bird C, Blom RE, Brennan C, Brusch D. Single-dose psilocybin for a treatment-resistant episode of major depression. *New England Journal of Medicine* (2022) 387:1637–1648.

11. Carhart-Harris RL, Bolstridge M, Day CMJ, Rucker J, Watts R, Erritzoe DE, Kaelen M, Giribaldi B, Bloomfield M, Pilling S. Psilocybin with psychological support for treatment-resistant depression: six-month follow-up. *Psychopharmacology (Berl)* (2018) 235:399–408.

12. Roseman L, Nutt DJ, Carhart-Harris RL. Quality of acute psychedelic experience predicts therapeutic efficacy of psilocybin for treatment-resistant depression. *Front Pharmacol* (2018) 8:309463.

13. Griffiths RR, Richards WA, McCann U, Jesse R. Psilocybin can occasion mystical-type experiences having substantial and sustained personal meaning and spiritual significance. *Psychopharmacology (Berl)* (2006) 187:268–283. doi: 10.1007/s00213-006-0457-5

14. Vogt SB, Ley L, Erne L, Straumann I, Becker AM, Klaiber A, Holze F, Vandersmissen A, Mueller L, Duthaler U. Acute effects of intravenous DMT in a randomized placebo-controlled study in healthy participants. *Transl Psychiatry* (2023) 13:172.

15. Gallimore AR, Strassman RJ. A model for the application of target-controlled intravenous infusion for a prolonged immersive DMT psychedelic experience. *Front Pharmacol* (2016) 7:205781.

16. Lawrence DW, Carhart-Harris R, Griffiths R, Timmermann C. Phenomenology and content of the inhaled N, N-dimethyltryptamine (N, N-DMT) experience. *Sci Rep* (2022) 12:8562.

17. Carbonaro TM, Gatch MB. Neuropharmacology of N, N-dimethyltryptamine. *Brain Res Bull* (2016) 126:74–88.

18. Good M, Joel Z, Benway T, Routledge C, Timmermann C, Erritzoe D, Weaver R, Allen G, Hughes C, Topping H. Pharmacokinetics of N, N-dimethyltryptamine in humans. *Eur J Drug Metab Pharmacokinet* (2023) 48:311–327.

19. Cameron LP, Olson DE. Dark classics in chemical neuroscience: N, N-dimethyltryptamine (DMT). *ACS Chem Neurosci* (2018) 9:2344–2357.

20. Holze F, Becker AM, Kolaczynska KE, Duthaler U, Liechti ME. Pharmacokinetics and pharmacodynamics of oral psilocybin administration in healthy participants. *Clin Pharmacol Ther* (2023) 113:822–831.

21. Holze F, Ley L, Müller F, Becker AM, Straumann I, Vizeli P, Kuehne SS, Roder MA, Duthaler U, Kolaczynska KE. Direct comparison of the acute effects of lysergic acid diethylamide and psilocybin in a double-blind placebo-controlled study in healthy subjects. *Neuropsychopharmacology* (2022) 47:1180–1187.

22. Dolder PC, Schmid Y, Haschke M, Rentsch KM, Liechti ME. Pharmacokinetics and concentration-effect relationship of oral LSD in humans. *International Journal of Neuropsychopharmacology* (2016) 19:pyv072.

23. Good M, Joel Z, Benway T, Routledge C, Timmermann C, Erritzoe D, Weaver R, Allen G, Hughes C, Topping H. Pharmacokinetics of N, N-dimethyltryptamine in humans. *Eur J Drug Metab Pharmacokinet* (2023) 48:311–327.

24. Shen H-W, Jiang X-L, C Winter J, Yu A-M. Psychedelic 5-methoxy-N, N-dimethyltryptamine: metabolism, pharmacokinetics, drug interactions, and pharmacological actions. *Curr Drug Metab* (2010) 11:659–666.

25. Liechti ME, Dolder PC, Schmid Y. Alterations of consciousness and mystical-type experiences after acute LSD in humans. *Psychopharmacology (Berl)* (2017) 234:1499–1510.

26. Schmid Y, Liechti ME. Long-lasting subjective effects of LSD in normal subjects. *Psychopharmacology (Berl)* (2018) 235:535–545.

27. Savage C, Savage E, Fadiman J, Harman W. LSD: Therapeutic effects of the psychedelic experience. *Psychol Rep* (1964) 14:111–120.

28. Gallimore AR, Strassman RJ. A model for the application of target-controlled intravenous infusion for a prolonged immersive DMT psychedelic experience. *Front Pharmacol* (2016) 7:205781.

29. Timmermann C, Roseman L, Williams L, Erritzoe D, Martial C, Cassol H, Laureys S, Nutt D, Carhart-Harris R. DMT models the near-death experience. *Front Psychol* (2018) 9:395026.

30. Shen H-W, Jiang X-L, C Winter J, Yu A-M. Psychedelic 5-methoxy-N, N-dimethyltryptamine: metabolism, pharmacokinetics, drug interactions, and pharmacological actions. *Curr Drug Metab* (2010) 11:659–666.

31. James E, Robertshaw TL, Hoskins M, Sessa B. Psilocybin occasioned mystical-type experiences. *Human Psychopharmacology: Clinical and Experimental* (2020) 35:e2742. doi: https://doi.org/10.1002/hup.2742

32. MacLean KA, Leoutsakos JMS, Johnson MW, Griffiths RR. Factor Analysis of the Mystical Experience Questionnaire: A Study of Experiences Occasioned by the Hallucinogen Psilocybin. *J Sci Study Relig* (2012) 51:721–737. doi: 10.1111/j.1468-5906.2012.01685.x

33. Kometer M, Vollenweider FX. "Serotonergic Hallucinogen-Induced Visual Perceptual Alterations.," In: Halberstadt AL, Vollenweider FX, Nichols DE, editors. *Behavioral Neurobiology of Psychedelic Drugs*. Berlin, Heidelberg: Springer Berlin Heidelberg (2018). p. 257–282 doi: 10.1007/7854_2016_461

34. Carhart-Harris RL, Roseman L, Haijen E, Erritzoe D, Watts R, Branchi I, Kaelen M. Psychedelics and the essential importance of context. *Journal of Psychopharmacology* (2018) 32:725–731. doi: 10.1177/0269881118754710

35. Brouwer A, Carhart-Harris RL. Pivotal mental states. *Journal of Psychopharmacology* (2021) 35:319–352.

36. Koslowski M, Johnson MW, Gründer G, Betzler F. Novel treatment approaches for substance use disorders: therapeutic use of psychedelics and the role of psychotherapy. *Curr Addict Rep* (2021)1–11.

37. Pearson CS, Siegel JS, Gold JA. Psilocybin-assisted psychotherapy for depression: Emerging research on a psychedelic compound with a rich history. *J Neurol Sci* (2021) 434: https://api.semanticscholar.org/CorpusID:245258099

38. Ross S, Agrawal M, Griffiths RR, Grob C, Berger A, Henningfield JE. Psychedelic-assisted psychotherapy to treat psychiatric and existential distress in life-threatening medical

illnesses and palliative care. *Neuropharmacology* (2022) 216:109174. doi: https://doi.org/10.1016/j.neuropharm.2022.109174

39. Schenberg EE. Psychedelic-assisted psychotherapy: a paradigm shift in psychiatric research and development. *Front Pharmacol* (2018) 9:323606.

40. Reiff CM, Richman EE, Nemeroff CB, Carpenter LL, Widge AS, Rodriguez CI, Kalin NH, McDonald WM, Work Group on Biomarkers and Novel Treatments a D of the APAC of R. Psychedelics and psychedelic-assisted psychotherapy. *American Journal of Psychiatry* (2020) 177:391–410.

41. Reiff CM, Richman EE, Nemeroff CB, Carpenter LL, Widge AS, Rodriguez CI, Kalin NH, McDonald WM. Psychedelics and psychedelic-assisted psychotherapy. *American Journal of Psychiatry* (2020) 177:391–410. doi: 10.1176/appi.ajp.2019.19010035

42. McNamee S, Devenot N, Buisson M. Studying harms is key to improving psychedelic-assisted therapy—participants call for changes to research landscape. *JAMA Psychiatry* (2023) 80:411–412.

43. Wolfgang AS, Hoge CW. Psychedelic-Assisted Therapy in Military and Veterans Healthcare Systems: Clinical, Legal, and Implementation Considerations. *Curr Psychiatry Rep* (2023) 25:513–532.

44. McCrone P, Fisher H, Knight C, Harding R, Schlag AK, Nutt DJ, Neill JC. Cost-effectiveness of psilocybin-assisted therapy for severe depression: exploratory findings from a decision analytic model. *Psychol Med* (2023) 53:7619–7626.

45. Marseille E, Bertozzi S, Kahn JG. The economics of psychedelic-assisted therapies: A research agenda. *Front Psychiatry* (2022) 13:1025726.

46. Luoma JB, Chwyl C, Bathje GJ, Davis AK, Lancelotta R. A meta-analysis of placebo-controlled trials of psychedelic-assisted therapy. *J Psychoactive Drugs* (2020) 52:289–299.

47. Hoener S, Wolfgang A, Nissan D, Howe E. Ethical considerations for psychedelic-assisted therapy in military clinical settings. *J Med Ethics* (2024) 50:258–262.

48. Johnston CB, Mangini M, Grob C, Anderson B. The safety and efficacy of psychedelic-assisted therapies for older adults: knowns and unknowns. *The American Journal of Geriatric Psychiatry* (2023) 31:44–53.

49. Bouchet L, Sager Z, Yrondi A, Nigam KB, Anderson BT, Ross S, Petridis PD, Beaussant Y. Older adults in psychedelic-assisted therapy trials: A systematic review. *Journal of Psychopharmacology* (2024)02698811231215420.

50. Beaussant Y, Nigam K. Expending perspectives on the potential for psychedelic-assisted therapies to improve the experience of aging. *The American Journal of Geriatric Psychiatry* (2023) 31:54–57.

51. Davis AK, Levin AW, Nagib PB, Armstrong SB, Lancelotta RL. Study protocol of an open-label proof-of-concept trial examining the safety and clinical efficacy of psilocybin-assisted therapy for veterans with PTSD. *BMJ Open* (2023) 13:e068884.

52. Davis AK, Averill LA, Sepeda ND, Barsuglia JP, Amoroso T. Psychedelic treatment for trauma-related psychological and cognitive impairment among US special operations forces veterans. *Chronic Stress* (2020) 4:2470547020939564.

53. Illingworth BJG, Lewis DJ, Lambarth AT, Stocking K, Duffy JMN, Jelen LA, Rucker JJ. A comparison of MDMA-assisted psychotherapy to non-assisted psychotherapy in treatment-resistant PTSD: A systematic review and meta-analysis. *Journal of psychopharmacology* (2021) 35:501–511.

54. Maia LO, Beaussant Y, Garcia ACM. The therapeutic potential of psychedelic-assisted therapies for symptom control in patients diagnosed with serious illness: a systematic review. *J Pain Symptom Manage* (2022) 63:e725–e738.

55. Iwata N, Ishigooka J, Kim W-H, Yoon B-H, Lin S-K, Sulaiman AH, Cosca R, Wang L, Suchkov Y, Agarkov A. Efficacy and safety of blonanserin transdermal patch in patients with schizophrenia: a 6-week randomized, double-blind, placebo-controlled, multicenter study. *Schizophr Res* (2020) 215:408–415.

56. Citrome L, Walling DP, Zeni CM, Starling BR, Terahara T, Kuriki M, Park AS, Komaroff M. Efficacy and safety of HP-3070, an asenapine transdermal system, in patients with schizophrenia: a phase 3, randomized, placebo-controlled study. *J Clin Psychiatry* (2020) 82:1417.

57. Citrome L, Zeni CM, Correll CU. Patches: established and emerging transdermal treatments in psychiatry. *J Clin Psychiatry* (2019) 80:21174.

58. Carrithers B, El-Mallakh RS. Transdermal asenapine in schizophrenia: a systematic review. *Patient Prefer Adherence* (2020)1541–1551.

59. Frampton JE. Rotigotine transdermal patch: a review in Parkinson's disease. *CNS Drugs* (2019) 33:707–718.

60. Sanford M, Scott LJ. Rotigotine transdermal patch: a review of its use in the treatment of Parkinson's disease. *CNS Drugs* (2011) 25:699–719.

61. Schnitzler A, Leffers K-W, Häck H-J. High compliance with rotigotine transdermal patch in the treatment of idiopathic Parkinson's disease. *Parkinsonism Relat Disord* (2010) 16:513–516.

62. Winblad B, Machado JC. Use of rivastigmine transdermal patch in the treatment of Alzheimer's disease. *Expert Opin Drug Deliv* (2008) 5:1377–1386.

63. Larkin HD. First donepezil transdermal patch approved for Alzheimer disease. *JAMA* (2022) 327:1642.

64. Kurz A, Farlow M, Lefevre G. Pharmacokinetics of a novel transdermal rivastigmine patch for the treatment of Alzheimer's disease: a review. *Int J Clin Pract* (2009) 63:799–805.

65. Lefevre G, Sędek G, Jhee SS, Leibowitz MT, Huang H, Enz A, Maton S, Ereshefsky L, Pommier F, Schmidli H. Pharmacokinetics and pharmacodynamics of the novel daily rivastigmine transdermal patch compared with twice-daily capsules in Alzheimer's disease patients. *Clin Pharmacol Ther* (2008) 83:106–114.

66. Abelin T, Müller P, Buehler A, Vesanen K, Imhof PR. Controlled trial of transdermal nicotine patch in tobacco withdrawal. *The Lancet* (1989) 333:7–10.

67. Mulligan SC, Masterson JG, Devane JG, Kelly JG. Clinical and pharmacokinetic properties of a transdermal nicotine patch. *Clin Pharmacol Ther* (1990) 47:331–337.

68. Davaran S, Rashidi MR, Khandaghi R, Hashemi M. Development of a novel prolonged-release nicotine transdermal patch. *Pharmacol Res* (2005) 51:233–237.

69. HURT RD, LAUGER GG, OFFORD KP, KOTTKE TE, DALE LC. Nicotine-replacement therapy with use of a transdermal nicotine patch—a randomized double-blind placebo-controlled trial. *Mayo Clinic Proceedings*. Elsevier (1990). p. 1529–1537

70. George TP, Ziedonis DM, Feingold A, Pepper WT, Satterburg CA, Winkel J, Rounsaville BJ, Kosten TR. Nicotine transdermal patch and atypical antipsychotic medications for

smoking cessation in schizophrenia. *American Journal of Psychiatry* (2000) 157:1835–1842.

71. Cameron LP, Nazarian A, Olson DE. Psychedelic microdosing: prevalence and subjective effects. *J Psychoactive Drugs* (2020) 52:113–122.

72. Polito V, Stevenson RJ. A systematic study of microdosing psychedelics. *PLoS One* (2019) 14: doi: 10.1371/journal.pone.0211023

73. Polito V, Liknaitzky P. The emerging science of microdosing: A systematic review of research on low dose psychedelics (1955–2021) and recommendations for the field. *Neurosci Biobehav Rev* (2022) 139:104706.

74. Fadiman J, Korb S. Might microdosing psychedelics be safe and beneficial? An initial exploration. *J Psychoactive Drugs* (2019) 51:118–122.

75. Hutten NRPW, Mason NL, Dolder PC, Kuypers KPC. Self-rated effectiveness of microdosing with psychedelics for mental and physical health problems among microdosers. *Front Psychiatry* (2019) 10:481074.

76. Ona G, Bouso JC. Potential safety, benefits, and influence of the placebo effect in microdosing psychedelic drugs: A systematic review. *Neurosci Biobehav Rev* (2020) 119:194–203.

77. Kuypers KPC. The therapeutic potential of microdosing psychedelics in depression. *Ther Adv Psychopharmacol* (2020) 10:2045125320950567.

78. Pastore MN, Kalia YN, Horstmann M, Roberts MS. Transdermal patches: history, development and pharmacology. *Br J Pharmacol* (2015) 172:2179–2209.

79. Citrome L, Zeni CM, Correll CU. Patches: established and emerging transdermal treatments in psychiatry. *J Clin Psychiatry* (2019) 80:21174.

80. Courtenay AJ, McAlister E, McCrudden MTC, Vora L, Steiner L, Levin G, Levy-Nissenbaum E, Shterman N, Kearney M-C, McCarthy HO. Hydrogel-forming microneedle arrays as a therapeutic option for transdermal esketamine delivery. *Journal of controlled release* (2020) 322:177–186.

81. Scheindlin S. Transdermal drug delivery: past, present, future. *Mol Interv* (2004) 4:308.

82. Tanner T, Marks R. Delivering drugs by the transdermal route: review and comment. *Skin Research and Technology* (2008) 14:249–260.

83. Rapoport AM, Freitag F, Pearlman SH. Innovative delivery systems for migraine: the clinical utility of a transdermal patch for the acute treatment of migraine. *CNS Drugs* (2010) 24:929–940.

84. Rifkin BD, Maraver MJ, Colzato LS. Microdosing psychedelics as cognitive and emotional enhancers. *Psychology of Consciousness: Theory, Research, and Practice* (2020) 7:316.

85. Olson DE. Psychoplastogens: a promising class of plasticity-promoting neurotherapeutics. *J Exp Neurosci* (2018) 12:1179069518800508.

86. Nichols DE, Walter H. The history of psychedelics in psychiatry. *Pharmacopsychiatry* (2021) 54:151–166.

87. Goodwin GM, Aaronson ST, Alvarez O, Atli M, Bennett JC, Croal M, DeBattista C, Dunlop BW, Feifel D, Hellerstein DJ, et al. Single-dose psilocybin for a treatment-resistant episode of major depression: Impact on patient-reported depression severity, anxiety, function, and quality of life. *J Affect Disord* (2023) 327:120–127. doi: https://doi.org/10.1016/j.jad.2023.01.108

88. Goodwin G, Aaronson S, Alvarez Bobo O, Arden P, Baker A, Bennett J, Bird C, Blom R, Brennan C, Brusch D, et al. Single-Dose Psilocybin for a Treatment-Resistant Episode of Major Depression. *New England Journal of Medicine* (2022) 387:1637–1648. doi: 10.1056/NEJMoa2206443

89. Palhano-Fontes F, Andrade KC, Tofoli LF, Santos AC, Crippa JAS, Hallak JEC, Ribeiro S, De Araujo DB. The psychedelic state induced by ayahuasca modulates the activity and connectivity of the default mode network. *PLoS One* (2015) 10:e0118143.

90. Turner EH. Esketamine for treatment-resistant depression: seven concerns about efficacy and FDA approval. *Lancet Psychiatry* (2019) 6:977 – 979. doi: 10.1016/S2215-0366(19)30394-3

91. Good M, Joel Z, Benway T, Routledge C, Timmermann C, Erritzoe D, Weaver R, Allen G, Hughes C, Topping H. Pharmacokinetics of N, N-dimethyltryptamine in humans. *Eur J Drug Metab Pharmacokinet* (2023) 48:311–327.

92. Szára St. Dimethyltryptamin: Its metabolism in man; the relation of its psychotic effect to the serotonin metabolism. *Experientia* (1956) 12:441 – 442. doi: 10.1007/BF02157378

93. Turner WJ, Merlis S. Effect of some indolealkylamines on man. *AMA Arch Neurol Psychiatry* (1959) 81:121–129.

94. Frecska E, Bokor P, Winkelman M. The therapeutic potentials of ayahuasca: possible effects against various diseases of civilization. *Front Pharmacol* (2016) 7:35.

95. Palhano-Fontes F, Barreto D, Onias H, Andrade KC, Novaes MM, Pessoa JA, Mota-Rolim SA, Osório FL, Sanches R, dos Santos RG, et al. Rapid antidepressant effects of the psychedelic ayahuasca in treatment-resistant depression: a randomized placebo-controlled trial. *Psychol Med* (2019) 49:655–663. doi: DOI: 10.1017/S0033291718001356

96. Barker SA, Borjigin J, Lomnicka I, Strassman R. LC/MS/MS analysis of the endogenous dimethyltryptamine hallucinogens, their precursors, and major metabolites in rat pineal gland microdialysate. *Biomedical Chromatography* (2013) 27:1690–1700.

97. Barker SA, McIlhenny EH, Strassman R. A critical review of reports of endogenous psychedelic N, N-dimethyltryptamines in humans: 1955–2010. *Drug Test Anal* (2012) 4:617–635.

98. Vargas M V, Dunlap LE, Dong C, Carter SJ, Tombari RJ, Jami SA, Cameron LP, Patel SD, Hennessey JJ, Saeger HN, et al. Psychedelics promote neuroplasticity through the activation of intracellular 5-HT2A receptors. *Science (1979)* (2023) 379:700 – 706. doi: 10.1126/science.adf0435

99. James E, Erritzoe D, Benway T, Joel Z, Timmermann C, Good M, Agnorelli C, Weiss BM, Barba T, Campbell G. Safety, tolerability, pharmacodynamic and wellbeing effects of SPL026 (dimethyltryptamine fumarate) in healthy participants: a randomized, placebo-controlled phase 1 trial. *Front Psychiatry* (2024) 14:1305796.

100. Strassman RJ, Qualls CR. Dose-Response Study of N, N-Dimethyltryptamine in Humans: I. Neuroendocrine, Autonomic, and Cardiovascular Effects. *Arch Gen Psychiatry* (1994) 51:85 – 97. doi: 10.1001/archpsyc.1994.03950020009001

101. Thomas G, Lucas P, Capler NR, Tupper KW, Martin G. Ayahuasca-assisted therapy for addiction: Results from a preliminary observational study in Canada. *Curr Drug Abuse Rev* (2013) 6:30 – 42. doi: 10.2174/15733998113099990003

102. Canal CE, Morgan D. 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) 4:556–576.

103. Ly C, Greb AC, Cameron LP, Wong JM, Barragan E V, Wilson PC, Burbach KF, Zarandi SS, Sood A, Paddy MR. Psychedelics promote structural and functional neural plasticity. *Cell Rep* (2018) 23:3170–3182.

104. Nardai S, László M, Szabó A, Alpár A, Hanics J, Zahola P, Merkely B, Frecska E, Nagy Z. N, N-dimethyltryptamine reduces infarct size and improves functional recovery following transient focal brain ischemia in rats. *Exp Neurol* (2020) 327:113245.

105. Szabó Í, Varga VÉ, Dvorácskó S, Farkas AE, Körmöczi T, Berkecz R, Kecskés S, Menyhárt Á, Frank R, Hantosi D, et al. N,N-Dimethyltryptamine attenuates spreading depolarization and restrains neurodegeneration by sigma-1 receptor activation in the ischemic rat brain. *Neuropharmacology* (2021) 192: doi: 10.1016/j.neuropharm.2021.108612

106. Cameron LP, Benson CJ, DeFelice BC, Fiehn O, Olson DE. Chronic, intermittent microdoses of the psychedelic N, N-dimethyltryptamine (DMT) produce positive effects on mood and anxiety in rodents. *ACS Chem Neurosci* (2019) 10:3261–3270.

107. Cheng D, Lei Z-G, Chu K, Lam OJH, Chiang CY, Zhang Z-J. N, N-Dimethyltryptamine, a natural hallucinogen, ameliorates Alzheimer's disease by restoring neuronal Sigma-1 receptor-mediated endoplasmic reticulum-mitochondria crosstalk. *Alzheimers Res Ther* (2024) 16:95.

108. Morales-Garcia JA, Calleja-Conde J, Lopez-Moreno JA, Alonso-Gil S, Sanz-SanCristobal M, Riba J, Perez-Castillo A. N, N-dimethyltryptamine compound found in the hallucinogenic tea ayahuasca, regulates adult neurogenesis in vitro and in vivo. *Transl Psychiatry* (2020) 10:331.

109. Kiilerich KF, Lorenz J, Scharff MB, Speth N, Brandt TG, Czurylo J, Xiong M, Jessen NS, Casado-Sainz A, Shalgunov V. Repeated low doses of psilocybin increase resilience to stress, lower compulsive actions, and strengthen cortical connections to the paraventricular thalamic nucleus in rats. *Mol Psychiatry* (2023) 28:3829–3841.

110. Bodkin JA, Amsterdam JD. Transdermal selegiline in major depression: a double-blind, placebo-controlled, parallel-group study in outpatients. *American Journal of Psychiatry* (2002) 159:1869–1875.

111. El-Tokhy FS, Abdel-Mottaleb MMA, El-Ghany EA, Geneidi AS. Transdermal delivery of second-generation antipsychotics for management of schizophrenia; disease overview, conventional and nanobased drug delivery systems. *J Drug Deliv Sci Technol* (2021) 61:102104.

112. Jiménez JH, Bouso JC. Significance of mammalian N, N-dimethyltryptamine (DMT): A 60-year-old debate. *Journal of Psychopharmacology* (2022) 36:905–919.

113. Cozzi N V, Daley PF. Synthesis and characterization of high-purity N, N-dimethyltryptamine hemifumarate for human clinical trials. *Drug Test Anal* (2020) 12:1483–1493.

114. Haq A, Goodyear B, Ameen D, Joshi V, Michniak-Kohn B. Strat-M® synthetic membrane: Permeability comparison to human cadaver skin. *Int J Pharm* (2018) 547:432–437.

115. McIlhenny EH, Riba J, Barbanoj MJ, Strassman R, Barker SA. Methodology for and the determination of the major constituents and metabolites of the Amazonian botanical medicine ayahuasca in human urine. *Biomedical Chromatography* (2011) 25:970–984.

116. Jones NT, Zahid Z, Grady SM, Sultan ZW, Zheng Z, Razidlo J, Banks MI, Wenthur CJ. Transient elevation of plasma glucocorticoids supports psilocybin-induced anxiolysis in mice. *ACS Pharmacol Transl Sci* (2023) 6:1221–1231.

117. Lane ME. Skin penetration enhancers. *Int J Pharm* (2013) 447:12–21.

118. Alper K, Cange J, Sah R, Schreiber-Gregory D, Sershen H, Vinod KY. Psilocybin sex-dependently reduces alcohol consumption in C57BL/6J mice. *Front Pharmacol* (2023) 13:1074633.

119. Polito V, Stevenson RJ. A systematic study of microdosing psychedelics. *PLoS One* (2019) 14: doi: 10.1371/journal.pone.0211023

Chapter 5: Conclusions and Future Directions

5.1 Psilocybin-Induced Stress Response: Elucidating the Role of CORT Signaling and Implications for Future Clinical Applications

In Chapter 2, and my first author publication, the study provides strong evidence that psilocybin-induced transient elevations in plasma glucocorticoids, more specifically corticosterone (CORT), play a crucial role in mediating both the post-acute and long-term anxiolytic effects of psilocybin in mice (200). Our findings show that administering psilocybin significantly increases plasma CORT levels. This increase is associated with reduced anxiety-like behaviors in the novelty-suppressed feeding (NSF) assay, assessed 4 hours post-injection. Notably, the anxiolytic effects observed 4 hours post-administration were reduced when glucocorticoid receptor activity was blocked or when CORT levels were suppressed by chronic oral CORT in drinking water or an acute bolus of CORT. This indicates that CORT release is a critical factor driving reductions in anxiety-like behavior in mice in the hours following drug clearance.

Additionally, chronic elevations in plasma glucocorticoids negated the long-term anxiolytic effects of psilocybin, underscoring the importance of a transient increase in CORT and the acute stress response for sustaining these benefits. Our research suggests that the mechanisms underlying psilocybin's therapeutic effects are intricately linked with the body's stress response system, specifically the hypothalamic-pituitary-adrenal (HPA) axis. The induction of a transient glucocorticoid surge appears to be a critical factor for the observed anxiolysis, supporting the hypothesis that psilocybin and similar serotonergic psychedelics may exert their therapeutic effects through modulation of the body's stress response (200). These findings point to HPA axis activation and changes in glucocorticoid and CORT concentration profiles over time as important translational factors for mechanistic consideration when investigating classical serotonergic psychedelic-assisted therapy for the treatment of psychiatric disorders. Given these insights, the

implications of these findings are multifaceted and pave the way for several future research directions. This research presents an exciting frontier in the quest for innovative treatments for stress-related disorders. A deeper understanding of this interaction can pave the way for more targeted and effective interventions, offering hope to countless individuals struggling with conditions like major depression, PTSD and generalized anxiety disorders.

In this context, Brouwer and Carhart-Harris (2021) introduce the concept of Pivotal Mental States (PiMS) as a groundbreaking construct for understanding rapid and profound psychological transformations. These states are described as hyper-plastic, meaning they are highly adaptable and conducive to learning, particularly under significant environmental pressures and stressors. The PiMS model suggests that chronic stress acts as a primer, preparing the brain for these states, while acute stress serves as a trigger (147). This interplay between stressors is crucial in facilitating psychological changes that can lead to either wellness or pathology.

Central to the PiMS model is the role of the serotonin 2A receptor (5-HT_{2A}R). Chronic stress is known to upregulate this receptor, enhancing its availability in the brain, while acute stress triggers serotonin release, activating this primed system (24,146,147,164,165,334). Psychedelic substances, which act as agonists of the 5-HT_{2A}R, can reliably induce PiMS. These drugs essentially hijack a natural mechanism that has evolved to facilitate significant psychological shifts when necessary.

PiMS are characterized by three key features: elevated cortical plasticity, an enhanced rate of associative learning, and a heightened capacity to mediate psychological transformation. These attributes make PiMS a powerful state for inducing change, but they also highlight the importance of the context in which these states occur. The outcomes of PiMS are highly dependent on both the immediate and broader environmental and psychological context (147). Properly managed, PiMS can lead to profound positive changes, but if mishandled, they can result in severe psychological distress or disorders.

The social and psychological context plays a critical role in determining the outcome of PiMS. Factors such as social isolation, feelings of defeat, and disconnection are significant in priming individuals for these states. When these contexts are managed therapeutically, such as through supportive environments and guided experiences, the potential for positive transformation increases (147). This understanding has significant implications for therapeutic practices, particularly in the realm of psychedelic therapy. Psychedelic therapy is viewed as a prototypical PiMS-focused intervention. By using controlled psychedelic experiences, therapists can facilitate psychological breakthroughs that might not be achievable through conventional methods. The success of such therapies underscores the importance of context, including the therapeutic setting, the intentions of the patient, and the integration of the experience into the patient's life. Proper contextual engineering can help guide the psychological changes induced by PiMS towards wellness rather than pathology.

From an evolutionary perspective, PiMS are seen as an adaptation designed to facilitate major psychological changes when necessary for survival. This model helps explain why these states can lead to such divergent outcomes, depending on the context. By understanding the biological and psychological mechanisms underlying PiMS, researchers and therapists can better harness these states for therapeutic purposes. Moreover, the PiMS model provides a comprehensive framework for understanding significant psychological transformations. It emphasizes the interplay between chronic and acute stress, the central role of the 5-HT_{2A}R, and the critical importance of context in shaping outcomes. This model not only enhances our understanding of psychological change but also offers practical insights for therapeutic interventions, particularly in the emerging field of psychedelic therapy.

In light of this PiMS model and the resulting insights, we can explore a couple of studies conducted after the publication of Chapter 1. These studies further investigate the role of acute physiological responses induced by psychedelic drugs and their increased potential contribution

to therapeutic efficacy. As we inquire further into this research, another critical question arises: whether the subjective experience of psychedelic drugs can occur without the stress response and, if absent, whether the experience retains its lasting effects.

This evidence of memory enhancement aligns with investigations into stress modulation through pharmacological interventions. For instance, the comprehensive study by Mason *et al.* (2023) examines the influence of psilocybin on the body's stress response, specifically focusing on its effect on the cortisol response to psychosocial stress in humans (170). The study observed a significant blunting of cortisol response to psychosocial stress in participants who received psilocybin compared to those who received a placebo. Typically, cortisol, a stress hormone released by the adrenal glands, spikes in response to stress as part of the body's fight-or-flight mechanism. The reduced cortisol response in the psilocybin group suggests a dampened stress response, indicative of a less reactive HPA axis (170). The study's findings suggest that psilocybin may recalibrate or suppress the activity of the HPA axis. This modulation potentially reflects a more balanced physiological response to stress, which could have implications for individuals with stress-related disorders. The study highlights that the modulation of the stress response by psilocybin is not merely transient but persists over time. This persistent effect suggests a long-term recalibration of the body's response to stress.

Building on the therapeutic insights gained from classical serotonergic psychedelics, such as psilocybin, the study of non-psychedelic substances that also influence stress responses, like ketamine, presents a fascinating parallel. Ketamine, a compound initially synthesized in 1962 and widely used as an anesthetic (355,356), has gained significant attention for its pharmacological versatility and therapeutic potential, particularly in psychiatry (357–362). Ketamine, recognized for its quick-acting antidepressant properties, acts as an NMDA receptor antagonist and is associated with elevated serum cortisol levels, suggesting it activates the HPA axis (363–369). This connection between ketamine and the HPA axis is particularly significant given that chronic

stress is widely recognized as a significant risk factor for depression, primarily through the HPA axis, which orchestrates the body's hormonal response to stress (163,189,370–373).

Building on this understanding, it is important to note that research has shown that individuals vary in their resilience or susceptibility to depression following stress, a process not entirely understood but linked to HPA axis activity (190,374,375). In a notable study on chronic social defeat stress (CSDS) in mice, an increase in plasma corticosterone (CORT) levels was observed shortly after exposure to stress, serving as a predictor of susceptibility to depression after prolonged stress. Mice classified as susceptible maintained higher CORT levels compared to their resilient counterparts, underscoring the role of this hormone in stress-related disorders. Interventions such as administering CORT directly or blocking glucocorticoid receptors (GR) with mifepristone altered susceptibility, with mifepristone notably reducing it. Furthermore, treatment with a single dose of ketamine not only countered depressive-like behaviors but also normalized CORT levels and restored GR function in the hippocampus, highlighting its potential for rapid therapeutic effects by modulating the HPA axis (159).

To further expand on these findings, another investigation using Wistar-Kyoto (WKY) rats examined the CORT response to ketamine administered at different times of the day. Employing an automated blood sampling system, researchers tracked CORT levels every 10 minutes for 28 hours post-ketamine infusion during both active and inactive phases. The findings indicated that ketamine consistently increased CORT levels regardless of the timing of administration, with a more significant, dose-dependent increase during the inactive phase. These results not only reinforce the understanding of ketamine’s rapid influence on the HPA axis but also suggest that the timing of ketamine administration could be crucial for optimizing its glucocorticoid-mediated effects in the treatment of depression (376).

The groundbreaking research by Georgiou *et al.* (2022) adds a novel dimension to our understanding of ketamine's pharmacological impact on depression by exploring the interaction

between the drug's effects and the HPA axis, specifically focusing on corticotropin-releasing factor (CRF) modulation in response to acute stress. The research highlights that the presence of male experimenters induces heightened stress in mice through the activation of CRF pathways in the entorhinal cortex, which in turn affects the behavioral and neural responses to ketamine. Intriguingly, this sex-dependent modulation of CRF can influence the antidepressant efficacy of ketamine. Through plasma corticosterone measurements, they found that exposure to male experimenters before ketamine administration significantly affected the CORT levels and the drug's antidepressant impact. This suggests a complex interplay where the environmental context (sex) interacts with pharmacological treatment (ketamine), mediated by acute stress responses involving CRF activation (377,378).

Moreover, these results reinforce the understanding of ketamine’s rapid influence on the HPA axis but also suggest that the timing of ketamine administration could be crucial for optimizing its glucocorticoid-mediated effects in the treatment of depression. The findings imply that not only are the neural and hormonal responses crucial in understanding ketamine's effects but also the conditions under which treatments are administered, thereby providing a nuanced perspective on how environmental factors can affect pharmacological outcomes. These research findings support and emphasize the importance of considering various environmental variables in preclinical studies to enhance the reproducibility and interpretation of pharmacological effects.

This emphasis on environmental factors is crucial because the complex interplay between psychedelic drugs, stress responses, and therapeutic outcomes offers a compelling avenue for psychiatric research and treatment strategies. Empirical evidence suggests that the stress induced by psychedelic drugs, notably through heightened physiological responses, may enhance memory encoding and the emotional impact of the experiences they induce, suggesting a potential mechanism through which these substances exert their lasting therapeutic effects. Moreover, parallels are drawn with non-psychedelic drugs like ketamine, which also modulate the

HPA axis but through different pharmacological pathways, underscoring the potential for diverse approaches in treating mood disorders. The modulation of stress responses, whether through psychedelics or alternatives like ketamine, represents a promising strategy in psychotherapy, potentially broadening the scope of effective treatments. Integrating neurobiological insights with clinical practice deepens our understanding of drug impacts on brain function. It paves the way for innovative treatments considering individual stress responses and environmental factors, ensuring more personalized and effective therapeutic interventions.

Further investigation is warranted to investigate and delineate the precise molecular and cellular mechanisms through which glucocorticoid signaling facilitates the anxiolytic effects of psilocybin. This could involve examining the role of specific glucocorticoid receptors in different brain regions and their interaction with serotonergic systems. Detailed studies could map the distribution and activity of these receptors in the hippocampus, amygdala, and prefrontal cortex, regions known to be critical in stress and anxiety responses. Additionally, investigating the downstream signaling pathways activated by glucocorticoid receptors and their cross-talk with serotonergic signaling can reveal more about the molecular underpinnings of psilocybin's effects.

Building on the need for mechanistic insights, our results highlight the potential of using glucocorticoid levels as biomarkers to predict and possibly enhance the therapeutic efficacy of psilocybin in clinical settings. Monitoring cortisol levels before, during, and after psilocybin administration could provide valuable insights into individual responses to treatment, helping identify patients more likely to benefit from the therapy. Future clinical trials could incorporate these measurements to tailor and optimize psilocybin-assisted therapies for anxiety and other stress-related disorders, potentially adjusting dosages and administration protocols based on cortisol responses to maximize therapeutic outcomes.

The development of biomarkers is crucial for advancing psilocybin research, but it is equally important to consider the context of 'set and setting' in psychedelic experiences. 'Set'

refers to the mental state and expectations of the individual, while 'setting' encompasses the physical and social environment in which the experience occurs. Given the profound impact these factors have on the outcomes of psychedelic therapy, future research needs to delve deeper into how various environmental stressors and psychological states, both before and during psilocybin administration, affect glucocorticoid responses and overall therapeutic results. To achieve a comprehensive understanding, studies should investigate specific environmental stressors such as noise, lighting, interpersonal interactions, and individual psychological conditions like anxiety, mood, and mindset. By examining how these variables influence the body's glucocorticoid responses—hormones critical in stress regulation—researchers can better grasp the complex interplay between the external environment, internal psychological state, and biological mechanisms activated by psilocybin.

Moreover, this understanding could lead to the refinement of therapeutic protocols. For instance, identifying optimal environmental conditions could involve creating a calm, supportive setting with controlled lighting and soothing music. Additionally, ensuring psychological support through pre-session counseling, real-time guidance during the session, and post-session integration could further enhance therapeutic outcomes. By tailoring these factors, therapists can maximize the therapeutic benefits of psilocybin while reducing the risk of adverse effects. This holistic approach not only emphasizes the importance of biomarkers but also recognizes the critical role of 'set and setting' in the efficacy of psychedelic treatments. Ultimately, such comprehensive research could pave the way for standardized, evidence-based protocols that are personalized to each individual's needs, thereby improving the safety and effectiveness of psilocybin-assisted therapies.

Furthermore, expanding the scope of our research, comparative studies involving other psychedelics such as LSD, DMT, and mescaline, which also influence the HPA axis, could provide broader insights into the generalizability of our findings across different substances. These studies

could examine whether similar glucocorticoid-mediated mechanisms underlie the anxiolytic effects of these psychedelics, helping to identify common therapeutic pathways. Understanding the commonalities and differences in their mechanisms of action could inform the development of more targeted and effective treatments, potentially leading to personalized therapeutic strategies based on individual neuroendocrine profiles.

To ensure the long-term safety and efficacy of these treatments, long-term studies are needed to assess the sustainability of psilocybin's anxiolytic effects and the role of glucocorticoids in maintaining these effects over extended periods. Such studies could investigate the persistence of therapeutic benefits and potential side effects associated with repeated or prolonged use of psilocybin. They could also explore how chronic alterations in glucocorticoid levels impact long-term outcomes, providing a comprehensive understanding of the balance between therapeutic efficacy and safety. Moreover, this study underscores the critical interplay between psilocybin and the body's glucocorticoid response in mediating its anxiolytic effects. By advancing our understanding of these mechanisms, we can better harness the therapeutic potential of psilocybin and other psychedelics for treating anxiety and related disorders. We have identified and provided a novel and potentially critical underlying mechanism of action for psilocybin. This not only broadens our scientific perspective but may also lead to more effective and even tailored therapeutic strategies for psychiatric disorders.

5.2 *In Vivo* Validation of Psilacetin as a Prodrug: Implications for Psychedelic Research and Future Clinical Applications

In Chapter 3, my second first-author publication, this study presents the first direct evidence supporting the hypothesis that psilacetin (O-acetyl psilocin) acts as a prodrug for psilocin *in vivo* (209). Our findings demonstrate that, when administered to C57Bl6/J mice, psilacetin leads

to robust metabolic conversion to psilocin, albeit with lower peripheral psilocin exposure compared to psilocybin on an equimolar basis. Specifically, psilacetin resulted in approximately 70% of the psilocin exposure observed with psilocybin (209). This confirms that psilacetin can be a viable alternative to psilocybin in pre-clinical research settings, offering the potential for more straightforward regulatory approval and broader accessibility for researchers.

The validation of psilacetin as a prodrug opens new avenues for pre-clinical psychedelic research. Its status as an unscheduled compound simplifies access and reduces regulatory barriers, facilitating broader and more rapid exploration of psilocin's mechanisms of action and therapeutic potential. Consequently, researchers can conduct more extensive and diverse studies without the complications associated with Schedule I substances. This advantage is crucial for accelerating the pace of discovery and understanding in the field of psychedelic science.

Building on this, future studies should focus on the comprehensive pharmacokinetic and pharmacodynamic profiling of psilacetin across different species, including humans. This will help understand the exposure of the central nervous system and the intrinsic pharmacological activity of psilacetin alongside its metabolite, psilocin. Such detailed profiles are crucial for effectively translating pre-clinical findings into clinical settings. Understanding these dynamics will ensure that researchers can optimize dosing regimens and maximize therapeutic efficacy.

Moreover, direct comparisons of psilacetin and psilocybin in clinical settings could offer valuable insights into their relative efficacies, safety profiles, and potential side effects. The subtle molecular differences between these compounds may lead to variations in their pharmacodynamics and pharmacokinetics, potentially influencing their therapeutic outcomes and side effect profiles. By conducting rigorous comparative studies, researchers can systematically evaluate the relative strengths and weaknesses of psilacetin and psilocybin. These studies should focus on various dimensions, including onset and duration of action, subjective experience, physiological responses, and long-term therapeutic effects. Such research will help determine if

psilacetin can match or surpass psilocybin in terms of efficacy for treating conditions like depression, anxiety, PTSD, and other psychiatric disorders.

Furthermore, this study's findings encourage exploring other novel psilocin prodrugs that might offer improved pharmacokinetic profiles, reduced side effects, or simpler synthetic pathways. By identifying and developing these alternatives, the therapeutic utility and accessibility of psychedelic treatments can be significantly enhanced. This continuous development is essential for addressing diverse patient needs and optimizing therapeutic outcomes. The search for better alternatives is a key driver of innovation in psychedelic therapy. In addition, the use of psilacetin and other synthetic alternatives to naturally derived psilocybin may alleviate ethical and sustainability concerns associated with the commercial exploitation of Psilocybe mushrooms. Researchers and clinicians can respect indigenous cultural practices and contribute to biodiversity conservation by opting for synthetic compounds. This approach aligns with the growing emphasis on ethical and sustainable research practices. The shift towards synthetic alternatives represents a responsible and forward-thinking strategy. By focusing on synthetic compounds like psilacetin, we can reduce reliance on natural sources, thereby promoting sustainability and ensuring a consistent supply for research and therapeutic use.

Finally, extensive research on the safety, efficacy, and optimal dosing regimens of psilacetin in human subjects is needed for clinical translation. Such research is crucial for validating psilacetin as a viable therapeutic option and for developing safe and effective treatment protocols. Additionally, the regulatory framework should evolve to accommodate the unique characteristics of synthetic psychedelics, promoting safe and effective use in medical settings. This regulatory evolution will be crucial for integrating psychedelic therapies into mainstream medical practice. Ensuring regulatory clarity and support will pave the way for broader acceptance and utilization of these therapies. This study not only substantiates the prodrug status of psilacetin but also highlights its potential as a research and therapeutic tool. By reducing regulatory hurdles

and expanding scientific understanding, psilacetin can significantly contribute to the advancement of psychedelic science and its application in mental health treatment. The findings underscore the importance of continued research and development in this promising field. These advancements represent a pivotal step towards unlocking the full therapeutic potential of psychedelics.

5.3 Transdermal Delivery of DMT: Advancements and Future Implications

In Chapter 4, and my third publication as co-second author, the development of a transdermal drug delivery system (TDDS) for N, N-dimethyltryptamine (DMT) represents a significant advancement in the field of psychedelic medicine (379). Our study successfully formulated and tested an extended-release transdermal patch (F5), demonstrating its capability to provide consistent, sub-hallucinogenic plasma concentrations of DMT over an extended period. This method of delivery addresses the challenges associated with rapid metabolic degradation and the need for invasive intravenous administration, making DMT therapy more accessible and potentially safer for outpatient use.

Moreover, the F5 formulation, with a bioavailability of 77% compared to intravenous administration, achieved a 20-fold extension in DMT half-life, maintaining plasma concentrations below 60 ng/mL. This concentration is crucial as it is below the threshold known to induce hallucinogenic effects, as confirmed by the translational head twitch response (HTR) assay in mice. Consequently, the ability to maintain therapeutic levels without hallucinogenic side effects opens new avenues for treating conditions like depression, anxiety, and potentially neurodegenerative disorders with fewer risks and lower medical supervision (379). This innovation not only broadens the potential applications of DMT but also makes it a more viable option for a broader range of patients, enhancing its appeal as a versatile therapeutic agent.

Given these promising results, several future directions and implications emerge. The next logical step is to conduct clinical trials to evaluate the efficacy, safety, and pharmacokinetics of the DMT transdermal patch in human subjects. These trials will be critical in validating the pre-clinical findings and ensuring that the benefits observed in early studies translate effectively to clinical settings. By delving into these aspects, researchers aim to establish a solid foundation for the safe and effective use of DMT in medical settings. Furthermore, beyond psychiatric disorders, the non-hallucinogenic dosing strategy could be explored for neuroprotective roles in conditions such as stroke and traumatic brain injury, as suggested by preliminary studies. This expansion of potential applications signifies a broader horizon for DMT, illustrating its versatile therapeutic promise beyond its traditional use. This expansion into neuroprotective applications could lead to significant breakthroughs in treating various neurological conditions, highlighting the versatility of DMT therapy. The potential to aid in recovery from stroke and traumatic brain injury underscores the importance of continuing this line of research, which could revolutionize current treatment paradigms.

Moreover, the observed variations in drug absorption and efficacy between males and females indicate a need for personalized approaches in dosing and administration. Recognizing and addressing these differences is crucial for optimizing therapeutic outcomes and ensuring patients receive the most effective and safe treatment tailored to their specific needs. Therefore, further research should focus on optimizing the formulation for different demographics to ensure maximum efficacy and safety for all patients, enhancing the therapeutic outcomes of DMT treatment. Tailoring these formulations to accommodate various physiological differences will be a key step in advancing personalized medicine and maximizing the benefits of DMT therapy for a broader patient population. Additionally, future research could explore the incorporation of permeation enhancers and alternative adhesives to improve the efficiency and consistency of drug delivery. Innovations in these areas could lead to more reliable and effective administration

methods, further solidifying the role of DMT in modern medical treatments. By investigating the potential of combining DMT with other therapeutic agents in a single patch, the treatment outcomes and patient convenience could be significantly enhanced, offering a more comprehensive approach to therapy. This integrated strategy could streamline treatment regimens, making it easier for patients to adhere to their therapies and potentially improving overall efficacy.

Lastly, with the growing interest in psychedelics for therapeutic use, establishing regulatory pathways will be essential to ensure that safe and effective products reach the market. Creating clear guidelines and standards will be crucial for successfully integrating these innovative treatments into mainstream medicine, ensuring patient safety, and fostering trust in these new therapeutic options. Collaboration with regulatory bodies will be crucial to navigate the approval process for this novel delivery system, ensuring it meets all safety and efficacy standards and facilitating its acceptance and use in mainstream medical practice. By addressing these future directions, the transdermal delivery system for DMT could significantly impact the field of mental health and beyond. This innovative approach provides a new, non-hallucinogenic treatment modality that aligns with the evolving landscape of psychedelic therapy, potentially offering significant benefits to a wide range of patients.

REFERENCES

1. Jones NT, Zahid Z, Grady SM, Sultan ZW, Zheng Z, Razidlo J, Banks MI, Wenthur CJ. Transient elevation of plasma glucocorticoids supports psilocybin-induced anxiolysis in mice. *ACS Pharmacol Transl Sci* (2023) 6:1221–1231.
2. Brouwer A, Carhart-Harris RL. Pivotal mental states. *Journal of Psychopharmacology* (2021) 35:319–352.
3. Murnane KS. Serotonin 2A receptors are a stress response system: implications for post-traumatic stress disorder. *Behavioural pharmacology* (2019) 30:151–162.
4. Calder AE, Hasler G. Towards an understanding of psychedelic-induced neuroplasticity. *Neuropsychopharmacology* (2023) 48:104–112. doi: 10.1038/s41386-022-01389-z
5. Aleksandrova LR, Phillips AG. Neuroplasticity as a convergent mechanism of ketamine and classical psychedelics. *Trends Pharmacol Sci* (2021) 42:929–942. doi: 10.1016/j.tips.2021.08.003
6. Vargas M V, Dunlap LE, Dong C, Carter SJ, Tombari RJ, Jami SA, Cameron LP, Patel SD, Hennessey JJ, Saeger HN, et al. Psychedelics promote neuroplasticity through the activation of intracellular 5-HT2A receptors. *Science (1979)* (2023) 379:700 – 706. doi: 10.1126/science.adf0435
7. Banks MI, Zahid Z, Jones NT, Sultan ZW, Wenthur CJ. Catalysts for change: the cellular neurobiology of psychedelics. *Mol Biol Cell* (2021) 32:1135–1144. doi: 10.1091/mbc.e20-05-0340
8. Mason NL, Szabo A, Kuypers KPC, Mallaroni PA, de la Torre Fornell R, Reckweg JT, Tse DHY, Hutten NRPW, Feilding A, Ramaekers JG. Psilocybin induces acute and persisting alterations in immune status in healthy volunteers: An experimental, placebo-controlled study. *Brain Behav Immun* (2023) 114:299–310. doi: https://doi.org/10.1016/j.bbi.2023.09.004
9. Li L, Vlisides PE. Ketamine: 50 years of modulating the mind. *Front Hum Neurosci* (2016) 10:612.
10. Mion G. History of anaesthesia: The ketamine story–past, present and future. *European Journal of Anaesthesiologyl EJA* (2017) 34:571–575.

11. Aan Het Rot M, Zarate Jr CA, Charney DS, Mathew SJ. Ketamine for depression: where do we go from here? *Biol Psychiatry* (2012) 72:537–547.

12. Corriger A, Pickering G. Ketamine and depression: a narrative review. *Drug Des Devel Ther* (2019)3051–3067.

13. Wan L-B, Levitch CF, Perez AM, Brallier JW, Iosifescu D V, Chang LC, Foulkes A, Mathew SJ, Charney DS, Murrough JW. Ketamine safety and tolerability in clinical trials for treatment-resistant depression. *J Clin Psychiatry* (2014) 76:10121.

14. Schwartz J, Murrough JW, Iosifescu D V. Ketamine for treatment-resistant depression: recent developments and clinical applications. *BMJ Ment Health* (2016) 19:35–38.

15. Oliver PA, Snyder AD, Feinn R, Malov S, McDiarmid G, Arias AJ. Clinical effectiveness of intravenous racemic ketamine infusions in a large community sample of patients with treatment-resistant depression, suicidal ideation, and generalized anxiety symptoms: a retrospective chart review. *J Clin Psychiatry* (2022) 83:42811.

16. Idvall J, Ahlgren I, Aronsen KF, Stenberg P. Ketamine infusions: pharmacokinetics and clinical effects. *Br J Anaesth* (1979) 51:1167–1173.

17. Johnston CJ, Fitzgerald PJ, Gewarges JS, Watson BO, Spencer-Segal JL. Ketamine decreases HPA axis reactivity to a novel stressor in male but not female mice. *bioRxiv* (2021)2021–2026.

18. Dutton M, Can AT, Lagopoulos J, Hermens DF. Stress, mental disorder and ketamine as a novel, rapid acting treatment. *European Neuropsychopharmacology* (2022) 65:15–29.

19. Besnier E, Clavier T, Tonon M-C, Selim J, Lefevre-Scelles A, Morin F, Tamion F, Dureuil B, Castel H, Compere V. Ketamine and etomidate down-regulate the hypothalamic–pituitary–adrenal axis in an endotoxemic mouse model. *Anesthesiology* (2017) 127:347–354.

20. Garcia LSB, Comim CM, Valvassori SS, Réus GZ, Stertz L, Kapczinski F, Gavioli EC, Quevedo J. Ketamine treatment reverses behavioral and physiological alterations induced by chronic mild stress in rats. *Prog Neuropsychopharmacol Biol Psychiatry* (2009) 33:450–455.

21. Wang W, Liu L, Yang X, Gao H, Tang Q-K, Yin L-Y, Yin X-Y, Hao J-R, Geng D-Q, Gao C. Ketamine improved depressive-like behaviors via hippocampal glucocorticoid receptor in chronic stress induced-susceptible mice. *Behavioural brain research* (2019) 364:75–84.

22. Birnie MT, Eapen A V, Kershaw YM, Lodge D, Collingridge GL, Conway-Campbell BL, Lightman SL. Time of day influences stress hormone response to ketamine. *J Neuroendocrinol* (2022) 34:e13194.

23. Khalili-Mahani N, Martini CH, Olofsen E, Dahan A, Niesters M. Effect of subanaesthetic ketamine on plasma and saliva cortisol secretion. *Br J Anaesth* (2015) 115:68–75.

24. Pittenger C, Duman RS. Stress, Depression, and Neuroplasticity: A Convergence of Mechanisms. *Neuropsychopharmacology Reviews* (2008) 33:88–109. doi: 10.1038/sj.npp.1301574

25. Chiba S, Numakawa T, Ninomiya M, Richards MC, Wakabayashi C, Kunugi H. Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. *Prog Neuropsychopharmacol Biol Psychiatry* (2012) 39:112–119. doi: 10.1016/j.pnpbp.2012.05.018

26. Vollmayr B, Henn FA. Stress models of depression. *Clin Neurosci Res* (2003) 3:245–251.

27. Hammen CL. Stress and depression: old questions, new approaches. *Curr Opin Psychol* (2015) 4:80–85.

28. Tafet GE, Bernardini R. Psychoneuroendocrinological links between chronic stress and depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2003) 27:893–903.

29. Breslau N, Davis GC. Chronic stress and major depression. *Arch Gen Psychiatry* (1986) 43:309–314.

30. Monroe S, Harkness K. Life Stress, the “Kindling” Hypothesis, and the Recurrence of Depression: Considerations From a Life Stress Perspective. *Psychol Rev* (2005) 112:417–445. doi: 10.1037/0033-295X.112.2.417

31. McEwen BS. Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain. *Physiol Rev* (2007) 87:873–904. doi: 10.1152/physrev.00041.2006

32. Feder A, Nestler EJ, Charney DS. Psychobiology and molecular genetics of resilience. *Nat Rev Neurosci* (2009) 10:446–457. doi: 10.1038/nrn2649

33. Wang W, Liu L, Yang X, Gao H, Tang Q-K, Yin L-Y, Yin X-Y, Hao J-R, Geng D-Q, Gao C. Ketamine improved depressive-like behaviors via hippocampal glucocorticoid receptor in chronic stress induced- susceptible mice. *Behavioural Brain Research* (2019) 364:75–84. doi: https://doi.org/10.1016/j.bbr.2019.01.057

34. Birnie MT, Eapen A V, Kershaw YM, Lodge D, Collingridge GL, Conway-Campbell BL, Lightman SL. Time of day influences stress hormone response to ketamine. *J Neuroendocrinol* (2022) 34:e13194. doi: https://doi.org/10.1111/jne.13194

35. Gould T, Georgiou P. Inconvenient truths and the usefulness of identifying unknown unknowns. *Nat Neurosci* (2022) 25:1122–1123. doi: 10.1038/s41593-022-01147-w

36. Georgiou P, Zanos P, Mou T-CM, An X, Gerhard DM, Dryanovski DI, Potter LE, Highland JN, Jenne CE, Stewart BW, et al. Experimenters' sex modulates mouse behaviors and neural responses to ketamine via corticotropin releasing factor. *Nat Neurosci* (2022) 25:1191–1200. doi: 10.1038/s41593-022-01146-x

37. Jones, Nathan T. Wagner, Laura Scarlett, Cameron 0. Hanh, Molly C. Wenthur CJ. In Vivo Validation of Psilacetin as a Prodrug Yielding Modestly Lower Peripheral Psilocin Exposure than Psilocybin. *Front Psychiatry* (2023) 14: doi: doi: 10.3389/fpsyt.2023.1303365

38. Witowski CG, Hess MR, Jones NT, Pellitteri Hahn MC, Razidlo J, Bhavsar R, Beer C, Gonzalez-Velazquez N, Scarlett CO, Wenthur CJ, et al. Novel extended-release transdermal formulations of the psychedelic N,N-dimethyltryptamine (DMT). *European Journal of Pharmaceutical Sciences* (2024) 199:106803. doi: https://doi.org/10.1016/j.ejps.2024.106803

APPENDIX

Supplementary Methods and Materials

Surgery

Skull screw EEG electrodes were chronically implanted in animals under isoflurane anesthesia (1.5 - 2%) using aseptic technique. Electrodes were placed bilaterally in the frontal (1.5 mm anterior to Bregma, 1.5 mm lateral to midline) and parietal (2.0 mm posterior to Bregma, 2.0 mm lateral to the midline) plates. Bilateral reference electrode screws were placed through the occipital plate and tied together to ground. EEG wires were stranded copper (0.012" diameter, 0.022" diameter including insulation; Cooner Wire, Chatsworth, CA). Wires were soldered to a 1-cm2 electrode interface board (EIB-16; Neuralynx, Bozeman, MT), which was fixed into place using dental cement (Fusio A3; Pentron; Orange, CA). Animals recovered for at least 5 days prior to the first recording day and were housed individually.

Drug Preparation and Administration

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 30-60s, and brought to pH 6-7 using 1 M NaOH. This material was filtered through a 0.2 um filter and administered intraperitoneally (IP) at doses between 0.3 – 3 mg/kg. Ketamine Hydrochloride (Spectrum Chemical Mfg. Corp.; Gardena, CA) was diluted in 0.9% sterile saline, filtered through a 0.2 um filter, and administered at a dose of 30 mg/kg IP. All IP injections were given at a volume of 10 mL/kg. Corticosterone (Sigma-Aldrich) was diluted in 10% ethanol (EtOH) or 4.5% (2-hydroxypropyl)-Beta-Cyclodextrin (Biosynth-Carbosynth) in water, vortexed for 1 min, and then sonicated for 3 min at 22 °C, before being diluted to either 1% EtOH or 0.45% Beta-cyclodextrin in the animals' drinking water. For

experimental batteries requiring chronic corticosterone exposure, mice were given ad libitum access to either corticosterone water (0 - 80 µg/mL) or vehicle (1% EtOH or0.45% (2-hydroxypropyl)-Beta-Cyclodextrin) in their home cage for 28 days. Both vehicle and corticosterone bottles were refreshed every 7 days for the duration of the 28-day period.

Open Field Test (OFT)

To test for drug-induced changes in locomotor, exploratory, and anxious behavior, mice were assessed in the OFT. Mice were injected IP with psilocybin (0 - 3 mg/kg) and then individually placed into a corner within an open-field apparatus (41×20×24 cm), at a time period from 5 min to 7 days afterward. The center zone was defined as the middle one-third (6×27cm) of the arena. The apparatus was illuminated at ~200 - 250 lux. Mice were allowed to explore freely for 10 minutes in acute experiments and 150 min in long-term experiments. Time spent in the center and total distance travelled were automatically quantified using the Any-Maze software. Each apparatus was cleaned before and after each test with Trifectant. All OFT measurements were run between 1-4 PM. For all stand-alone OFT experiments, this represented the dark phase of the cycle. In behavioral batteries where the OFT was given subsequent to the NSF, this represented the light phase of the cycle.

Head Twitch Response (HTR)

To measure acute unconditioned responses to psilocybin, mice were assessed using an automated HTR detection platform adapted from previous approaches (*Chakraborty et al., 2020; Yasmin et al., 2020).* In this study, mice already implanted with chronic skull screw EEG electrodes were anesthetized with isoflurane (1.5 - 2%) to attach a neodymium magnet to the exposed dental cement from the EEG implant, at least 1 day prior to recording. Following

recovery, individual animals were placed into a clear acrylic cylinder (15.24 cm height × 15.24 cm diameter) wrapped with ~300 rotations of 30 Gauge copper magnet wire, (Essex, Fort Wayne, IN) inside of a dark sound-attenuation chamber, connected to a flexible tether (ZC16) to record EEG while allowing free range of motion, and their behavior was recorded using an infrared camera (240×320 pixels) controlled by Synapse (Tucker Davis Technologies, Alachua, FL [TDT]) for 1 h prior to drug administration. Magnetometer signals were amplified near the source with a homemade custom circuit, and the signal was routed to an RZ5D (filtered at 0.2-1000 Hz, then digitized at 3,051.8 Hz). After this time period, the animals were administered 3 mg/kg IP psilocybin and recorded for an additional 4 h. Changes in the local magnetic field induced by head twitches (~ 60-90 Hz signal) were assessed using in-house MATLAB code. Automated results were compared to observed HTRs for internal validation. All HTR responses were recorded during the dark phase of the light cycle.

Forced Swim Test (FST)

To measure the post-acute effects of drug treatment on immediate threat response, mice were assessed in the FST. Mice were injected IP with either psilocybin (3 mg/kg), ketamine (30 mg/kg) or saline. Animals were individually placed into a clear Plexiglas swim tank (46×10cm) for a period of 6 min. Water temperature was maintained at 26°C and the tanks were illuminated at 40-42 lux. Immobility time and distance travelled were quantified for the final 4 min of the test using Any-Maze software. At the completion of the testing period, animals were removed from the water, dried and placed into a clean cage with a heating pad to facilitate rapid recovery of normal body temperature. The animals were monitored in this chamber for 15-minutes before being placed back into group housing. All FST measurements were run between 1-4 PM, during the dark phase of the cycle.

Sucrose Preference Test (SPT)

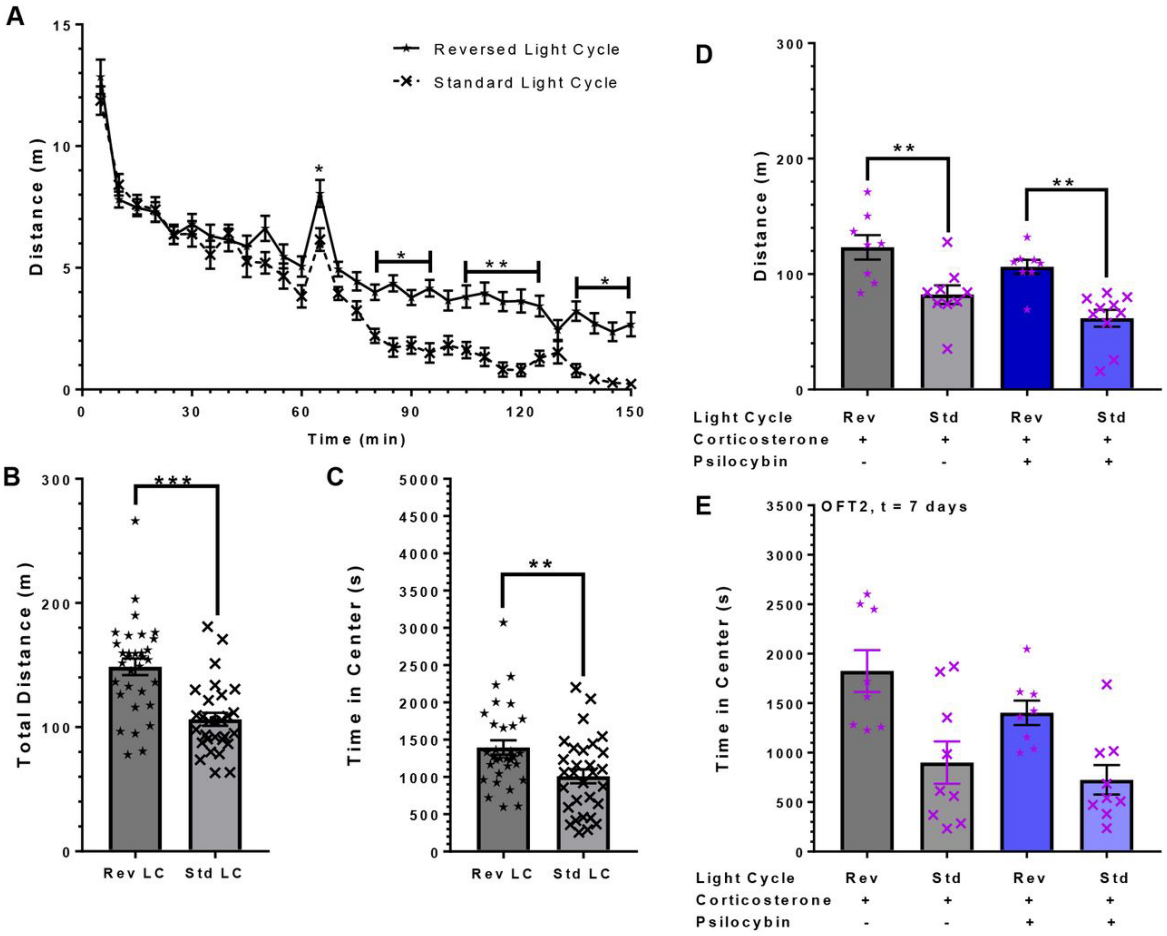
The SPT was conducted as a measure of hedonic responding both acutely and chronically. In this test, mice were placed into individual housing (41×20×24cm) to habituate for 48 h and then provided two identical bottles with either a 1% sucrose solution or water. At this time, mice had ad libitum access to standard chow and the 2 bottles provided. Animals underwent three 16 h restriction periods during which they had access to the 1% sucrose solution and water, with food and water provided between these periods. Immediately after the final restriction period, two identical bottles containing either 1% sucrose solution or water were placed into each cage and consumption was measured for a period of up to 15 days. The bottles were weighed at each test period, and sucrose preference was calculated as: sucrose weight / (sucrose weight + water weight) *100.

Novelty Suppressed Feeding (NSF)

In order to measure animal behavior in a task combining motivated behavior with anxious responding, mice were assessed within the NSF. Animals underwent a sequential food reduction of 2-Days at 20% and 1-day at 80% and food deprivation (16 - 24 h,). Mice then received an IP injection of saline or drug 4-5 h prior to performing the NSF test. For the test, a food pellet soaked in 50% sucrose solution was placed into a glass petri dish that served as the feeding zone (9×0.375 cm) and centered within a novel cage environment (61×41×37cm) that was brightly illuminated. Mice were then placed into a corner of the apparatus and allowed to explore for 10 minutes. Latency to first feed was recorded by a trained and a blinded observer, and movement and distance traveled were monitored via the Any-Maze software. Pellet weights were also obtained immediately before and after each test. After testing, the mice were returned to their housing and given normal food and water. Each apparatus was cleaned before and after each

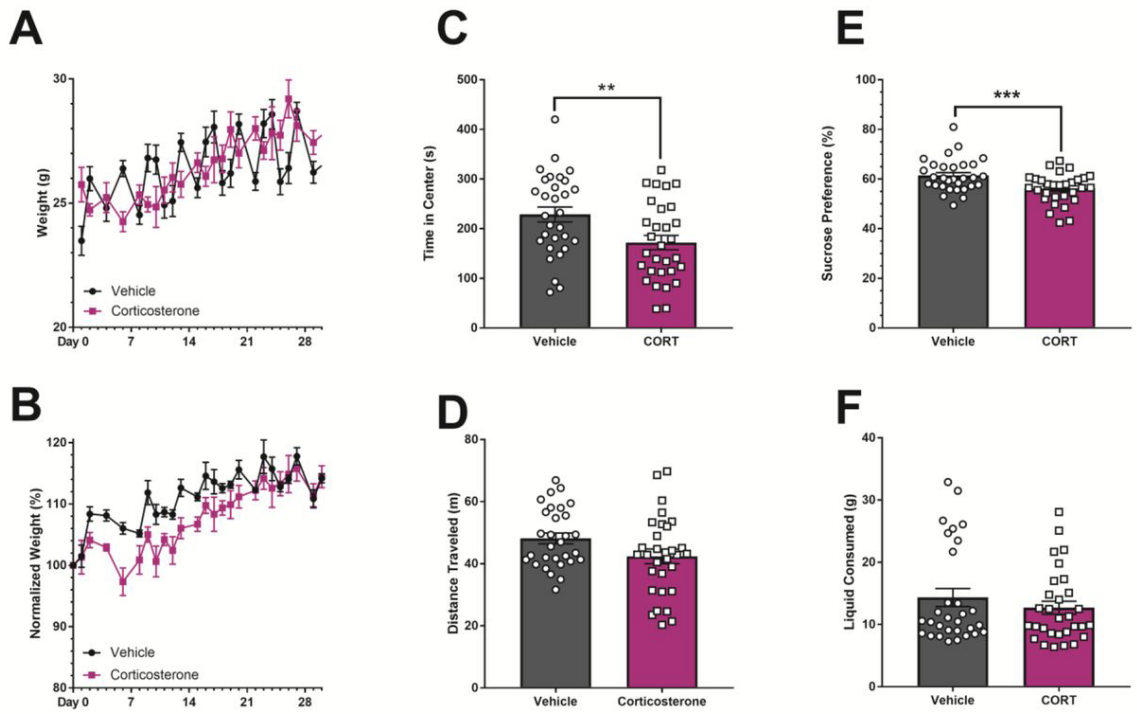
test with Trifectant. All NSF measurements were run between 11-4 PM, during the light phase of the light cycle.

Appendix Figure 1: Light Cycle Reversal Increases Signal Window for Locomotor and Center Time Measurements Across Treatment Conditions.



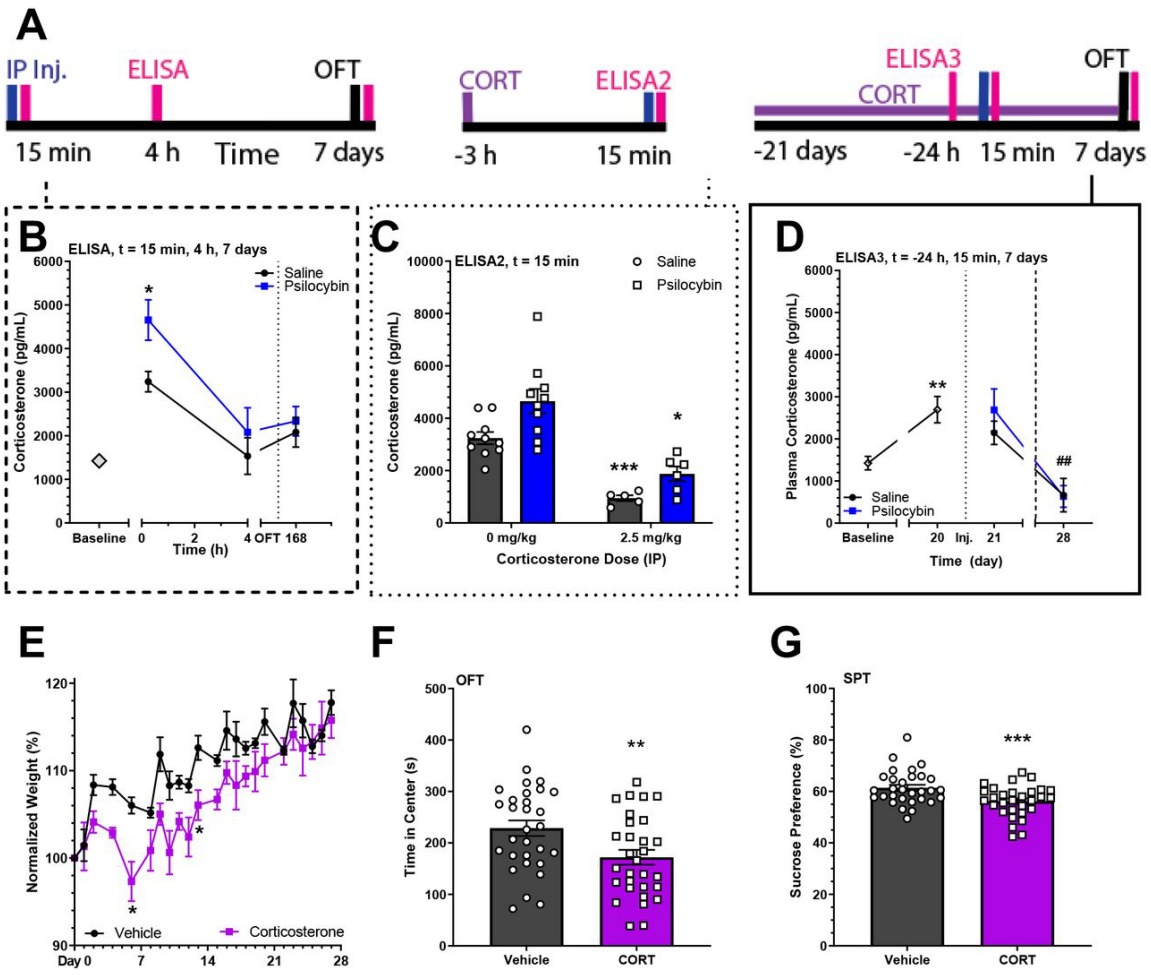
Appendix Figure 1: A) Distance traveled across 5 min time periods for 60 min prior to and 90 min after saline injection, across different light cycle conditions. (Standard, n = 30, Reversed, n = 32). *: $p<0.05$, ** $p<0.01$, Two-Way ANOVA with Sidak's. B) Total distance traveled in the same test. ***: $p<0.001$, Student's t-test. C) Time in center of open field apparatus in the same test. **: $p<0.01$, Student's t-test. D) Total distance traveled in an open field test from 5-155 min after drug administration to corticosterone-exposed animals, across light cycle conditions. **: $p<0.01$, ANOVA with Sidak's. E) Time in center for the open field apparatus in the same test. One outlier removed from CORT + Psilocybin (ROUT). All error bars presented as Mean ± SEM.

Appendix Figure 2: Chronic Corticosterone Exposure Via Drinking Water Yields Behavioral and Physiologic Changes in Mice.



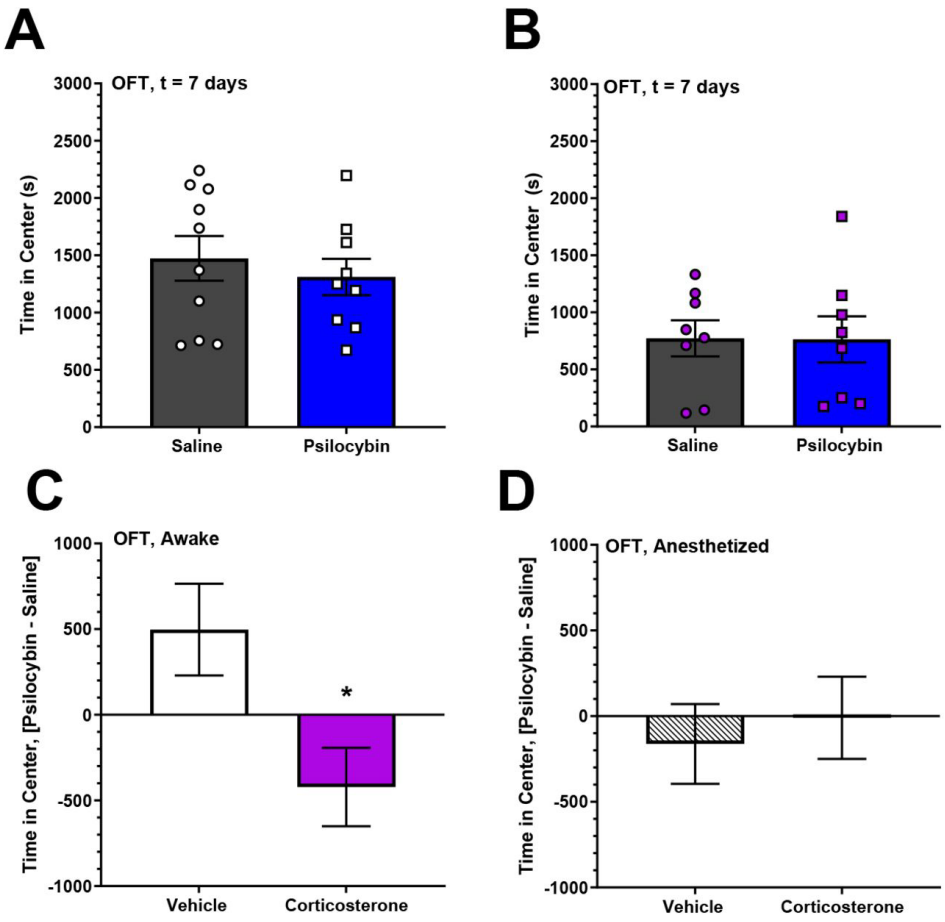
Appendix Figure 2: A) Weight measurements across the 28-day corticosterone exposure period (Vehicle, n = 30; Corticosterone, n = 30). B) Baseline-normalized changes in weight across the same period. C) Time spent in the center of the open field apparatus following 28-day corticosterone exposure period. **: $p<0.01$, T-test. D) Total distance traveled in the same open field test. E) Sucrose preference following a 28-day corticosterone exposure period. ***: $p<0.001$, T-test. F) Average of total liquid consumed on days 30 – 31 after chronic corticosterone exposure. All error bars presented as mean ± SEM.

Appendix Figure 3: Chronic Corticosterone Exposure Suppress Stress and Psilocybin-Induced Acute Glucocorticoid Release.



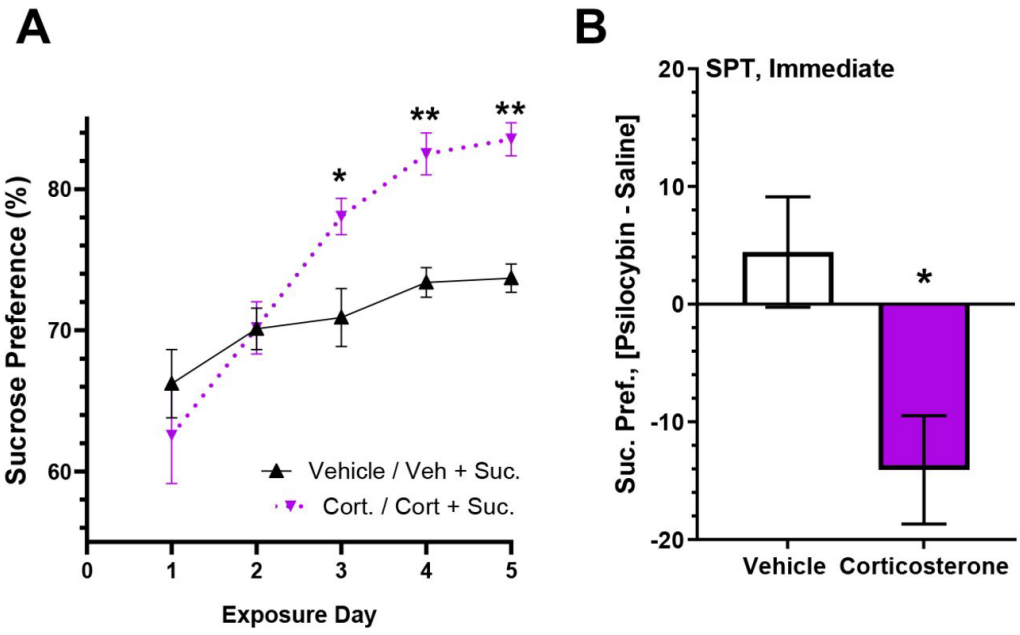
Appendix Figure 3: A) Experimental timelines for measurement of plasma corticosterone. IP Inj: intraperitoneal injection, ELISA: enzyme linked immunosorbent assay, OFT: Open Field Test; CORT: corticosterone. Vertical lines indicate acute intervention; horizontal lines indicate ongoing exposure. B) ELISA for plasma corticosterone concentrations at baseline and at various time-points following drug administration. Dotted line denotes open field test. Two-Way ANOVA with Sidak's, * = $p < 0.05$ vs. Saline, 15 min. (n = 16 baseline, n = 10, saline, psilocybin). C) ELISA for plasma corticosterone concentrations at 15 min following drug administration in the presence of IP corticosterone pretreatment. Two-Way ANOVA with Sidak's, * = $p < 0.05$, *** = $p < 0.001$ vs Saline + 0 mg/kg Corticosterone. D) ELISA for plasma corticosterone concentrations at baseline, following chronic oral corticosterone exposure (80 µg/mL), at 15 min following drug administration, and following exogenous corticosterone withdrawal. Student's t-test, ** = $p < 0.01$ vs Baseline. ## = $p < 0.01$ vs Vehicle. Dotted line denotes drug injection. (n = 16 baseline, day 20; n = 8, day 21 and 28). E) Normalized weight during oral corticosterone exposure. (n = 8) * = $p < 0.05$ vs. Vehicle. F) Time spent in the center of an open field arena following 28 days of oral corticosterone exposure. ** = $p < 0.01$. G) Sucrose preference following 28 days of oral corticosterone exposure. *** = $p < 0.001$. All experiments use 3 mg/kg psilocybin IP. All data presented as mean ± SEM.

Appendix Figure 4: Brief Isoflurane Anesthesia Eliminates the Interaction Between Corticosterone Exposure and Psilocybin's Long-Term Effects on Anxious Responding.



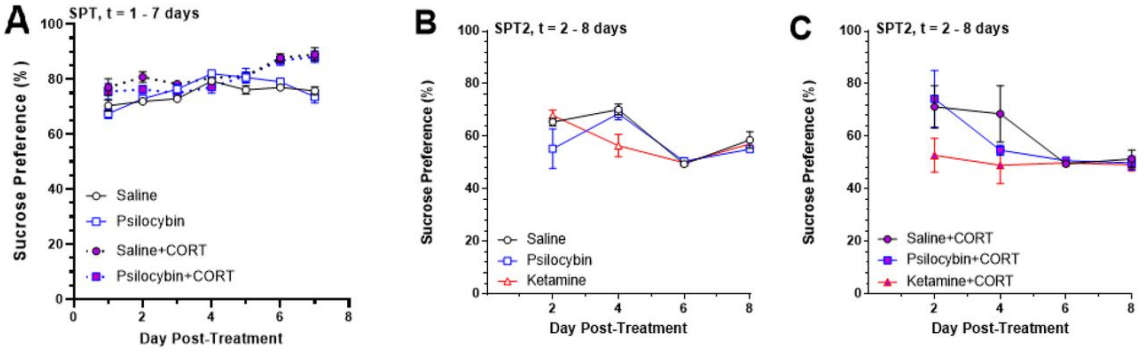
Appendix Figure 4: A) Time spent in the center during a 150 min open field test at 7 days after psilocybin treatment. B) F) Time spent in the center during a 150 min open field test following oral corticosterone or vehicle exposure at 7 days after psilocybin treatment. C) Difference between psilocybin mean response and saline mean response for time in center during a 150 min open field test at 7 days after psilocybin treatment. * = $p < 0.05$. D) Difference between psilocybin mean response and saline mean response for time in center during a 150 min open field test at 7 days after psilocybin treatment., in animals exposed to brief (3-5 min) isoflurane anesthesia at 15 min after psilocybin treatment. All experiments use 3 mg/kg psilocybin IP. Data presented as Mean ± SEM. For C and D, this represents the difference between the means of the psilocybin and saline treated animals, with the errors for these means added in quadrature.

Appendix Figure 5: Psilocybin Acutely Blocks Avoidance of Corticosterone Water Rather Than Decreasing Preference for Sucrose Water.



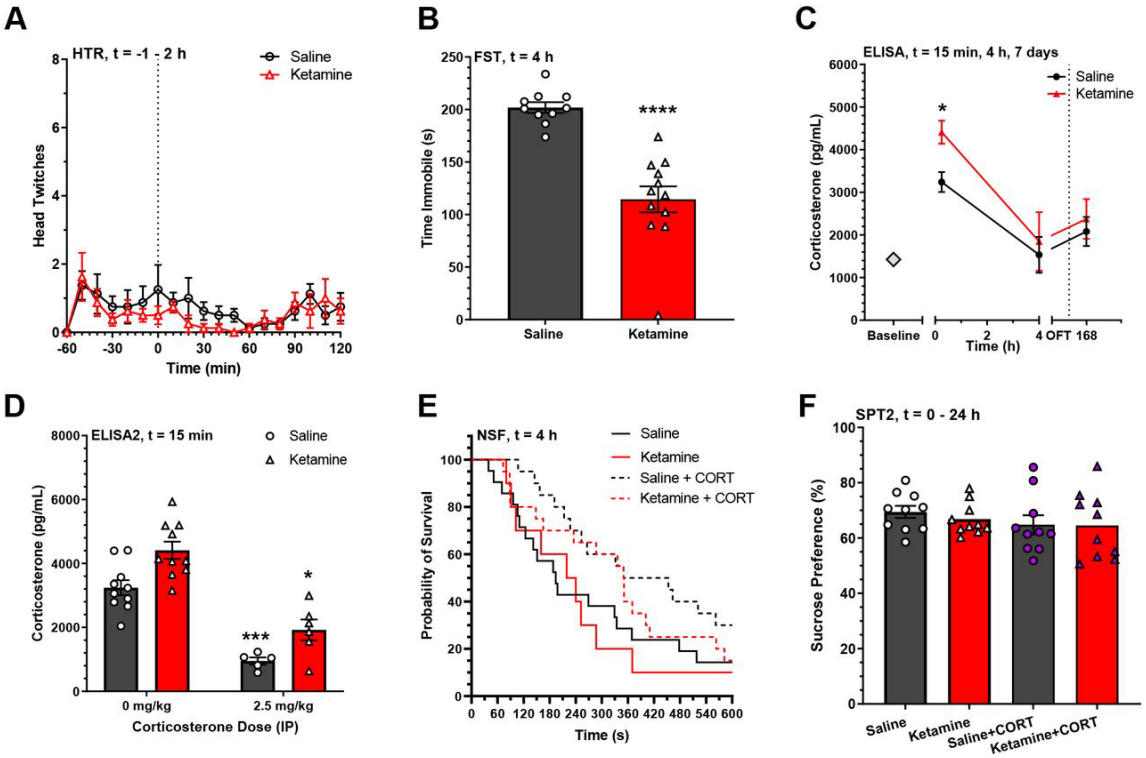
Appendix Figure 5: A) Sucrose preference on Days 1 – 5 of exposure to two-bottle choice with water containing either Vehicle / Vehicle+Sucrose or Corticosterone / Corticosterone+Sucrose (n = 15-16). * = $p < 0.05$, ** = $p < 0.01$, Two-Way RM ANOVA with Sidak's. B) Difference in sucrose preference between animals treated with 3 mg/kg of psilocybin vs saline, starting 15 min after drug administration, during exposure to two-bottle choice with water containing either Vehicle / Vehicle+Sucrose or Corticosterone / Corticosterone+Sucrose (n = 16, vehicle; n = 8, corticosterone). * = $p<0.05$, Student's t-test. All error bars presented as Mean ± SEM.

Appendix Figure 6: No Long-Term Effects of Psilocybin and Ketamine on Sucrose Preference



Appendix Figure 6: A) Sucrose preference for 1-7 days in drug-treated animals with ongoing chronic vehicle or corticosterone exposure (n=7-8). B) Sucrose preference of vehicle-treated animals upon retest beginning at 1, 3, 5, and 7 days after treatment (n= 2-3). C) Sucrose preference for 0-24 h period in drug-treated animals with prior chronic corticosterone exposure (n= 2-3). All error bars presented as Mean ± SEM.

Appendix Figure 7: Effects of 30 mg/kg Ketamine on Study Measures.



Appendix Figure 7: A) Time course of automated head twitch response detections across each 10 min time period from 1 h before to 2 h after drug administration. (n=8, saline, ketamine) B) Immobility time in the forced swim test at 4 h following drug administration. ****: $p < 0.0001$. C) ELISA for plasma corticosterone concentrations at baseline and at various time-points following drug administration. Dotted line denotes open field test. Two-Way ANOVA with Sidak's, * = $p < 0.05$ vs. Saline, 15 min. (n = 16 baseline, n = 10, saline, ketamine). D) ELISA for plasma corticosterone concentrations at 15 min following drug administration in the presence of IP corticosterone pretreatment. Two-Way ANOVA with Sidak's, * = $p < 0.05$. E) Survival curves of latency to feed for saline and ketamine as matched across corticosterone exposure conditions (n = 10, ketamine; n = 20, all others). F) Sucrose preference following vehicle or corticosterone-exposure at 0 – 24 h after ketamine administration. All data presented as Mean ±SEM.