Psychedelics and novel non-hallucinogenic analogs for treating neuropsychiatric disorders

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WHAT IS THE REALITY OF ANY FEELING?

An Unquiet Mind, Kay Redfield Jamison

Abstract

PSYCHEDELICS AND NOVEL NON-HALLUCINOGENIC ANALOGS FOR TREATING NEUROPSYCHIATRIC DISORDERS

Psychedelic medicine is gaining rapid popularity for treating neuropsychiatric disorders such as depression, anxiety, PTSD and addiction. Unlike current medications on the market, these compounds appear to act rapidly and in treatment-resistant populations. However promising, these therapeutics are hindered by the fact that they cause intense hallucinations, which can be dangerous and decrease accessibility to this type of medical care. We sought to determine if hallucinations are necessary to achieve the therapeutic effects, or if these two phenomena are dissociable. Using rodent behavioural paradigms, cell culture assays and human survey data, we demonstrate that both psychedelic microdosing and novel non-hallucinogenic psychedelic analogs may have therapeutic effects.

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Chapter 1

NEUROPLASTICITY AND MENTAL HEALTH

1.1 Prevalence and Impact of Mental Health Disorders

Depression, anxiety and post-traumatic stress disorder (PTSD) are considered stress-related illnesses and are among the leading causes of disability worldwide.[1] In fact, over the last decade, cases have increased, and suicide rates now surpass the number of deaths caused by motor vehicle accidents.[2] Finally, the number of diagnoses for these conditions have substantially increased since the beginning of COVID-19.[3]

1.2 Current Medications are Inadequate to Treat Stress-Related Illnesses

In 2019, 18.5% of adults experienced depressive symptoms.[4] Depression characterized by persistent sad or "empty" moods, feelings of hopelessness, irritability, feelings of guilt, decreased energy or fatigue, difficulty concentrating, difficulty sleeping, changes in appetite and weight and in severe cases, thoughts of suicide or death.[5] Depression occurs with higher rates and severity of depression and anxiety in women.[4] It is associated with diminished quality of life and increased disability.[6] Currently, traditional antidepressants are the first-line treatment for depression.[7]

Like depression, generalized anxiety disorder (GAD) has a prevalence of 15% in the United States, and is characterized by restlessness, being easily fatigued, difficultly concentrating, irritability, muscle tension or sleep disturbance.[8, 9] Given that both depression and anxiety have similar neuropathology, it is unsurprising that treatments for GAD are also antidepressants[10] such as selective serotonin reuptake inhibitors (SSRIs)[11, 12] or serotonin and norepinephrine reuptake inhibitors (SNRIs).[13]

For decades, scientists thought low serotonin levels contribute to this depression—called the serotonin or monoamine hypothesis of depression—has led to the perpetuation of drugs that increase serotonin availability.[14] There are different types of antidepressants on the market, including tricyclics, SSRIs, and SNRIs, all which generally aim to increase serotonin concentrations in the synaptic cleft. Until recently, there has not been a mechanistically novel pharmaceutical compound to treat depression in almost 30 years (**Figure 1.2**). Side effects associated with these medications include reduced libido, weight gain, fatigue, and hypotension to

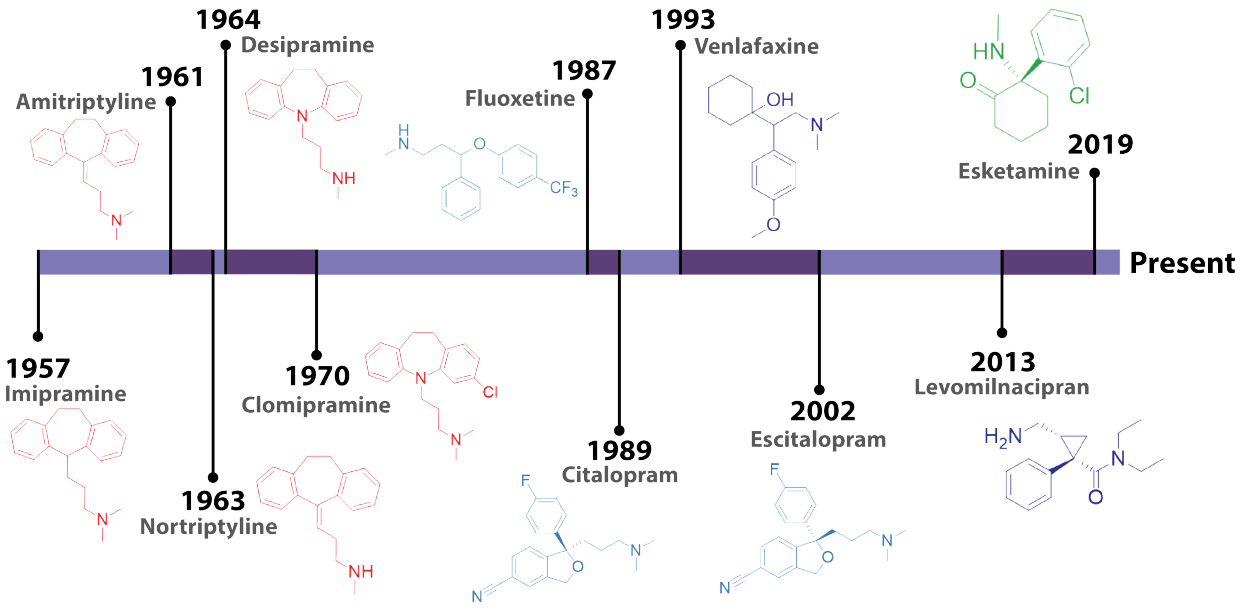

Figure 1.2-1. *Timeline of approved medications for depression and anxiety.*

Red *represents tricyclics;* ***light blue*** *represents SSRIs;* ***dark blue*** *represents SNRIs;* ***green*** *represents psychoplastogens. Image created by Lindsay Cameron and Lee Dunlap.*

name a few.[15] Many patients discontinue treatment due to such side effects.[16] This theory has not stood the test of time, with the primary caveat being that these drugs work rapidly to increase serotonin levels in the brain, but clinically take many weeks to start working.

Given that antidepressants are the most common therapeutic medications in the United States,[17] it is shocking how ineffective they are. Approximately 30-40% of patient do not actually respond to medications on the market, and these patients are termed treatment-resistant.[18, 19] Though many patients discontinue treatment due to side effects, the majority of patients discontinue due to this lack of efficacy.[20] Finally, these drugs take ~6 weeks to begin working, leaving no therapeutic options for those who are severely depressed or suicidal.[21, 22]

More recently, scientists have supported the neurotrophic hypothesis of depression—a hypothesis which posits that a dearth of neurotrophins may result in atrophy and decreased function of cortical neurons.[23]

1.3 The neurotrophic hypothesis for depression and the prefrontal cortex

Depression, anxiety and post-traumatic stress disorder (PTSD) are stress-related disorders characterized by atrophy of neurons in the prefrontal cortex (PFC), which has been found in rodent studies, as well as human post-mortem tissue.[24, 25, 26, 27] Further, patients have shown decreased BOLD response in PFC regions,[28] and increased cortical thinning.[29, 30, 31, 32, 33] These decreases in activation in vmPFC are correlated with impaired fear responses.[34] Ultimately, these disorders are thought to arise from a complex interaction of genetic, biochemical, and environmental (stress) variables.[35]

Neurotrophins such brain-derived neurotrophic factor (BDNF)—which play crucial roles in growth and plasticity—are vital for the functioning of this brain region, and the neurotrophin

hypothesis of depression posits that low levels of such neurotrophic factors may lead to neuronal atrophy in the PFC, subsequently manifesting as depression.[23, 36] Exposure to chronic stress—physical or social—decreases levels of BDNF in the PFC and hippocampus.[37, 38] In patients with depression, there are significantly decreased levels of BDNF,[39, 40, 41, 42] which subsequently increase again after treatment.[43, 44, 45, 46, 47] Post-mortem studies of patients who received chronic (but not acute) treatment with traditional antidepressants demonstrate increases in BDNF.[37, 43, 44] Infusion of BDNF induces rapid increases in function and growth of neurons.[48,49] Binding of BDNF to the tyrosine kinase receptor B (TrkB) activates cell pathways related to survival and differentiation.[50] In neurons, it promotes branching of dendrites and initiation/stabilization of synaptic contacts.[49,50] Murine models expressing mutant TrkB receptors are resistant to treatment with traditional antidepressant compounds.[51]

It should be noted that although substance use disorder (SUD) is not always considered a stress-related disorder, stress-related disorders puts one at risk for developing SUD[52, 53] and these conditions commonly co-occur.[54, 55] Exposure to stressors enhances drug self-administration and cause reinstatement of drug-seeking behaviour in animals who previously took the substances.[56] Though studies have demonstrated that various addictive substances—opiates, amphetamines, nicotine, etc.—all work on the limbic system in different ways, they all are associated with atrophy of prefrontal cortical neurons in the PFC.[57] Of course, this calls into question if this atrophy is a cause, a result or simply correlated with drug seeking behaviour. Studies have demonstrated that low serum levels of BDNF are highly correlated with alcohol and drug dependence.[42]

The prefrontal cortex plays an integral role in the top-down regulation of emotion. As mentioned above, atrophy of neurons in the prefrontal cortex is highly associated with depression. Conversely, regrowth of neurons in this area is seen with treatment and is correlated with

amelioration of symptoms. There is ample evidence to suggest that the PFC is also implicated in drug seeking behaviour. Despite the fact that many addictive compounds exist with different mechanisms of action (opiates, amphetamines, cocaine, alcohol, etc), all are associated with atrophy and change of function of neurons in the prefrontal cortex.[58]

The PFC consists mainly of excitatory pyramidal cells (80-90% of the population), with a smaller proportion of GABAergic interneurons (10-20%).[59, 60] Each of these cell types can be further subdivided based on molecular markers, morphology, as well as physiological and molecular characteristics. Layer V pyramidal cells extend projections to subcortical areas and modulate their activity. Indeed, optogenetic activation of PFC terminals in the dorsal raphe (DR) increases motivated behaviours,[61] activation of PFC terminals in the amygdala promotes anxiolytic behaviours,[62] and activation of PFC terminals in the NAc reduces drug seeking behaviour.[63] Furthermore, infusions of BDNF to the infralimbic cortex in the absence of fear extinction learning is sufficient for enhancing extinction learning,[64] suggesting that perhaps simply increasing BDNF levels in this region is enough for the expression of extinction behaviour.

The PFC plays an integral role in modulating emotion-related behaviours, so it is unsurprising that atrophy of neurons in this region results in the development of pathologies like depression, anxiety, PTSD and SUD.

1.4 Ketamine and mental health

Within the last few decades, data has emerged that sub-anesthetic infusion of ketamine is capable of promoting rapid-acting antidepressant responses, within hours of administration.[65, 66, 67, 68, 69, 70, 71] In addition, ketamine infusions appear to be effective for treatment-resistant populations.[72] Compared to traditional antidepressants that take approximately 6 weeks to demonstrate efficacy,

and do not work in treatment-resistant populations, ketamine revolutionized the field of psychiatry. Compounds that are fast-acting therapeutics, like ketamine, capable of *rapidly* promoting structural and functional neural plasticity are referred to as psychoplastogens.

Pioneering studies by the Duman lab demonstrate that ketamine rapidly restores spine growth and function in the prefrontal cortex.[73] While depressed patients show reduced activity in the PFC,[74] this is rapidly reversed after ketamine treatment.[71, 75, 76] In rodents, chronic stress causes neuronal atrophy in the PFC,[77, 78] which is rapidly reversed after ketamine infusion. Furthermore, ketamine restores neuronal function through increases in glutamate transmission in the PFC of rodent models.[73, 79, 80] Ketamine likely works through a BDNF-mediated mechanism, as infusion a BDNF-sequestering antibody into the PFC blocks ketamine's antidepressant effects.[81] Optogenetic inhibition of the PFC blocks ketamine's antidepressant-like behavioural effects in rodent models, while optogenetic stimulation replicates it.[82] Moda-Sava and colleagues demonstrate that the spine growth in the PFC caused by ketamine administration is necessary for sustained antidepressant effects.[83] In 2019, the FDA approved Esketamine: a nasal spray version of *S*-ketamine to help with treatment-resistant depression (**Figure 1.2**).[84]

As promising as ketamine sounds, there are three major issues with its use as a therapeutic. Firstly, ketamine is addictive,[85, 86, 87, 88, 89] and evidence shows that there is overlap in the circuitry between its antidepressant and addictive effects.[90] Secondly, due to this abuse potential, ketamine is administered in clinics under supervision. For this reason, ketamine therapy is expensive and therefore inaccessible to populations who may need it most. Ketamine is not something you can take home and keep in your medicine cabinet. Finally, the therapeutic effects of ketamine are transient, lasting only 7-14 days.[66, 69]

In sum, the discovery of ketamine as a rapid-acting antidepressant has revolutionized the field of psychiatry. However, there still appears to be some drawbacks associated with this therapy. We can harness the information that scientists have learned from ketamine to develop better and longer-lasting therapeutics.

1.5 Psychedelics for treating mental health disorders

Studies with psychedelics have produced similar results as ketamine in being rapid-acting and effective in treatment-resistant populations. Much of what is known of psychedelic therapy stems from studies done in the Brazilian churches Santo Daime, Barquinha and União do Vegetal, where ayahuasca—containing the psychedelic *N,N*-dimethyltryptamine (DMT)—is administered for religious ceremonies. Studies in these groups revealed that ayahuasca is useful for treatment-resistant and recurrent depression.[91, 92]

Studies using other psychedelics such as psilocybin have also demonstrated to be effective rapidly and in treatment-resistant populations.[93, 94, 95, 96] Treatment with psychedelic compounds is able to increase BDNF mRNA in the cortex.[97] Remarkably, these effects are reported to last for years after a single administration.[98] Moreover, there is low abuse potential for psilocybin,[99] and no physical dependence or withdrawal.[100] In fear extinction studies, a model of post-traumatic stress disorder (PTSD), MDMA facilitates fear extinction learning.[101] Around this time, MDMA was granted the Breakthrough Therapy Designation by the FDA for treating PTSD[102, 103] and showed promising effects in Phase 3 trials in humans.[104]

Human work using psychedelic substances is fraught with administrative hurdles, not to mention the fact that having a true placebo group is very difficult: for example, if patients are administered LSD or saline, they will very likely know which substance they got based on if they

have a hallucinatory experience. From this, they can extrapolate if they will anticipate therapeutic responses. Using rodent models may circumvent these issues since they do not have a preconceived notion of acquiring a therapeutic effect from the substance.

When I began my PhD, there were a handful of studies that demonstrated the efficacy of psychedelic substances as rapid-acting antidepressants that were effective in treatment-resistant populations. Few people had any mechanistic insight into how these compounds worked to achieve this in the brain. For my PhD, I endeavoured to understand if these studies held up in rodent models (in lieu of placebo-controlled human studies), how they worked, and if hallucinations were necessary for their therapeutic action.

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Chapter 2

PSYCHEDELICS INCREASE PLASTICITY IN THE MEDIAL PREFRONTAL CORTEX AND REDUCE DEPRESSIVE-RELATED BEHAVIOURAL PHENOTYPES IN RODENTS

Psychedelic compounds have been used by humans for thousands of years and may be some of the oldest drugs known to our species. These compounds have been used for spiritual ceremonies, and often leave individuals feeling "spiritually enlightened."[1] Now, these compounds are gaining traction in the clinic for treating depression[2,3,4,5] and anxiety[6]; however mechanistic studies of their action on the nervous system are lacking.

For many of the studies in this dissertation, we chose to study the compound DMT. The structure of DMT constitutes the core structure of several tryptamine, ergoline and iboga psychedelics (**Figure 2.2.1-2**), and because of this many of the effects we observe in these studies can be hypothesized to extend to the entire family of psychedelics.

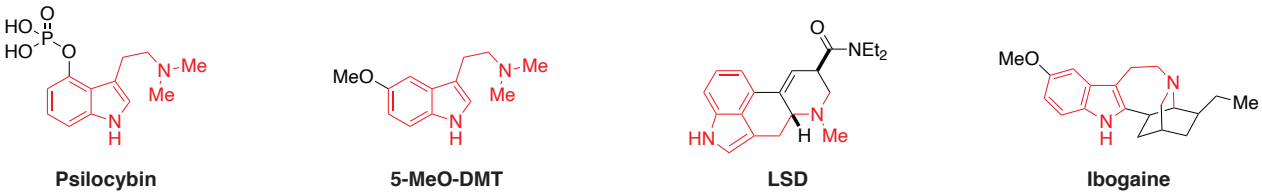

Figure 2.2.1-1. *DMT (highlighted in red) constitute the skeleton of classic psychedelic compounds.*

2.1 Effects of psychedelics in rodent models of mental health

There are several studies in humans suggesting that psychedelics and ayahuasca may have therapeutic potential for treating anxiety and depression. Given the challenges of studying psychedelics in humans with a proper placebo control, our group opted to test these compounds in rodent models. Using rodent models can be incredibly advantageous for two reasons: firstly, rodents do not have preconceived expectations of a drug's effect, which partially allows one to study the drugs' efficacy without placebo effects. Secondly, tools that are available for studying rodents are far superior for assessing changes on a cellular and circuit level than are currently available in humans. Prior to our study in 2018, there were very few rodent studies assessing the effects of psychedelics for mood and anxiety. An important study by Pic-Taylor and colleagues found that ayahuasca was both incredibly safe at high doses (at least 50x) and produced anxiolytic and anti-depressive like responses.[7] These animals displayed no significant signs of weight or alterations to the organs. In fact, the largest problem in this study was due to the solubility of the compound and the volume that had to be administered, which would cause stomach expansion, compression of the diaphragm and subsequent asphyxiation. Ayahuasca is a mixture of many compounds including three different types of harmala alkaloids—potent MAO inhibitors—so our study sought to determine the effects of DMT alone.

2.2 Effects of acute DMT treatment on animal behaviors relevant to depression and anxiety

2.2.1 Acute DMT demonstrates antidepressant efficacy in rodents

The forced swim test (FST) is a common test used to test antidepressant efficacy. Typically, an animal is placed in a tank of water for a period of 6 minutes, and the time the animal spends either swimming (motivated behavior) or immobile/floating (unmotivated behavior) is quantified. Things like chronic stress or chronic corticosterone paradigms which induce depressive-like states typically increase the amount of immobility in this test and conversely antidepressants increase the swimming (motivated) behaviours.

In this study, drug-naïve animals were first subjected to a pretest in which they spent 6 minutes in a Plexiglas cylinder full of water, then dried off and returned to their home cage. Animals the received a dose of 10 mg/kg of DMT or VEH at 23.5, 6 and 1 h prior to the test phase

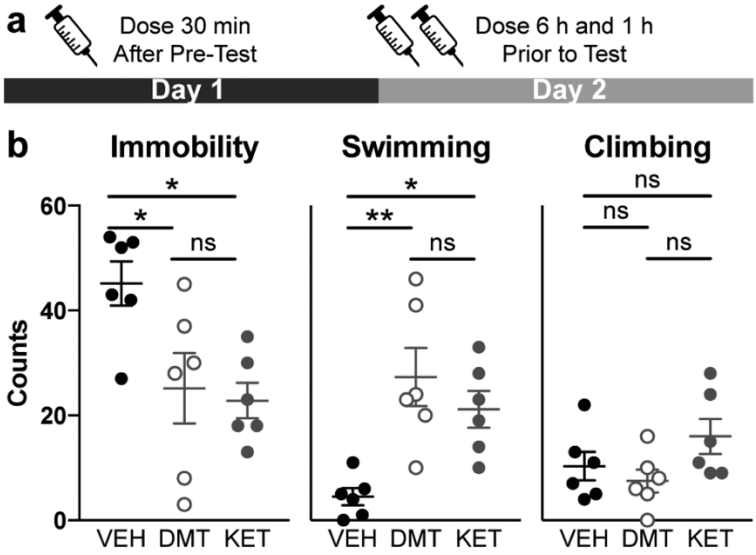

Figure 2.2.1-1. *Acute doses of DMT (10 mg/kg) elicit an antidepressant response in the forced swim test comparable to ketamine (10 mg/kg). (a) Experimental design of the forced swim test. (b) Quantification of different forced swimming behaviors.*

*N = 6 rats per condition. Data represents mean ± SEM. *p < 0.05, **p < 0.01. One-way ANOVA with Tukey post-hoc test. VEH = vehicle (saline), DMT = N,N-dimethyltryptamine, KET = ketamine, ns = not significant.*

(**Figure 2.2.1-2a**). Importantly, animals fully returned to normal home cage and grooming behaviour by 30 mins after dosing. This dosing paradigm has proven effective for a wide range of antidepressant compounds.[8] Indeed, DMT administration decreased the time spent immobile comparable to the fast-acting antidepressant ketamine, and significantly compared to vehicle (**Figure 2.2.1-2b**).

2.2.2 Effects of DMT treatment on animal behaviors relevant to anxiety

Drug-naïve rats were given either DMT (10 mg/kg) or VEH 1 h before a novelty-induced locomotion (NIL) assay for 45 minutes. Animals were then given 2 days off to ensure the drug was completely cleared from their system and subsequently reinjected (consistent with what they were dosed previously) and exposed to the elevated plus maze (EPM) for 5 minutes.

In the NIL paradigm, animals that were administered DMT moved significantly less distance than animals that had been administered VEH (**Figure 2.2.2-1b,c**). In addition, they display reduced number of rearings and small decrease in the time spent engaging in stereotypies (**Figure 2.2.2-1e-h**). There was no difference in the amount of time the animals spent in the center vs the margins of the apparatus (**Figure 2.2.2-1d**).

In the EPM task, animals that were injected with DMT spent less time in the open arms and had overall fewer entries to these areas (**Figure 2.2.2-1i,j**). The total distance and velocity between treatment groups was not significant in this task (**Figure 2.2.2-1k,l**). Together, these data suggest that acute DMT may be mildly anxiogenic.

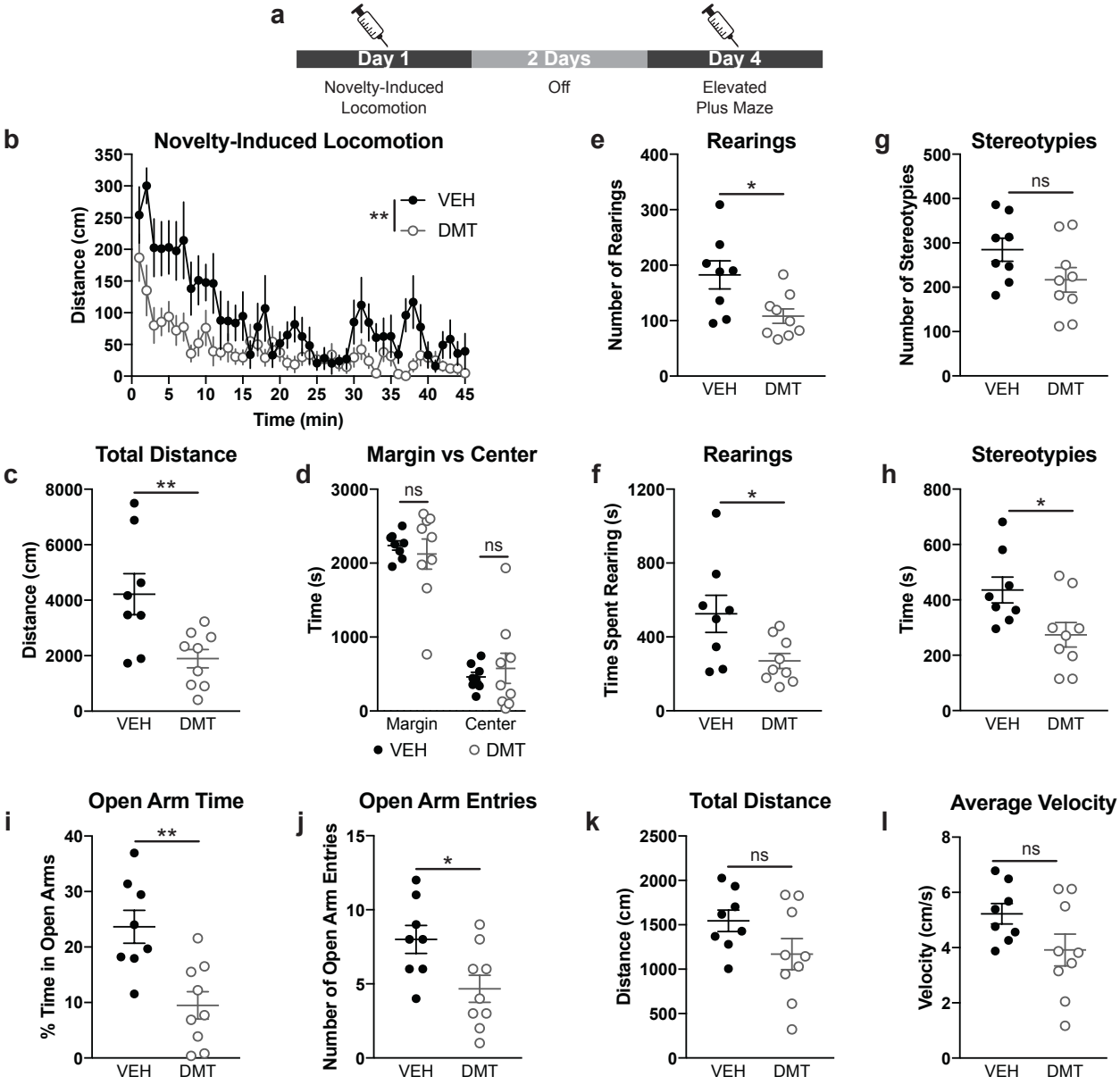

Figure 2.2.2-1. *Exploratory behavior and anxiety is impacted by an acute dose of DMT (10 mg/kg). (a) Timeline of behavioral tests. Drug-naïve animals were dosed 1 h prior to the novelty-induced locomotion test. After the completion of that experiment, these same animals were given 2 days of rest before being administered DMT 1 h prior to the elevated plus maze test. (b) Novelty-induced locomotion was quantified as total distance traveled in 1 min bins over time. Two-way repeated measures ANOVA. (c) total distance traveled over the entire 45 min experiment. Unpaired two0tailed t-test. (d) The proportion of time spent on the margin of the arena versus the center was determined. Two-way ANOVA with Sidak post-hoc test. (e,f) The number of vertical movements (e) (i.e., rearing) and total time spent rearing (f) were quantified. Unpaired two-tailed t-test. (g,h) The number of stereotypies (g) and total time spent engaged in stereotypies (h) were quantified. Unpaired two-tailed t-test. (i−l) Anxiety levels were measured using the elevated plus maze. The percentage of time spent in the open arms (i) as well as the number of open arm entries (j) was quantified. There was no difference between the treatment groups with respect to the total distance moved (k) or average velocity (l). Unpaired two-tailed t-test.*

*N = 8 rats per condition. Data represents mean ± SEM. *p < 0.05, **p < 0.01. VEH = vehicle, DMT = N,N-dimethyltryptamine, KET = ketamine, ns =not significant.*

2.2.3 Effects of DMT treatment on animal behaviors relevant to fear learning

To determine if acute psychedelic administration was able to facilitate fear conditioning, we administered DMT (10 mg/kg) prior to fear conditioning training. After 1 h, the rodents' behaviour returned to normal and we put the animals in a chamber and administered 3 tone-shock pairings (80 dB white noise, 30 s presentations paired with a 0.8 mA, 2 s shock, **Figure 2.2.3-1a**). We found that although DMT-treated animals froze more immediately after training this effect did not persist (**Figure 2.2.3-1b**). There was no difference in freezing levels during context or cue testing. This suggests that acute DMT administration does not enhance the persistence of the fear memory.

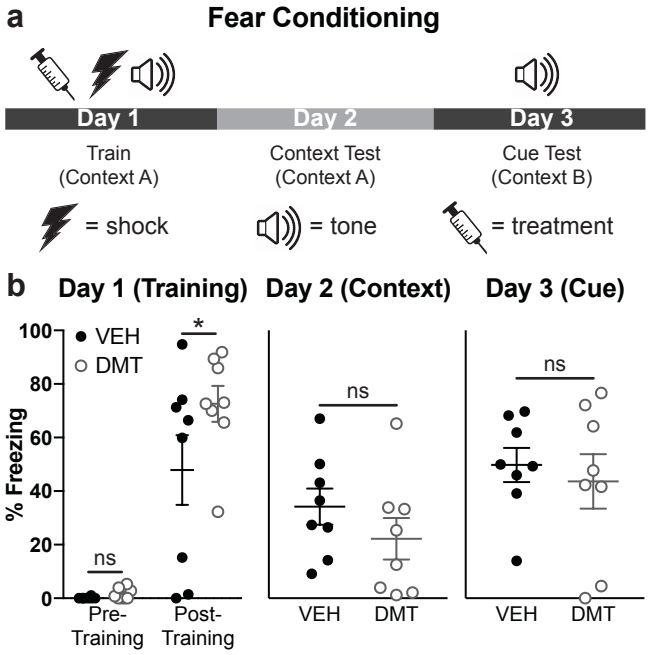

Figure 2.2.3-1. *Acute dose of DMT (10 mg/kg) prior to fear conditioning does not affect contextual or cued fear memory. (a) Experimental design for the fear conditioning experiment. (b) DMT increased immediate freezing following foot shocks, but had no effect on either contextual or cued fear memory. Pre-training and post-training represent the 2 min immediately before and after the presentation of shocks, respectively. Two-way ANOVA with Sidak post-hoc test. Contextual freezing was determined over the course of the entire 10 min session. Unpaired two-tailed t-test. Cued freezing was assessed as the percentage of time spent freezing during the eight tone presentations. Unpaired two-tailed t-test.*

*N = 8 rats per condition. Data represents mean ± SEM. *p < 0.05, **p < 0.01. VEH = vehicle, DMT = N,N-dimethyltryptamine, KET = ketamine, ns =not significant.*

Next, we sought to determine if acute DMT administration enhanced cued fear extinction (**Figure 2.2.3-2a**). Animals were trained with 3 tone-shock pairings (described previously), then after a day of rest, animals were administered DMT (10 mg/kg) 1 h prior to tone extinction training. We found no difference in extinction training between animals treated with VEH or DMT (**Figure 2.2.3-2b,c**). On the 4th day, animals were tested to see if they remembered the extinction training from the previous day. We found that animals that were treated with acute DMT retained the extinction/safety memory better than those that were administered VEH (**Figure 2.2.3-2d,e**). Whether this results from weakening of the fear memory or strengthening of the safety memory remains unclear, but the overall effect is a decreased fear response after DMT administration.

Finally, we examined the effects of DMT in contextual fear extinction. After a fear conditioning (6 shocks of 1.0 mA, 2 s each, no tone pairings), animals were given a day off, then administered an acute dose of DMT (10 mg/kg) 1 h prior to contextual fear extinction training for 3 days (**Figure 2.2.3-2f**). Freezing levels were measured each day. On the test day, animals administered DMT did not show differences from animals administered VEH (**Figure 2.2.3-2g,h**). This suggests acute doses of DMT do not facilitate context fear extinction.

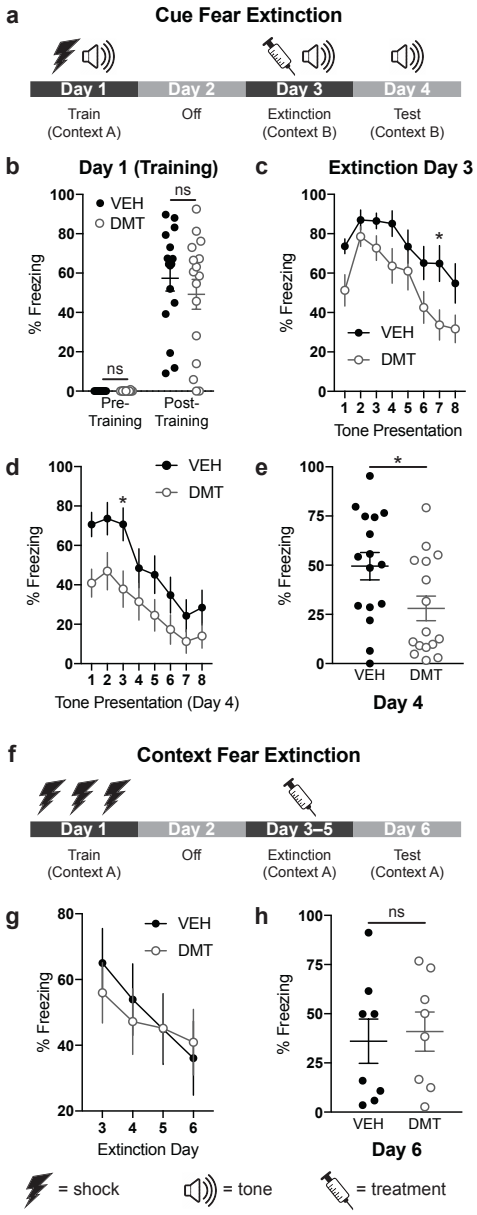

Figure 2.2.3-2. *Acute dose of DMT (10 mg/kg) facilitates cued, but not contextual, fear extinction. (a) Experimental design for the cued fear extinction experiment (N = 16 (VEH), N = 16 (DMT)). Following cued fear conditioning in context A, animals were dosed and subjected to one session of cued extinction training in context B on day 3. On day 4, the DMT-treated group demonstrated significantly lower freezing responses in the absence of drug. (b) Fear conditioning prior to drug treatment demonstrates that there is no difference between the two treatment groups, two-way ANOVA with Sidak post-hoc test. (c) Cued extinction during training day 3 demonstrates that administration of DMT 1h prior to training does not impair the initial recall of the fear memory but does enhance within session extinction. Two-way ANOVA with Sidak post-hoc test. (d) Percentage of time spent freezing during each of the eight auditory presentations on the test day (day 4). (e) Total percentage of time spent freezing during all eight auditory presentations on the test day (day 4). (f) Experimental design for the context extinction experiment (N = 8 (VEH) N = 8 (DMT)). Animals were fear conditioned in context A, and dosed prior to contextual extinction training (days 3−5). On day 6, contextual fear memory was assessed in the absence of drug. (g) Percentage of time spent freezing during the entire 10 min session on each of the extinction days. Both treatment groups effectively extinguish contextual fear memories over time. The extinction day had a significant effect of freezing levels (p = 0.0073) as analyzed using a repeated measures two-way ANOVA. (h) Individual data points for the contextual extinction test in the absence of drug on day 6.*

*N = 8 rats per condition. Data represents mean ± SEM. *p < 0.05. VEH = vehicle, DMT = N,N-dimethyltryptamine, KET = ketamine, ns =not significant.*

2.3 Psychedelics increase structural and functional plasticity in the medial prefrontal cortex

Studies have demonstrated that compounds capable of restoring neuronal structure and function have tremendous therapeutic value.[9] Given the therapeutic responses we saw in rodent behavioural paradigms, we sought to determine if there were changes to the structure and function to the PFC, an area of the brain responsible for motivation by exerting top-down control to other brain regions involving fear and reward.[10]

2.3.1 Psychedelics increase growth of cortical neurons

Using primary neuronal cultures, we demonstrate that cortical neurons can grow in response to application of psychedelics, including DMT. Dendritic outgrowth (as measured by Sholl analysis, **Figure 2.3.1-1**), spine number and synapse number are all increased *in vitro* after application of compound (**Figure 2.3.1-2a-d**).[11] In addition, we found that spine number was also increased in

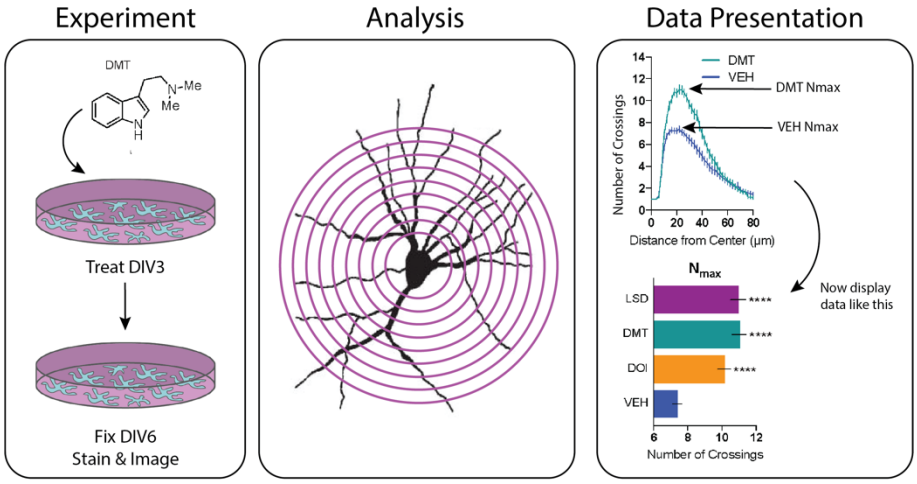

Figure 2.3.1-1*. Schematic of Sholl analysis. Rat embryonic cortical neurons are cultured in a dish, treated with drug on their 3rd day in vitro (DIV3), fixed and stained on DIV6. Sholl analysis is done by making concentric circles from the centre of the cell body at regular intervals. Each time the circle intersects with a neuronal process, that is counted. If neurons are more branched, they will have more intersections. This can be plotted as the number of intersections by the distance from the centre of the cell. The maximum number of intersections at a given distance is referred to as the Nmax.*

mPFC neurons in vivo, after a 24 h treatment with 10 mg/kg DMT (**Figure 2.3.1-2e,f**).[11] This effect was comparable to ketamine-treated animals with no differences between apical and basal spine growth (**Figure 2.3.1-2f**).

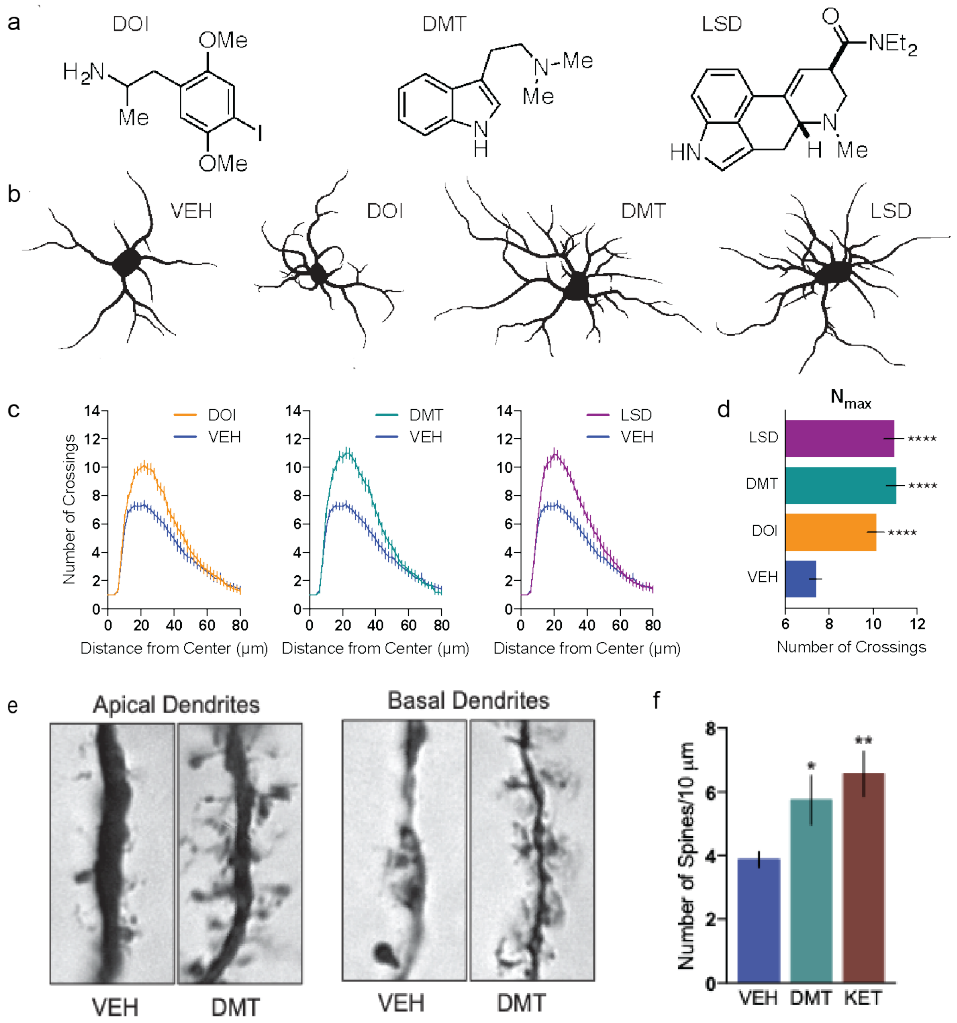

Figure 2.3.1-2. *Psychedelics promote structural growth in cortical neurons. (a) Chemical structures of psychedelics. (b) Representative tracings of cortical neurons (DIV6) treated with compounds. (c) Sholl analysis demonstrates that psychedelics increase dendritic arbor complexity. N = 39–41 neurons. (d) Maximum number of crossings (Nmax) of the Sholl plots in (c), one-way ANOVA with Dunnett's post-hoc test. (e) Representative images of Golgi-Cox-stained pyramidal neurons from the PFC of rats 24 h after receiving a 10 mg/kg dose of DMT. (f) Quantification of spines from (e), demonstrating that DMT (10 mg/kg) increases spinogenesis in vivo to a comparable extent as ketamine (10 mg/kg). N = 11–17 neurons.*

*Data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, as compared to vehicle control using a one-way ANOVA with Dunnett's post-hoc test. Scale bars, 30mm. VEH = vehicle, DMT = N,N-dimethyltryptamine, DOI = 2,5-dimethoxy-4-iodoamphetamine, LSD = lysergic acid diethylamide, KET = ketamine, ns =not significant.*

Data in (c, d) was generated by Calvin Ly (Ly et al., Cell Reports*, 2018), though I have replicated this as well since the paper's publication*[11]

2.3.2 Psychedelics increase the function of neurons cortical neurons

To determine if psychedelic compounds changed the function of cortical neurons in addition to structure, we injected female Sprague-Dawley rats (~8 weeks old) with either saline or DMT (10 mg/kg). Twenty-four hours after injection, animals were transcardially perfused with ACSF and cells were patched and data was analyzed for spontaneous excitatory post synaptic currents (sEPSCs). We found that both the frequency and amplitude of sEPSCs were increased after treatment with DMT (**Figure 2.3.2-1**).[11]

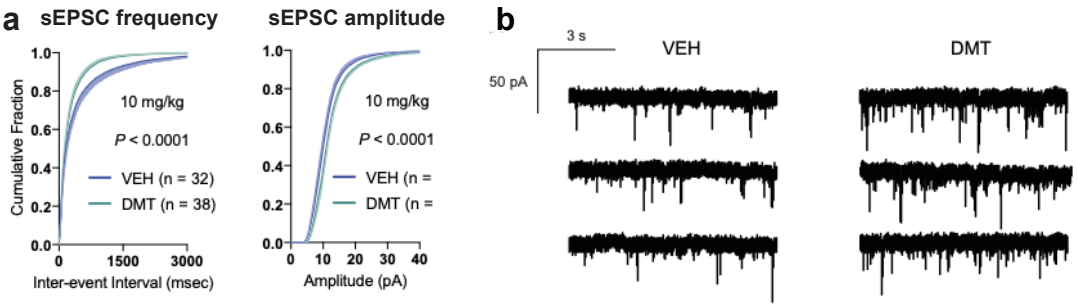

Figure 2.3.2-1. *Psychedelics promote functional plasticity. (a) Whole-cell voltage-clamp recordings of layer V pyramidal neurons from slices obtained 24 hr after DMT treatment (10 mg/kg and 1 mg/kg) demonstrate that DMT increases both spontaneous excitatory postsynaptic current (sEPSC) frequency and amplitude). (b) Representative traces for the 10 mg/kg experiments quantified in (a).*

N = 11–38 neurons from 3 animals. Probability distributions were compared using a Kolmogorov-Smirnov test. VEH = vehicle, DMT = N,N-dimethyltryptamine.

2.4 Conclusion and Discussion

The results from this study suggest that acute DMT (10 mg/kg) has antidepressant effects and facilitates fear extinction. We postulate that this is due to increasing the growth and function of neurons in the prefrontal cortex.

The antidepressant-like effects found in this study are congruent with the previous study by Pic-Taylor using ayahuasca.[7] This also mirrors human studies in which ayahuasca has been shown to decrease depression.[2,3,4,5]

Interestingly, we found that acute DMT treatment was anxiogenic. Pic-Taylor and colleagues found ayahuasca to be anxiolytic[7]; however both these studies suffer from impaired locomotion of animals at the high doses. Alternatively, the difference in anxiety-like behaviours may be attributed to the fact that this group used an ayahuasca brew—containing many harmala alkaloids as well—which themselves were able to produce an anxiolytic effect, or the combination. Unfortunately, the exact dose of DMT in this study is difficult to determine based on the nature of the tea, so this may also be attributed to differences in dose.

Effects of DMT may be acutely anxiogenic, however it is unclear if humans—who would have a cognitive understanding that they would undergo a psychedelic experience—would experience this same reaction. Studies using ayahuasca indicate significant decreases in hopelessness and panic-like parameters in humans using a double-blind investigation.[5] As stated before, it is extraordinarily difficult to properly run a placebo-controlled experiment when studying psychedelics in humans.

The DMT study facilitating fear extinction learning is designed to mirror current human clinical trials in which patients are given a psychedelic coupled with psychotherapy (ie. a psychoplastogen with 'training'). Though no trials exist investigating DMT or ayahuasca for PTSD

treatment, it has been suggested by experts[12] and there are anecdotal reports of individuals self-medicating using the tisane.[13] Several psychedelics, including MDMA,[14] psilocybin[15] and ketamine[16] have demonstrated efficacy in accelerating fear extinction learning in rodents. In humans, MDMA was given breakthrough therapy status by the FDA in 2017 and has been proven to be highly efficacious in human clinical trials.[17]

Increases in dendritogenesis, spinogenesis and synaptogenesis were seen after acute DMT treatment. Complementary to this, increases in sEPSCs can be due to an increase in the number of synapses—which would be congruent with the spinogenesis data—but may also suggest an increase in presynaptic release frequency. Both changes in structure and in function have been found after treatment with ketamine in similar paradigms.[18]

2.4.1 Advances in the field of psychedelic medicine

Our paper demonstrates therapeutic efficacy of psychedelics in rodent models of antidepressant efficacy and related neuropsychiatric disorders. Since the release of this paper, several more studies have followed up, demonstrating therapeutic efficacy in rodent behavioural paradigms for other psychedelic compounds, particularly psilocybin.[19] Of interest, a recent paper by the Kwan group demonstrated that not only did a 1 mg/kg dose of psilocybin create increases in spine density in vivo which persist and are correlated with antidepressant responses.[19] This group also performed miniature excitatory postsynaptic current (mEPSC) recordings and found both an increase in amplitude and frequency in layer 5 pyramidal neurons in the cortex.[19]

In this same vein, a recent unpublished article by de la Fuente Revenga and colleagues in BioRxiv demonstrates that psychedelics facilitate fear extinction learning and that this effect is

dependent on the presence of the 5HT2A receptor.[20] Further discussion of psychedelics' mechanism of action can be found in **Chapter 5: Mechanism of Action**.

Human studies continue to demonstrate long-lasting efficacy for both regular and ayahuasca-naïve individuals.[21] Furthermore, there is evidence to suggest that DMT is capable of promoting neurogenesis in human hippocampal neurons in vitro and in vivo.[22]

2.5 Methods

2.5.1 Animals

Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA, USA), were housed two per cage, and were given ad libitum access to food and water. Lights in the vivarium were turned on at 07:00 hours and turned off at 19:00 hours. All experiments were performed on 8- to 14-week-old male rats. Studies were performed during the light-on phase, with experiments taking place between 08:00 and 18:00 hours. All experimental procedures involving animals were approved by the UC Davis Institutional Animal Care and Use Committee (IACUC) and adhered to principles described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The University of California, Davis is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

2.5.2 Drugs

The DMT utilized in these studies was synthesized in our laboratory using the following procedure. To an ice-cold solution of tryptamine (0.50 g, 3.1 mmol), 37% formaldehyde$_{(aq)}$ (0.66 ml, 8.1 mmol, 2.6 equiv), and glacial acetic acid (0.89 ml, 15 mmol, 5.0 equiv) in MeOH (49 ml) was added sodium cyanoborohydride (0.39 g, 6.2 mmol, 2.0 eq.). The reaction was stirred at room temperature for 5 h before being diluted with 1M $NaOH_{(aq)}$ (100 ml) and CH_2Cl_2 (50 ml). The phases were separated and the aqueous phase was extracted twice with CH_2Cl_2 (50 ml). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The unpurified material was dissolved in acetone (15 ml) and added to a boiling solution of fumaric acid (0.26 g, 2.2 mmol, 0.7 equiv) in acetone (50 ml). A precipitate formed

immediately, and the solution was cooled to room temperature prior to being filtered. The resulting white solid was triturated with cold solvent, filtered, and dried under reduced pressure to yield the pure compound as the fumarate salt (2:1 DMT:fumaric acid) (white solid, 0.48 g, 62%): TLC R_f (DMT free base) = 0.35 (9:1 CH_2Cl_2:MeOH with 1% $NH_4OH_{(aq)}$); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.8 (s, 1H), 7.5 (d, 1H , J = 7.9 Hz), 7.3 (d, 1H, J = 7.9 Hz), 7.1 (s, 1H), 7.0 (t, 1H, J = 7.9 Hz), 6.9 (dd, 1H, J = 7.9 Hz), 6.5 (s, 1H), 2.9 (t, 2H, J = 8.6 Hz), 2.8 (t, 2H, J = 8.6 Hz), 2.4 (s, 6H) ppm; ^{13}C NMR (100 MHz, DMSO) δ 168.5, 136.7, 135.5, 127.5, 123.2, 121.4, 118.7, 111.9, 111.5, 100.0, 58.8, 43.9, 22.1 ppm; IR (diamond, ATR**)** ν 3483, 3146, 3107, 3045, 2927, 2881, 1561, 1226, 749cm^{-1}. The compound was stored at -20ºC in the dark prior to use. The prepared DMT was judged to be analytically pure by both LC-MS and NMR spectroscopy. For each experiment, a solution of DMT•fumarate (2:1) in 0.9% sterile saline was freshly prepared and passed through a 0.2 μm syringe filter. For all experiments, DMT•fumarate (2:1) was administered at 10 mg/kg via intraperitoneal injection using an injection volume of 1 mL/kg. Ketamine•HCl was purchased from Fagron and administered at 10 mg/kg via intraperitoneal injection using an injection volume of 1 mL/kg.

2.5.3 Novelty-Induced Locomotion (NIL)

Rats were administered either DMT or vehicle 1 hr before exposure to the open-field apparatus. Animals were allowed to acclimate to the test room for 10 mins prior to being gently placed into the center of a Digiscan (Model RXYZCM(16)CCD) open field chamber and allowed to freely explore the chamber for 45 mins. At the conclusion of the test, animals were returned to their home cages and the test chambers cleaned with 10% Nolvasan. Horizontal motion, rotations, and stereotypies (repetitive beam breaks) were recorded in 1-min intervals for the duration of the test.

The margin of the arena was defined as being 10 cm from the wall. The open field chamber measured 16.5” L x 16.5” W x 11.25” H and was illuminated to between 25 and 30 lux.

2.5.4 Elevated Plus Maze (EPM)

The EPM apparatus consisted of a plus-shaped black plastic platform positioned 50 cm above the ground. Two opposite arms of the maze were bordered by vertical walls measuring 12.5” high, with the other two arms possessing unprotected edges. Rats were administered DMT or vehicle 1 hr before being placed into the center of the maze facing an open arm and allowed to explore freely for 5 min. At the conclusion of the test, rats were returned to their home cages and the apparatus was cleaned with 10% Nolvasan. Animal movement was recorded and analyzed during the trial using EthoVision XT (version 9) software.

2.5.5 Fear Conditioning (FC)

On day 1, animals were administered either DMT or vehicle 1 hr prior to conditioning. They were placed in a fear conditioning apparatus (Med Associates) for 3.5 min prior to three presentations of auditory cues (80 dB white noise, 30 s), each co-terminating with a foot shock (0.8 mA, 2 s.) and spaced 90 s apart. After the last shock, the animals remained in the chambers for an additional 2 min before being returned to their home cages. During fear conditioning, the apparatus was illuminated to 100 lux and did not contain any additional odor cues. On day 2, contextual fear memory was assessed by exposing the animals to the conditioning context for 10 min before returning them to their home cages. On day 3, cued fear memory was assessed by exposing the animals to a novel context (lights off, A-frame insert, floor insert, additional vanilla odor) for 2 min prior to eight presentations of auditory cues (80 dB white noise, 30 s) spaced 30 s apart.

Freezing responses for cue testing (day 3) are presented as the percentage of time spent freezing over mins 2–6 (i.e., the first 4 presentations). Fear conditioning experiments were performed between the hours of 08:00–11:00. Freezing behavior was scored using Med Associates Video Freeze software (motion threshold = 18 au, detection method = linear, minimum freeze duration = 30 frames). The apparatus was cleaned with 70% EtOH in between trials.

2.5.6 Cued Fear Extinction

On day 1, drug-naïve animals were fear conditioned as described above, but in the absence of drug, and allowed to rest on day 2. On day 3, the animals were administered either DMT or vehicle 1 hr prior to extinction training. Extinction training consisted of exposure to a novel context (lights off, A-frame insert, floor insert, additional vanilla odor) for 2 mins prior to 8 presentations of auditory cues (80 dB white noise, 30 s) spaced 30 s apart. After the extinction training, animals were returned to their home cages. The procedure was repeated on day 4 in the absence of drug. Fear extinction experiments were performed between the hours of 08:00–11:00. Freezing responses for cue testing (day 4) are presented as individual blocks (block 0 = first 2 mins, blocks 1–8 = each minute following a presentation) and as the percentage of time spent freezing over mins 2–6 (i.e., the first 4 presentations). Freezing behavior was scored using Med Associates Video Freeze software (motion threshold = 18 au, detection method = linear, minimum freeze duration = 30 frames). The apparatus was cleaned with 70% EtOH in between trials.

2.5.7 Contextual Fear Extinction

First, animals were subjected to an optimal foreground contextual fear conditioning protocol. Rats were placed into the fear conditioning apparatus for three mins before being subjected to six foot

shocks (1.0 mA, 2 s) spaced 58 s apart. After initiation of the last shock, the animals remained in the chambers for an additional 2 min before being returned to their home cages. The animals were allowed to rest on day 2. On day 3, these drug-naïve rodents were administered either DMT or vehicle 1 hr prior being placed in the fear conditioning context for 10 min. This procedure was repeated on days 4 and 5. On day 6, the animals were placed in the fear conditioning context without receiving any injections. Contextual fear conditioning experiments were performed between the hours of 08:00–11:00. Freezing behavior was scored using Med Associates Video Freeze software (motion threshold = 18 au, detection method = linear, minimum freeze duration = 30 frames) and reported in 1 min blocks and as the percentage of time spent freezing over the entire 10 min session. The apparatus was cleaned with 70% EtOH in between trials.

2.5.8 Forced Swim Test

The FST apparatus consisted of a clear Plexiglas cylinder measuring 80 cm tall, 20 cm in diameter and filled with 30 cm of 24 ± 1ºC water. Fresh water was used for every rat. Animals were subjected to a pre-test phase in which they were placed in the cylinder for 15 mins before being dried and returned to their home cage. Twenty-four hours later, rats were again placed in the FST apparatus for 5 mins and their activity was video recorded. Each rat received three administrations of DMT, ketamine, or vehicle at 23.5, 6, and 1 hr before the test phase. Each video was scored for immobility, swimming, and climbing behavior by a trained observer.

2.5.9 Data Analysis

Statistical analyses were performed using GraphPad Prism (version 7.0a) on aggregated data, but not on data presented as line graphs (i.e., Figure 2(b), Figure 4(b), and Figure 4(e)). Comparisons

of DMT- and vehicle-treated groups were accomplished using a two-tailed student's t-test. As data from contextual extinction experiments (Figure 4(c) and 4(f)) displayed a bimodal distribution, a Mann-Whitney Test was used instead. To analyze data from the FST (Figure 5(b)), which involved multiple comparisons, a one-way analysis of variance (ANOVA) was utilized with Tukey's post hoc test. All data are represented as means SEM, NS = not significant, *$p < 0.05$, **$p < 0.01$.

2.5.10 Golgi-Cox Staining

Female Sprague Dawley rats (~8 weeks old) were given an intraperitoneal injection of DMT, ketamine, or vehicle and sacrificed via decapitation 24 h later. Tissue was prepared following the protocol outlined in the FD Neurotechnologies Rapid GolgiStain Kit (FD Neurotechnologies) with slight modifications. Brains were stored in solution C for 2 months prior to slicing into 120 μm sections using a vibratome. These slices were placed onto microscope slides that were pre-coated with (3- aminopropyl)triethoxysilane. Slices were air dried for a week before staining. Slides were immersed in water twice for 2 minutes, DE solution for 10 minutes, and then water for 2 minutes. After this, slides were immersed sequentially in 25% ethanol for 1 minute, 50% ethanol for 4 minutes, 75% ethanol for 4 minutes, 95% ethanol for 4 minutes, and 100% ethanol for 4 minutes. Slides were then briefly dipped into xylenes before being mounted using DPX Mountant For Histology (Sigma), air-dried, and imaged on a Zeiss AxioScope. Spines were traced in three dimensions using Neurolucida software (version 10) at 100x magnification. Data acquisition and analysis was performed by an experimenter blinded to treatment conditions. Data represents individual neurons taken from 3 different animals per treatment.

2.5.11 Electrophysiology

Female Sprague Dawley rats (~8 weeks old) were given an intraperitoneal injection of DMT or vehicle. After 24 h, rats were anesthetized with isofluorane and transcardially perfused with ice-cold artificial cerebrospinal fluid (ACSF), containing 119 mM NaCl, 26.2 mM NaHCO3, 11 mM glucose, 2.5 mM KCl, 1 mM NaH2PO4, 2.5 mM CaCl2 and 1.3 mM MgSO4. Brains were rapidly removed and 300 μm coronal slices from the mPFC were cut on a Leica VT1200 vibratome (Buffalo Grove, IL) with ice-cold ACSF solution. Slices were incubated in 32 °C NMDG solution for 10 minutes, transferred to room temperature ACSF, and held for at least 50 minutes before recording. All solutions were vigorously perfused with 95% O2 and 5% CO2. Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded at -70 mV in 32 °C ACSF. Cells were patched with 3–5 MΩ borosilicate pipettes filled with intracellular solution containing 135 mM cesium methanesulfonate, 8 mM NaCl, 10 mM HEPES, 0.3 mM Na-GTP, 4 mM Mg-ATP, 0.3 mM EGTA, and 5 mM QX-314 (Sigma, St Louis, MO). Series resistance was monitored throughout experiments; cells were discarded if series resistance varied more than 25%. All recordings were obtained with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Analysis was performed with the Mini Analysis program (Synaptosoft, Decatur, GA) with a 4 pA detection threshold. Data represents individual neurons taken from 3 different animals per treatment. Data acquisition and analysis was performed by experimenters blinded to treatment conditions.

2.6 Contributions & Collaborations

I couldn't have done this alone. Thank you to all my collaborators who helped me get this far. Contributions are outlined below.

Charles J. Benson performed behavioural experiments and LPC executed all data analyses with supervision from DEO. Calvin Ly generated neuronal cultures, treated with psychedelic compounds and performed Sholl analysis with supervision from DEO. LPC completed the Golgi-Cox staining and analysis. Lee E. Dunlap synthesized the DMT necessary for this experiment.

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University of California, Davis

Jon Wong, Eden Barragan, LPC and John A. Gray performed the electrophysiology studies with input from LPC and DEO.

Gray Lab

University of California, Davis

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Chapter 3

PSYCHEDELIC MICRODOSING

Psychedelic microdosing—the practice of taking subhallucinogenic doses of psychedelic compound every 2-3 days—is an increasingly popular practice, with individuals who participate in the practice claiming improvements in cognitive and emotional function. The process was described by Ayelet Waldman in her book *A Really Good Day: How Microdosing Made a Mega Difference in My Mood, My Marriage, and My Life*[1] and James Fadiman's *The Psychedelic Explorer's Guide: Safe, Therapeutic, and Sacred Journeys*.[2] Both of these sources describe the routine of microdosing and anecdotally claim improvements in cognitive and emotional function. The practice of psychedelic microdosing has become very popular, especially in places like Silicon Valley[3] and as such, studies investigating both the therapeutic claims as well as the dangers associated with microdosing are imperative.

3.1 Human experiences with psychedelic microdosing

To investigate the claims associated with human psychedelic microdosing, we launched an online survey to determine the prevalence and subjective effects of individuals who partake in psychedelic microdosing.[4] The survey was described as an anonymous research study on "Recreational Drug and Alcohol Use" to prevent biasing the study towards participants with extensive knowledge and/or experience with psychedelics. Participants were recruited via

snowball sampling through a number of outlets including social media (e.g., Facebook, Twitter, Instagram), our research group's website (www.olsonlab.org), drug- and nondrug-related online forums (Reddit, Craigslist), as well as distribution of pamphlets across the UC Davis campus and community events (e.g., local farmers market). Participants could choose to skip questions if they preferred. From April to August 2018, we sampled 2347 people.

It is important to understand that the following human survey data (in this Section 3.1) is from individuals who are both self-medicating and self-reporting. There is no placebo-controlled group in this study.

3.1.1 Prevalence of psychedelic microdosing

We found that many participants (59.41%, N = 2183) were familiar with the concept of psychedelic microdosing (**Figure 3.1.1-1a**), and a surprising number of individuals said they previously (13%) or actively (4%) participate in the practice (N = 2200, **Figure 3.1.1-1b**).

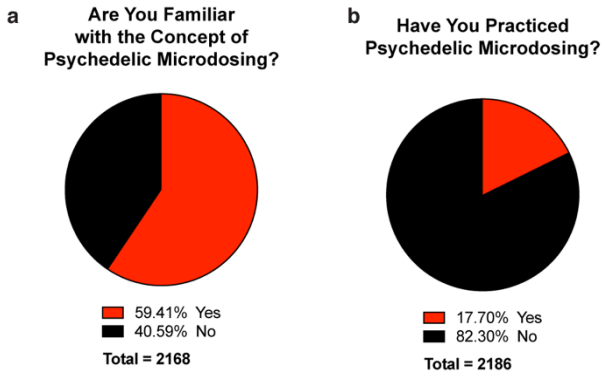

Figure 3.1.1-1*. Prevalence of psychedelic microdosing. (a) 59% of respondents indicated they are familiar with the practice of psychedelic microdosing. (b) 13% of respondents indicated they had microdosed at some point in their life and 4% indicate they currently microdose psychedelic compounds.*

3.1.2 Demographics of individuals who participate in psychedelic microdosing

Of the individuals that have experience with microdosing, the average age was 33.26 (N = 290, **Table 3.1-1**). Of the microdosers that reported gender, males were significantly more likely to report engaging in this practice (64.16%, **Table 3.1-1**, $\chi 2 = 31.81$, df = 3, $p < 0.001$). Although anecdotal reports suggest that microdosing may be more prevalent in the tech industry, we found this was not the case. In fact, we found no association with occupation and microdosing history (**Table 3.1-1**).

Education was significantly associated with microdosing practices, with less educated individuals reporting more frequent use (N = 1705, **Table 3.1-1**, $\chi 2 = 26.49$, df = 1, $p < 0.01$).

Participants were divided into lower-income (<$50,000), middle-income ($50,000-$99,999), and upper-income ($100,000+) households according to the definition of each group provided by the Pew Research Center.[5] Participants from lower socioeconomic statuses were significantly more likely to engage in psychedelic microdosing (N = 1699, **Table 3.1-1**, $\chi 2 = 11.43$, df = 2, $p<0.01$).

Interestingly, 27% of veterans surveyed reported experimenting with psychedelic microdosing. This is a significantly greater proportion of veterans who microdose compared to the general population (**Table 3.1-1**, $\chi 2=5.16$, df=1, p=0.02).

Table 3.1-1. *Demographic characteristics of respondents (N=2437).*

	Total (n = 2437) % (n)	Non-Microdosers (n = 2054) % (n)	Microdosers (n = 383) % (n)
Age (mean, SD)	(1687)	35.62, 14.12	33.26, 14.15
No Response	n = 750	n = 657	n = 93
Gender			
Male	50.09 (855)	47.17 (667)	64.16 (188)
Female	48.74 (832)	51.76 (732)	34.13 (100)
Other	0.47 (8)	0.53 (5)	1.02 (3)
Prefer not to say	0.70 (12)	0.71 (10)	0.68 (2)
No Response	n = 730	n = 640	n = 90
Employment			
Non-Tech Industry	40.99 (698)	84.53 (590)	15.47 (108)
Tech Industry	9.04 (154)	79.87 (123)	20.13 (31)
Student	30.48 (519)	80.27 (427)	17.72 (92)
Retired	6.93 (118)	79.66 (94)	20.34 (24)
Unemployed	12.57 (214)	82.24 (176)	17.76 (38)
No Response	n = 734	n = 644	n = 90
Veteran Status			
Non-veteran	95.48 (1625)	96.03 (1355)	92.78 (270)
Veteran	4.52 (77)	3.97 (56)	7.22 (21)
No Response	n = 735	n = 643	n = 92
Education Level			
No High School Diploma	2.17 (37)	1.91 (27)	3.44 (10)
High School or equivalent	31.73 (541)	29.28 (414)	43.64 (127)
Associate or Bachelor degree	42.99 (733)	43.64 (617)	39.86 (116)
Master degree or higher	23.11 (394)	25.18 (356)	13.05 (38)
No Response	n = 732	n = 640	n = 92
Income level			
< $49,999	66.89 (1133)	64.96 (914)	75.00 (219)
$50,000 - $99,999	21.01 (357)	21.89 (308)	16.78 (49)
> $100,000	12.30 (209)	13.15 (185)	8.22 (24)
No Response	n = 738	n = 647	n = 91

3.1.3 Common drugs used for psychedelic microdosing

LSD and psilocybin were the most common drugs used for psychedelic microdosing (48.58% and 26.18% respectively, N = 317, **Table 3.1-2**). Individuals who selected "Other" (11.67%, n = 37, N = 317) reported using marijuana, cocaine, or a combination of psychedelic compounds (i.e., LSD and psilocybin or LSD and ayahuasca). We did not collect information regarding the dose or dosing regimen because previous work has demonstrated that users largely estimate the dosage reported.[6,7] Additionally, dosage can vary according to factors that cannot be controlled in survey studies, such as the compound used, the purity of the substance used, and the weight or body composition of the individual.

Table 3.1-2*. Psychedelics used in psychedelic microdosing (N = 383).*

	% (n)
LSD	48.58 (154)
DMT	1.58 (5)
Psilocybin	26.18 (83)
MDMA	11.99 (38)
Poly-Drug Use	7.89 (25)
Other	3.78 (12)
No Response	n = 66

3.1.4 Subjective effects of psychedelic microdosing in humans

People who participate in microdosing were likely to report an improvement in depressive symptoms (Improvement: 71.84%, Worsening: 4.77%, No Effect: 23.42%; N = 316, **Table 3.1-3**, $\chi 2 = 227.32$, df = 2, $p<0.001$). In addition, we found that although both males and females were likely to report an improvement in symptoms, males were more likely to report positive outcomes ($\chi 2 = 13.57$, df = 2, $p < 0.001$). This difference may have been driven by a greater proportion of

improvement in males, and a greater percentage of females reporting worsening of symptoms (11.00% of responses, compared to 1.61% in males).

***Table 3.1-3**. Effects of psychedelic microdosing by gender (N = 383).*

	Total sample (n = 383) % (n)	Male (n = 188) % (n)	Female (n = 100) % (n)	Gender not specified (n = 95) % (n)
Depression				
Improvement	71.84 (227)	77.96 (145)	65.00 (65)	56.67 (17)
No Effect	23.42 (74)	20.43 (38)	24.00 (24)	40.00 (12)
Worsening	4.75 (15)	1.61 (3)	11.00 (11)	3.33 (1)
No Response	n = 67	n = 2	n = 0	n = 65
Anxiety				
Improvement	56.55 (177)	61.20 (112)	53.00 (53)	40.00 (12)
No Effect	30.35 (95)	28.42 (52)	29.00 (29)	46.67 (14)
Worsening	13.10 (41)	10.38 (19)	18.00 (18)	13.33 (4)
No Response	n = 70	n = 5	n = 0	n = 65
Memory				
Improvement	38.85 (122)	43.48 (80)	30.00 (30)	40.00 (12)
No Effect	46.50 (146)	48.37 (89)	43.00 (43)	46.67 (14)
Worsening	14.65 (46)	8.15 (15)	27.00 (27)	13.33 (4)
No Response	n = 69	n = 4	n = 0	n = 65
Focus/Attention				
Improvement	58.97 (184)	66.30 (122)	44.44 (44)	62.07 (18)
No Effect	26.28 (82)	22.83 (42)	32.32 (32)	27.59 (8)
Worsening	14.74 (46)	10.87 (20)	23.23 (23)	10.34 (3)
No Response	n = 71	n = 4	n = 1	n = 66
Sociability				
Improvement	66.56 (209)	70.11 (129)	60.00 (60)	66.67 (20)
No Effect	22.29 (70)	21.74 (40)	21.00 (21)	30.00 (9)
Worsening	11.15 (35)	8.15 (15)	19.00 (19)	3.33 (1)
No Response	n = 69	n = 4	n = 0	n = 65

Microdosers were also likely to report improvements in anxiety (Improvement: 56.55%, Worsening: 13.10%, No Effect: 30.35%; N = 313, **Table 3.1-3**, $\chi 2 = 89.89$, df = 2, $p < 0.001$). There was a trend for a larger improvement in males, however this was not significant.

Individuals who responded to the survey item also reported significant improvements to memory (N = 313, **Table 3.1-3**, $\chi 2 = 89.89$, df = 2, $p < 0.001$). There was a significant difference in the observed difference between males and females ($\chi 2 = 19.00$, df = 2, $p < 0.001$), though this was likely to have been driven by a greater proportion of males reporting improvements and/or females reporting worsening of symptoms.

Changes in attentional focus were also reported to be significant (N = 314, **Table 3.1-3**, $\chi 2 = 52.07$, df = 2, $p < 0.001$). Again, this change was seen to be greater in males than females ($N_T=283$, $\chi 2=13.93$, df=2, $p<0.001$).

Finally, individuals who microdose reported a change in sociability (N = 314, **Table 3.1-3**, $\chi 2 = 161.85$, df = 2, $p < 0.001$), with males reporting significantly greater improvements than females ($\chi 2 = 7.38$, df = 2, $p = 0.025$).

3.1.5 Physical changes associated with psychedelic microdosing

Individuals could elaborate on any physical changes they experienced with a text response, which were subsequently analyzed for thematic content. Two-thirds (65.63%) of responses were related to positive physical or emotional outcomes (e.g., “working out so body composition is improving”, “slight euphoria w/ on-set that lasts throughout initial peak 2-3 hrs”, “better workouts?”) and 31.25% of responses cited negative outcomes (e.g., “occasional ‘swimmy’ vision”, “Memory is pretty bad”, “sweats”). Approximately half (49.21%) of the responses were not related to changes in weight.

3.1.6 Deterrents to microdosing

Individuals who discontinued the practice of psychedelic microdosing were asked why. Interestingly, the most cited reasons were related to the risk associated with taking illegal substances (N = 59, 24.28%), the difficulty of obtaining the drug (N = 55, 22.63%), or the cost (N = 20, 8:23%). Interestingly, few people stopped the practice due to not achieving the effects they wanted (N = 12, 4.94%) or side effects (N = 10, 4.12%).

For the 36.80% of individuals that responded "Other" were prompted to elaborate with a text response. Themes that surfaced from these responses included: a desire to microdose infrequently (e.g., "It isn't something I want to do everyday," "It's not something I felt like doing all the time"), a fear of dependency (e.g., "Did not want to become addicted," "The physical euphoria brought on a sense of dependency that isn't present on a normal dose"), aging ("Getting older," "Gotten older, health issues"), and unstandardized dosing regimens ("Psilocybin is too easy to overdose without a standardized preparation. DMT has proved easier to administer in a threshold dose," "LSD I got wasn't properly perforated making the proper dosing hard to do").

Table 3.1-4*. Deterrents from microdosing (N = 383).*

	Total Responses % (n)
Too expensive	8.23 (20)
Too difficult to obtain materials	22.63 (55)
Too risky due to legal concerns	24.28 (59)
Wasn't getting the effects I was hoping for	4.94 (12)
Side effects	4.12 (10)
Other	35.80 (87)
No Response	n = 140

This psychedelic microdosing survey was useful for collecting information on the demographics of individuals who participate in the practice and quantify the perceived changes. It must be openly acknowledged that these individuals are both self-medicating and self-reporting. For a further discussion of these results, please see Section **3.3 Conclusion and Discussion**.

3.2 Psychedelic microdosing in rodent models

At the beginning of this project, there were no academic articles on psychedelic microdosing and only a couple by the time this manuscript was published. We chose to study psychedelic microdosing in rodents because they do not have a preconceived notion of having a therapeutic outcome in response to a psychedelic drug, and therefore we can bypass the issue of placebo groups that commonly plagues human clinical trials.

For this study, we gave rats low doses (1 mg/kg) of DMT or VEH on a chronic (~2 months), intermittent (every three days) schedule.[8] After two weeks (5 doses), we began to test animals in behavioral paradigms from least to most stressful that were designed to test differences in mood, anxiety and cognitive function. A full schematic of the behavioral battery is outlined in **Figure 3.1.6-1**. Both male and female young adult Sprague Dawley rats were used. For assays in which there was no difference in sexes, data sets were combined.

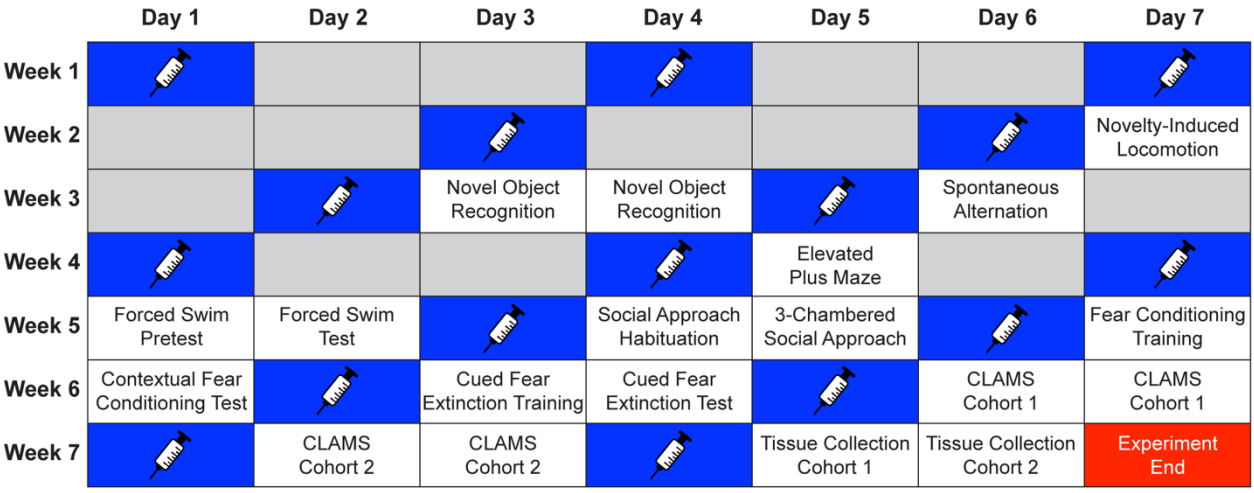

Figure 3.1.6-1. *Experimental design for testing the effects of chronic, intermittent, low doses of DMT on rats. Blue boxes indicate the days when drug was administered. Behavioral testing was performed on the days between doses. Gray boxes indicate days the animals spent in their home cages with no testing being performed. CLAMS = Comprehensive Lab Animal Monitoring System.*

There are several pieces of evidence that suggest that the 1 mg/kg dose used in this study was subhallucinogenic. Firstly, this dose only produces ~20% correct lever responding in drug discrimination tasks using Sprague Dawley rats trained to discriminate DMT from saline. Furthermore, it produces even fewer lever responses in rats trained to discriminate other hallucinogens such as (−)-2,5-dimethoxy-4-methylamphetamine (DOM, 0.5 mg/kg) or LSD (0.1 mg/kg) from saline.[9] Secondly, a 1 mg/kg dose not produce drastic changes in body posture or behavior that is characteristic of a fully hallucinogenic 10 mg/kg dose.[10] Finally, the 1 mg/kg dose is calculated to be non-hallucinogenic based on allometric scaling[11] of what is known to be a subhallucinogenic dose in humans.[12]

3.2.1 Chronic, intermittent, subhallucinogenic doses of DMT do not produce changes in anxiety, as tested in NIL or EPM tests

We tested both NIL and EPM to evaluate differences (if any) in animals that had been chronically administered subhallucinogenic doses of DMT. We found no significant difference in the total

distance travelled, the time spent in the margin vs the center, the time spent rearing or the number of time spent engaging in stereotypies (**Figure 3.2.1-1a**). There was a significant increase in the number of rearings in animals that underwent the microdosing regime, which may be reflective as an anxiolytic behaviour. When these behaviours are z-scored using the method developed by Guilloux et al.[13]— a method which increases sensitivity and reliability of behavioural phenotyping—there is no difference in anxiety behaviours between control and DMT-treated animals ($p = 0.08$, **Figure 3.2.1-1c**).

In the EPM, DMT-treated animals did not display any signs of anxiety, as measured by both the time and number of entries to the closed or open arms (**Figure 3.2.1-1b**). These measurements were also z-scored and revealed no significant effect between treatment groups (**Figure 3.2.1-1d**). Importantly, the treatment groups and sexes displayed similar levels of locomotion, as measured by the total distance travelled and velocity, so no ambiguity can be attributed to locomotor deficits (**Figure 3.2.1-1e**).

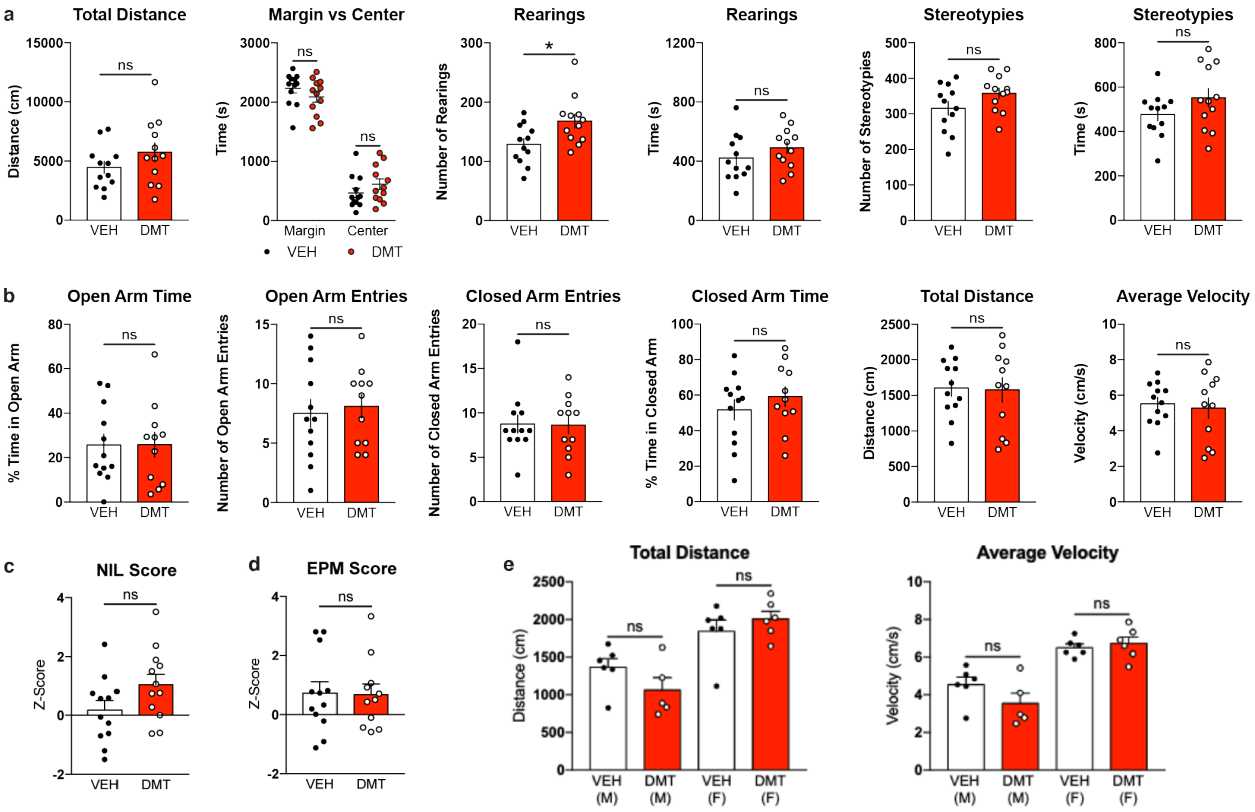

Figure 3.2.1-1. *Chronic, intermittent, low doses of DMT do not produce anxiogenic-like effects in rats. (a) Results from NIL assays for male and female animals treated with vehicle or microdosed with DMT. (b) DMT-treated and vehicle-treated groups display similar phenotypes in the NIL. (c) Results from EPM assays for male and female animals treated with vehicle or microdosed with DMT. (d) DMT-treated and vehicle-treated groups display similar phenotypes in the EPM. (e) Control experiments demonstrate that there is no difference in locomotor activity between the two treatment groups in the EPM.*

*N = 12 rats per condition. Data represents mean ± SEM. *p < 0.05. VEH = vehicle (saline), DMT = N,N-dimethyltryptamine, EPM = elevated plus maze, NIL = novelty-induced locomotion, M = males, F = females, ns = not significant.*

3.2.2 Effects of psychedelic microdosing on animal behaviours relevant to fear learning

After several weeks of psychedelic microdosing, we also tested contextual and cued fear memory following fear conditioning (**Figure 3.2.2-1a**). Prior to fear conditioning, rodents exhibited no freezing in response to the tone (**Figure 3.2.2-1b**). Following training, both VEH and DMT-treated animals exhibited similar amounts of freezing ($p = 0.13$, **Figure 3.2.2-1c**), suggesting that fear acquisition is no different between treatment groups. The day afterwards, contextual fear

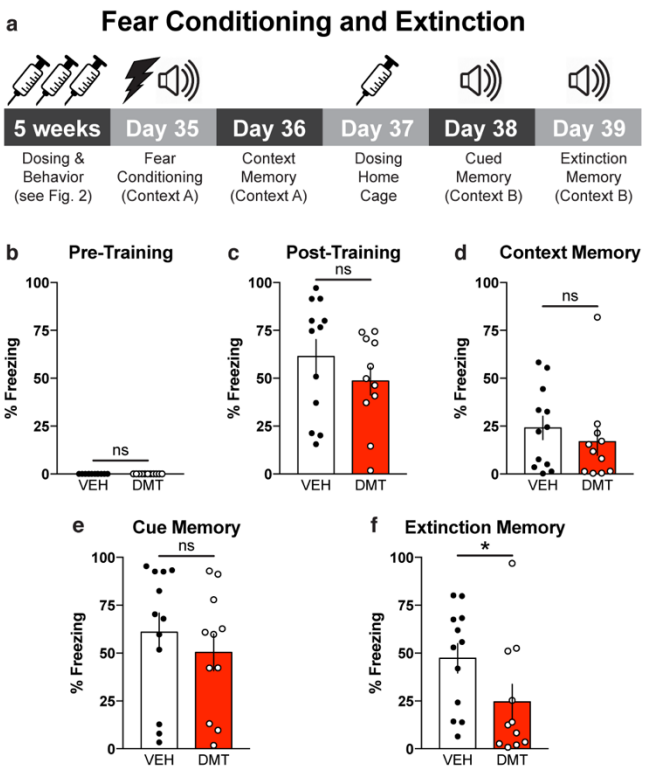

Figure 3.2.2-1. *Chronic, intermittent, low doses of DMT enhance fear extinction in rats. (a) Experimental design for the fear conditioning and extinction experiments. (b, c) DMT- and vehicle-treated groups displayed comparable levels of freezing in the 2 min period before (b) receiving foot shocks. Two-way ANOVA with Sidak's post-hoc test. (c) DMT- and VEH-treated groups display similar freezing levels after receiving foot shocks. Two-tailed unpaired t-test. (d, e) Neither contextual fear memory (d) nor cued fear memory (e) were impaired by chronic,intermittent treatment with low doses of DMT. Two-tailed unpaired t-test. (f) DMT-treated animals exhibited enhanced cued extinction memory as compared to vehicle-treated controls. Two-tailed unpaired t-test.*

*N = 11–12 rats per condition. Data represents mean ± SEM. *p < 0.05, **p < 0.01. VEH = vehicle (saline), DMT = N,N-dimethyltryptamine, ns = not significant.*

memory was assessed and no difference in contextual fear memory was observed ($p = 0.32$, **Figure 3.2.2-1d**). The following day, animals were administered DMT, as per their usual dosing regime, and allowed to rest in their home cage. The following day, animals were subjected to cue extinction training, which consisted of a novel context (context B) and 8 tone presentations with no foot shocks. Twenty-four hours later, animals were subjected to the same novel context (context B) and freezing rates were assessed to determine if animals remembered the extinction training from the previous day. Indeed, animals treated with chronic, intermittent low doses of DMT exhibited decreased freezing when testing fear extinction memory ($p = 0.03$, **Figure 3.2.2-1f**).

3.2.3 Effects of psychedelic microdosing on animal behaviours relevant to depression

To determine if psychedelic microdosing had anti-depressant like effects in rodents, we subjected the rodents to a forced swim test, comprising of the typical pretest followed by the test portion the next day. Though both male and female DMT-treated animals exhibited decreased immobility counts, only females were significant (p = 0.02, **Figure 3.2.3-1a,b**). Both males and females treated with psychedelic exhibited an increase in swimming (p = 0.04) and climbing behaviour (p = 0.05) compared to the VEH-treated groups (**Figure 3.2.3-1c,d**). Each of these effects predict that chronic, intermittent, low doses of DMT may have antidepressant efficacy.

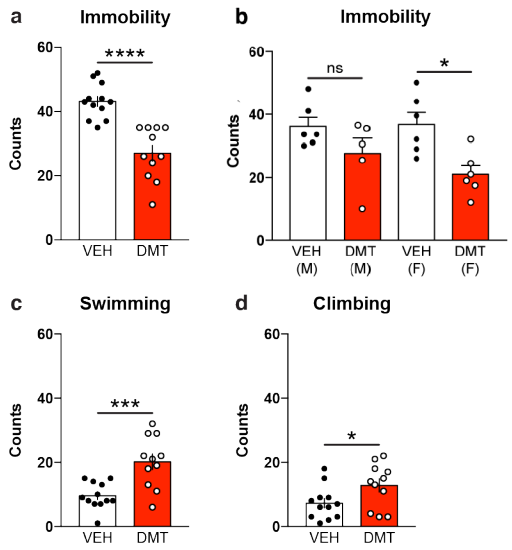

Figure 3.2.3-1. *Chronic, intermittent, low doses of DMT produce antidepressant-like effects in rats. (a) Psychedelic microdosing in rodents reduced immobility (p = 0.03), (b) though this was only significant in females (p = 0.02, males p = 0.37). This microdosing paradigm increased climbing (c), and increased swimming (d) in the FST.*

*N = 11–12 rats per condition. Data represents mean ± SEM. *p < 0.05. VEH = vehicle (saline), DMT = N,N-dimethyltryptamine, FST = forced swim test,, M = males, F = females, ns = not significant.*

3.2.4 Effects of psychedelic microdosing on animal behaviours relevant to working memory and sociability.

We tested the effects of chronic, intermittent low doses of DMT on working memory using the spontaneous alternation (SALT) and short-term memory using novel object recognition (NOR) paradigms. In SALT, deficits in spatial working memory or attention typically manifest as fewer alternations in this paradigm. However, we saw no difference between the VEH-treated and DMT-treated animals ($p = 0.93$, **Figure 3.2.4-1a, b**).

Similarly, NOR is typically used to assess short term memory. Both groups spent more time exploring the novel object, and no differences were observed between treatment groups (familiar $p = 0.65$, novel $p = 0.56$, **Figure 3.2.4-1c,d**).

Finally, we assessed the impact of psychedelic microdosing on social preference using the 3-chamber social approach assay in which the time spent with an object or another animal (drug-naïve conspecific) is quantified. This assay revealed a preference for social behaviour for both treatments groups, which were not significant when compared ($p = 0.20$, **Figure 3.2.4-1e**).

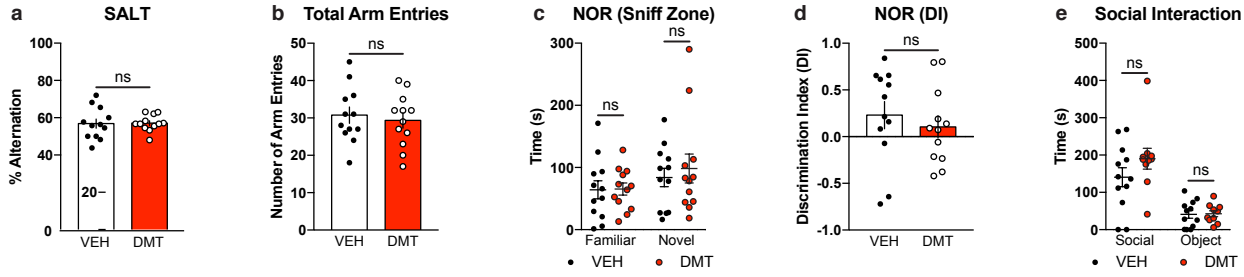

Figure 3.2.4-1. *Chronic, intermittent, low doses of DMT do not improve cognitive function or increase social interaction in rats. (a–b) In the spontaneous alternation behavior (SALT) paradigm, DMT- and vehicle-treated groups displayed comparable levels of percent alternation (a) and total arm entries (b). Two-tailed unpaired t-test. (c–d) DMT- and vehicle-treated groups displayed no differences in novel object recognition as determined by the total time spent in the "sniff zone" of either the novel or familiar objects (c) or by comparing the discrimination indices (DI) between the groups (d). A DI > 1, < 1, = 0 indicates a preference for the novel object, a preference for the familiar object, or no preference, respectively. Two-way ANOVA with Sidak's post-hoc test (c) and two-tailed unpaired t-test (d). (e) No differences were observed between treatment groups in the 3-chambered social approach with respect to the amount of time they spent in the "sniff zone" of either the conspecific or object. Two-way ANOVA with Sidak's post-hoc test.*

*N = 11–12 rats per condition. Data represents mean ± SEM. *p < 0.05. VEH = vehicle (saline), DMT = N,N-dimethyltryptamine, SALT = spontaneous alternation, NOR = novel object recognition, M = males, F = females, ns = not significant.*

3.2.5 Effects of psychedelic microdosing on cortical structure and function

At the end of the behavioural battery, we collected brain tissue to assess changes to neuronal structure. As indicated previously, stress-related disorders tend to decrease the spine density pyramidal neurons in the prefrontal cortex,[14] while psychedelics tend to increase the spine density in the prefrontal cortex.[21] We predicted that chronic, intermittent low doses of DMT would increase spine density in the prefrontal cortex, a phenomenon that would be consistent with the improvements in mood and anxiety. Interestingly, we found that while males had no change in spine density ($p = 0.98$), females exhibited a *decrease* in spine density which was significant ($p = 0.03$, **Figure 3.2.5-1a,b**).

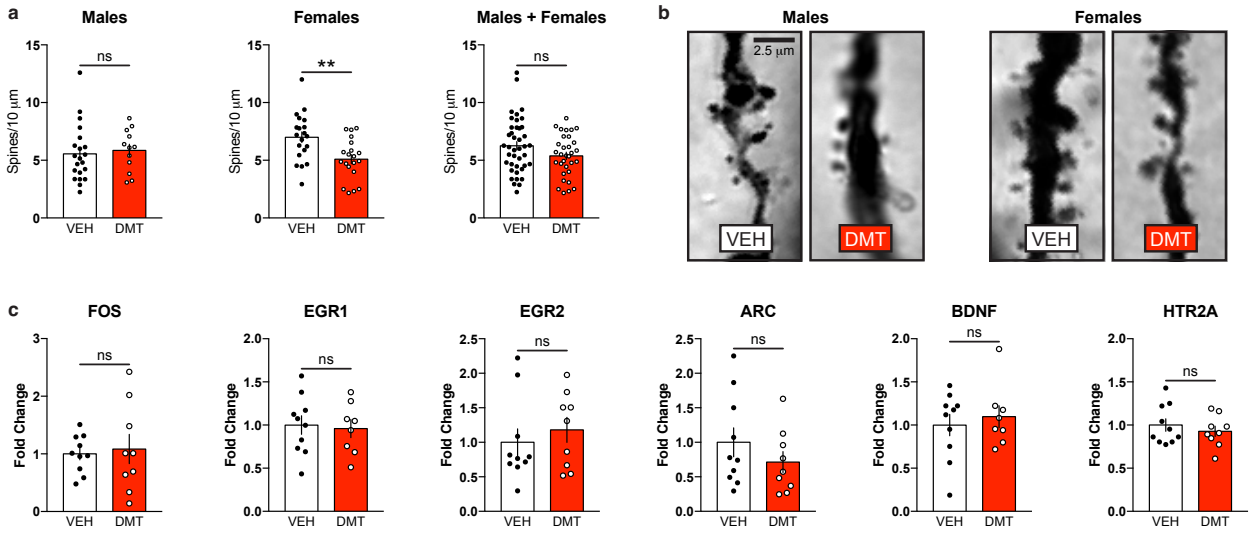

Figure 3.2.5-1. *Chronic, intermittent, low doses of DMT may affect structural plasticity in females. (a) Dendritic spine density on layer V pyramidal neurons is reduced following psychedelic microdosing in females as measured via Golgi-Cox staining. DMT: N= 20 cells from 2 animals; VEH: N = 20 cells from 2 animals. This effect was not present in males. DMT: N=12 cells from 2 animals; VEH: N = 21 cells from 2 animals. (b) Representative images of Golgi−Cox stained layer V pyramidal neurons in the PFC of rats. (c) There are no changes in early immediate genes after 2 months of psychedelic microdosing.*

*Data represents mean ± SEM. *p < 0.05. Two-tailed unpaired t-test. VEH = vehicle (saline), DMT = N,N-dimethyltryptamine, ns = not significant.*

We used the other half of the tissue through ddPCR analysis to look at changes in gene expression for several immediate early genes, including FOS, EGR1, EGR2, ARC, BDNF and HTR2A. We found no significant changes in immediate early gene expression (**Figure 3.2.5-1**).

3.2.6 Effects of psychedelic microdosing on metabolism and weight

Over the course of the study, we observed that males treated with DMT—but not females—exhibited significantly more weight-gain than their VEH-treated counterparts (182% versus 165%, $p = 0.003$, **Figure 3.2.6-1a,b**). To investigate this phenomenon, we completed a metabolic analysis using a Comprehensive Lab Animal Monitoring System (CLAMS). Despite gaining more weight, the DMT-treated male animals tended to eat less food (though this is not significant, $p = 0.1349$) than the VEH-treated controls (**Figure 3.2.6-1c**). There was no difference in female food consumption ($p = 0.77$). These results cannot be explained by changes in activity, heat dissipation or respiratory exchange rate (RER, **Figure 3.2.6-1d,e**).

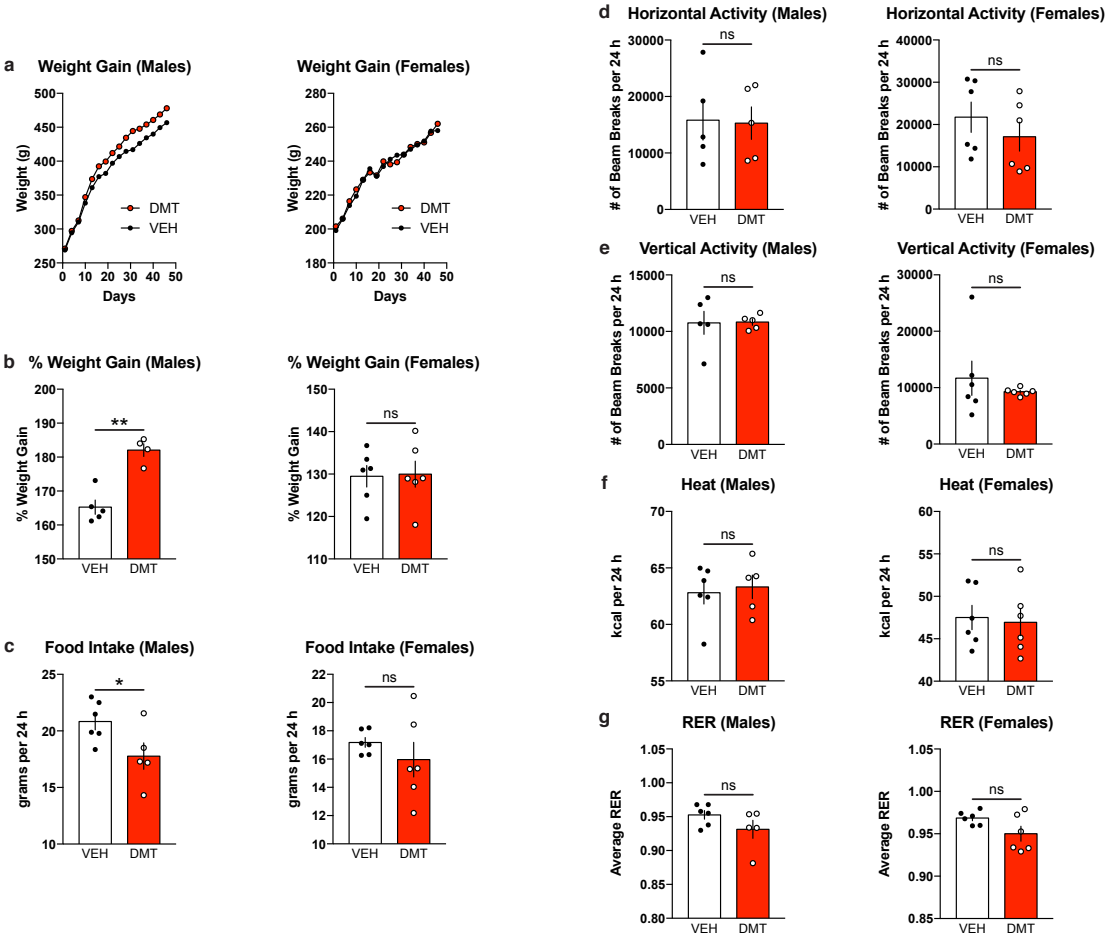

Figure 3.2.6-1. *Males, but not females, exhibit significant physical changes after chronic, intermittent, subhallucinogenic doses of DMT. (a) DMT administered at 1 mg/kg every third day causes accelerated weight gain in male, but not female rats. Error bars removed for clarity. (b) Percent weight gain over the course of a 7-week microdosing experiment. (c) Average food intake over the course of 48 h following 7 weeks of DMT microdosing was not affected by DMT treatment. (d–f) Microdosing did not impact horizontal activity (d), vertical activity (e), dissipation of heat (f), or respiratory exchange rate (RER) (g).*

*Data represents mean ± SEM. *p < 0.05. Two-tailed unpaired t-test. VEH = vehicle (saline), DMT = N,N-dimethyltryptamine, RER = respiratory exchange rate, ns = not significant.*

We reasoned that perhaps there were changes to the body composition makeup of the rodents. For example, muscle weighs more than fat, and different fats also have different densities and weights. For this study, we used a second cohort of animals and saw a similar trend with weight gain in male psychedelic-treated rats ($p = 0.03$, **Figure 3.2.6-2a**). This difference in weight was detectable as early as 1 week into the experiment. After 4 weeks of this intermittent dosing regimen, animals were sacrificed and fat pads were dissected and quantified as a percentage of total body weight (**Figure 3.2.6-2b**). There were no differences observed between treatment group

for white (epidydimal, mesenteric, retroperitoneal, subcutaneous) or brown adipose tissue. This suggests that DMT-induced body weight gain may be independent of adipose tissue content or composition.

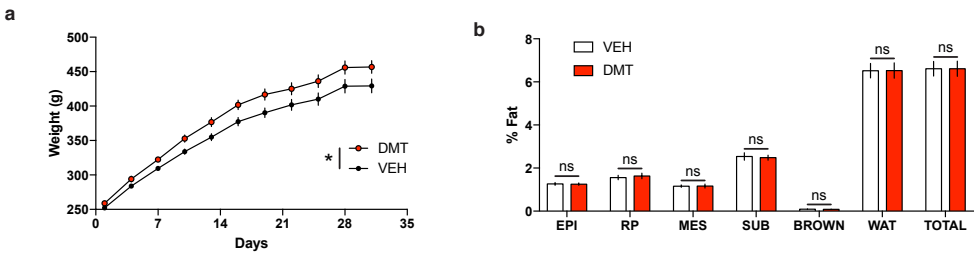

Figure 3.2.6-2. *Chronic, intermittent, low doses of DMT increase weight in male rats, but not adiposity. (a) Male rats were weighed and treated with 1 mg/kg DMT or vehicle every third day for 4 weeks. A significant difference was observed between the two groups (p = 0.026, two-way repeated measures ANOVA). (b) At the conclusion of 4 weeks of dosing, the animals were sacrificed and their fat pads dissected and weighed. Data are presented for each fat pad (or combination of fat pads) as a percentage of the total body weight. No significant differences were observed between treatment groups.*

*N = 12 per group. Data represents mean ± SEM. *p < 0.05. Two-tailed unpaired t-test. VEH = vehicle (saline), DMT = N,N-dimethyltryptamine, EPI = epididymal, RP = retroperitoneal, MES = mesenteric, SUB = subcutaneous, BROWN = brown fat, WAT = sum of white adipose tissue (EPI + RP + MES + SUB), TOTAL = sum of all fat pads, ns = not significant.*

Finally, we measured steroid levels in the serum of DMT-animals using mass spectrometry-based metabolomics profiling (**Figure 3.2.6-3**). A principal components analysis revealed large sex differences in steroid profiles, as expected. No statistical differences were found between treatment groups. Taken together, there is a clear and significant increase in body weight, but the mechanism by which this occurs remains unknown.

a

Name	MRM	RT	DP	CE	EP	CXP
17α-Hydroxyprogesterone	331.2/109.1	8.49	40	30	10	13
20-hydroxyprogesterone	317.1/299.1	11.07	50	24	10	15
aldosterone	361.2/343.1	1.48	10	24	10	25
allo-pregnanolone	301.1/283.2	15.7	30	17	10	20
androstanediol	275.1/257.2	6.83	20	20	10	16
androstenedione	287.2/109.1	7.71	50	35	10	15
beta-pregnanolone	301.2/283.1	11.97	30	18	10	18
cortexolone	347.2/109.1	3.94	50	43	10	15
cortexone	331.1/109.1	7.26	40	39	10	15
corticosterone	347.2/121.1	3.59	50	35	10	15
dehydroepiandrosterone	271.1/253.1	7.88	30	15	10	11
dehydroepiandrosterone sulfate	271.1/253.2	3.35	40	20	10	25
dihydroprogesterone	317.1/299.1	15.8	40	15	10	10
estradiol	255/159	5.7	35	22	10	18
estriol	271.1/253.1	1.33	30	15	10	11
progesterone	315.1/109.1	13.8	40	30	10	15
testosterone	289.2/109	6.37	20	28	10	12
trans-androsterone	273.1/255.2	9.09	50	18	10	25

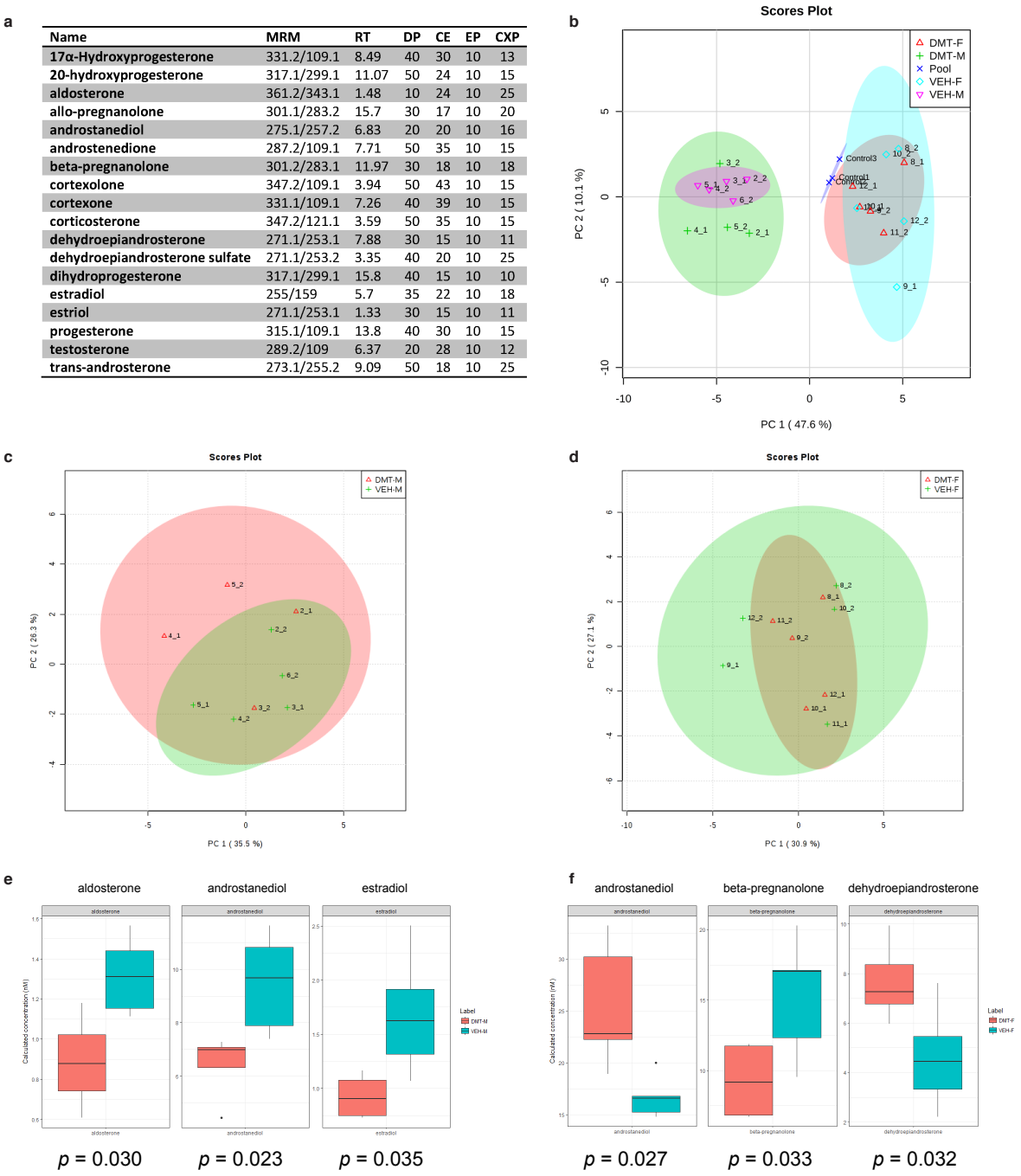

Figure 3.2.6-3. *Chronic, intermittent, low doses of DMT produce minimal changes in serum steroid levels. (a) We attempted to quantify 30 steroids in the serum of DMT-microdosed rats, however, 12 were below the limit of detection. The 18 steroid analytes detected, MRM transitions, retention times (RT), declustering potentials (DP), collision energies (CE), entrance potentials (EP), and cell exit potentials (CEP) are listed. (b) Principal components analysis revealed large differences between male and female samples. Data collected for pooled samples at the beginning, middle, and end of the mass spectrometry experiment were consistent with each other and demonstrate minimal assay drift. (c–d) Principal components analysis for male (c) and female (d) samples show that DMT-treatment produced minimal changes in serum steroid profiles. Principal components analyses were log10 transformed and pareto scaled. (e–f) Levels of three steroids in male (c) and female (d) samples following DMT microdosing are shown. For box plots: horizontal line = median, 50% quartile; lower hinge = 25% quartile; upper hinge = 75% quartile; lower whisker = smallest observation greater than or equal to lower hinge – 1.5 x IQR; upper whisker = largest observation less than or equal to upper hinge + 1.5 x IQR; Dots = outliers defined by being either greater than Q3 – 1.5 x IQR or less than Q1 – 1.5 x IQR. Q3 = third quartile; Q1 = first quartile; IQR = interquartile range = Q3 – Q1.*

3.3 Conclusion and Discussion

These studies—conducted in both humans and rodents—suggest that psychedelic microdosing may have therapeutic properties. Though there are a few subtle differences between the human and rodent data, the reports are strikingly congruent.

3.3.1 Comparison of human and rodent microdosing experience

Both humans and rodent studies suggest psychedelic microdosing may have there is therapeutic potential for treating depression and anxiety. Interestingly, there is a distinct lack of anxiogenic effects (potentially even verging on the side of anxiolytic, $p = 0.08$) after psychedelic microdosing in rodents (**Figure 3.2.1-1**). This contrasts to the fact that a single high dose of DMT may cause significant anxiogenic effects in both NIL and EPM tests (see Section **2.2.2: Effects of DMT treatment on animal behaviors relevant to anxiety**).[10] This mirrors the data collected by human participants which suggests that psychedelic microdosing may have anxiolytic properties (**Table 3.1-3**). There is the potential that the anxiolytic response in humans is greater by having a more cognitive understanding of the practice in addition to potential placebo effects.[15]

Interestingly, our rodent study suggests that psychedelic microdosing may not prevent the establishment of learned fear, but can be used to facilitate cued fear extinction (**Figure 3.2.2-1f**). Given that fear conditioning and extinction is a model for PTSD, it is perhaps unsurprising that a significant portion of veterans report participating in this practice (**Table 3.1-1**). It should be noted that other psychedelic compounds, such as 3,4-methylenedioxymethamphetamine (MDMA) are excellent candidates for treating PTSD.

We predict that these therapeutic effects relevant to depression, anxiety and PTSD are the result of strengthening of specific circuits anchored in the PFC that exert top-down control over

other brain regions involved in fear and motivated behaviours.[16,17, 18, 19] These behaviours far outlast the short half-life of DMT (approximately 15 mins),[20] which suggests that the persistent effects from these drugs are due to changes in neural circuits.

3.3.2 Excitability and function of cells in the mPFC

Changes in brain structure and function are likely to depend on the total length of psychedelic treatment.

Increases in function, as measured by sEPSCs, were measured at after a single 1 mg/kg dose of DMT in rats, suggesting that indeed this sub-hallucinogenic dose may be sufficient for enhancing neural activity (**Figure 3.3.2-1**).[21] If true, this could strengthen top-down control of circuits involved in emotion, thus contributing to the therapeutic responses we see in these studies.

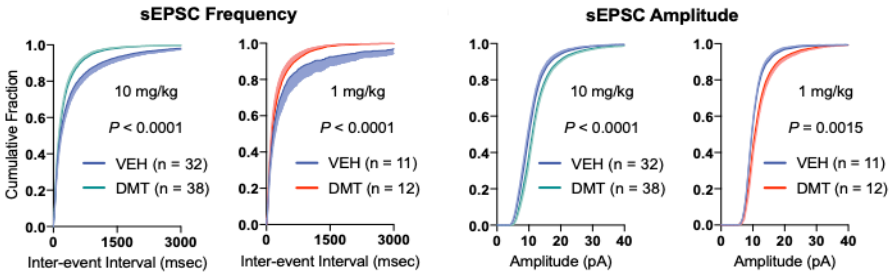

Figure 3.3.2-1. DMT increases both frequency (a) and amplitude (b) of sEPSCs in mPFC 24 h after dosing with a hallucinogenic (10 mg/kg) or subhallucinogenic (1 mg/kg) dose.

However, this was after a single dose and does not consider repeated dosing regimens. It appears that after multiple months of microdosing, this practice may result in a decrease in spine density (**Figure 3.2.5-1**), likely due to a homeostatic mechanism. This may be caused by excitotoxicity of these cells, resulting in retraction. Psychedelics are known to engage mTOR signaling,[21] and overactivation of mTOR is proposed to contribute to both autism spectrum disorder (ASD)[22] as well as Alzheimer's disease (AD).[23]

In the same vein, fully-hallucinogenic doses of LSD administered over 11 days appears to have antidepressant-like effects,[24] however when it is administered chronically (>3 months), animals develop a persistent state of hyperactivation, anhedonia and social deficits.[25] It should also be noted that the anxiolytic and antidepressant properties of these compounds were assessed earlier in this behavioural battery, and while increasing the stress of the paradigms. It is possible that this dosing regimen enhanced therapeutic properties for these earlier tests but as the animals are put through increasingly stressful situations, resilience to the stress decreases.

Immediate early genes involved in growth and activation are not found after prolonged dosing of DMT. Given the electrophysiology data, it is likely that immediate early genes are activated after a single acute dose, and this response is desensitized when DMT is administered chronically; in other words, this points again to a homeostatic mechanism. Further work assessing the effects of a single dose on immediate early genes and spine density are warranted, particularly given the electrophysiology data in **Figure 3.3.2-1**.

There are no changes to 5HT2A receptor gene expression levels (**Figure 3.2.5-1c**) despite chronic administration of a 5HT2A receptor agonist for 2 months. This is congruent by previous work by Smith and colleagues which suggests that the 5HT2A receptor does not desensitize in the presence of DMT,[26] which may explain why tolerance to DMT does not develop in humans.[27] These data indicate that desensitization may occur at the level of cell excitability rather than at the level of the 5HT2A receptor.

It is possible that structural changes in the mPFC after chronic, intermittent low doses of a psychedelic (a decrease in spines, **Figure 3.2.5-1**) is unrelated to the therapeutic effects of psychedelic microdosing (**Figure 3.2.1-1**, **Figure 3.2.2-1**, **Figure 3.2.3-1**). Indeed, studies with

long-term intermittent use of ayahuasca (a tisane which contains DMT) is correlated with the thinning of the posterior cingulate cortex in humans,[28] without increased risk for mental illness.[29]

In all, it appears that psychedelic microdosing may have therapeutic effects, as seen in the rodent microdosing study and the subjective human reports. That said, there is still much work to be done to establish the potential risks and benefits associated with this practice.

3.3.3 Advances in the field of psychedelic microdosing

Here I will comment on advancements in the field of psychedelic microdosing since the completion of my studies.

A study by Higgens et al. describe enhanced motivation in "low performing" rats using two different food motivation tasks when microdosed with ketamine (1–3 mg/kg) or psilocybin (0.05–0.1 mg/kg),[30] suggesting the practice may produce antidepressant effects in populations with anhedonia. This is comparable to our findings that psychedelic microdosing may enhance motivated behaviours in assays such as the forced swim test.

Horsley and colleagues examined psilocin and ketamine microdosing and assess anxiety in the EPM and found that higher doses of psilocybin produced an anxiogenic profile (0.075 mg/kg psilocin and 3 mg/kg ketamine).[31] This differs from our findings that microdosing is anxiolytic in rodent models, and may be due to differences in drugs administered, animal models used, or overall length of dosing regimen. The Spanagal lab investigated microdosing in a model of alcohol relapse but found it did not have long-lasting effects.[32]

There are several studies in humans, but for this dissertation, I will focus on studies that have investigated psychedelic microdosing on mental health specifically. Firstly, Hutten et al. demonstrated that LSD showed positive effects on mood, friendliness, arousal and decreasing

attentional lapses.[33] At higher doses (20 mcg), LSD increased confusion and anxiety in volunteers. Other studies indicate that at similar doses, drug disliking and anxiety increase,[34,35] and that patients can cycle between patterns of euphoric and depressive mood states.[36] Interestingly, this cycling is thought to induce cognitive flexibility.[37] Other online observational studies conclude lower measures of dysfunctional attitudes and negative emotionality while higher scores on wisdom, open-mindedness and creativity.[38]

There are no studies to my knowledge which explicitly explore the relationship between psychedelic microdosing and exercise or physical health. There are anecdotal reports of improving workouts and individuals having more motivation for a healthier lifestyle.[39,40,41]

A foundational study was released in eLife earlier this year, which suggests that though psychedelic microdosing improves mood, it is not significant from the placebo-treated group.[42] Impressively, they were able to use a Citizen Science-like platform and successfully blinded participants to the treatment they received. Since this study did not provide the study drugs—participants were required to source their own substances—so the purity and exact dose was estimated by participants. This study investigated a large cohort of healthy individuals (240), however it is well-known that patients with more severe depressive symptoms are more likely to be responsive to medication.[43] For this reason, it is possible that psychedelic microdosing may still hold therapeutic value for those suffering from mood disorders, though this warrants further investigation.

3.4 Methods

3.4.1 Survey Participants

A total of 2368 individuals responded to the survey. Some responses were excluded because they were under 18 years of age (n = 9), and because they did not provide their consent (n = 12). Thus, 2347 respondents were used in the remaining analyses. A total of 383 respondents reported having practiced microdosing, either previously or currently. Responses for previous and current microdosers were combined, with the exception of the question regarding discontinuing the practice, which is only relevant to past users. Given the sensitive nature of the survey material, participants could decline to answer any question. Thus, we report the proportion of responses for each result below. The total number of potential responses for a given question is denoted as N Question (N_Q); The total number of responses for a given question is denoted as N Total (N_T); the subset of answers for a given question are denoted as 'n'. Gender representation was relatively equal with 49% of participants being female (N_T = 1707, n = 832). Participants ranged from 18 to 99 years of age (mean = 35) and individuals represented various education and income levels, as well as diverse occupational experiences.

3.4.2 Recruitment and Survey Distribution

An anonymous online survey was used to sample 2347 people from April through August of 2018. Participants were recruited via snowball sampling through a number of outlets including social media (e.g., Facebook, Twitter, Instagram), our research group's website (www.olsonlab.org), drug- and nondrug-related online forums (Reddit, Craigslist), and distribution of pamphlets across the UC Davis campus and community events (e.g., local farmers market). To prevent biasing the

study towards participants with extensive knowledge and/or experience with psychedelics, the survey was described as an anonymous research study on "Recreational Drug and Alcohol Use." The survey was also designed such that each IP address could only take the survey once to avoid collecting multiple responses from the same participants. Individuals voluntarily participated in the study and did not receive compensation. The survey was approved by the University of California, Davis Institutional Review Board (IRB).

3.4.3 Microdosing Survey Design

After providing informed consent, participants were asked a series of questions related to their familiarity with psychedelic microdosing as well as their personal experience with the practice. Psychedelic microdosing was defined as "using multiple sub-hallucinogenic doses of a psychedelic compound on an intermittent basis for a minimum of at least 2 weeks." Individuals who indicated that they currently microdose or have previously microdosed ($n = 89$ and $n = 294$ respectively, $N_T = 2200$) were asked which drugs they used to microdose and what changes, if any, they perceived related to depression, anxiety, memory, focus/attention, sociability, and physical factors. Changes were reported for each construct using the following response options: improvement (positive effects), no effect, no noticeable effect and worsening of symptoms (negative effects). For clarity, "no effect" and "no noticeable effect" responses were combined. Additionally, individuals who reported previously engaging in microdosing were asked about factors that deterred them from continued engagement in this practice. The survey can be viewed in its entirety at 10.6084/m9.figshare.9757901.

3.4.4 Statistical Analysis for Human Survey Study

As participants could decline to answer any question, we report the number of responses, as well as the proportion of responses, for each result below. All statistical comparisons were made using completed responses and excluded cases where the question was not answered. Group responses were compared using Pearson's chi-squared test with a significance level of $p < 0.05$. Given the exploratory nature of this work, descriptive statistics were used to characterize overall trends in the prevalence of microdosing and the use of psychedelic substances across various groups in our study. Regression models were used with 95% confidence intervals reported to identify and quantify the strength of associations. Statistical analyses were performed using RStudio (version 1.0.143).

3.4.5 Drugs used in Animal Microdosing Study

Solid DMT•fumarate (2:1) was prepared as described previously[10] and stored in the dark at -20ºC prior to use. For each administration, a solution of DMT•fumarate (2:1) in 0.9% sterile saline was freshly prepared and passed through a 0.2 μm syringe filter. For all experiments, DMT•fumarate (2:1) was administered at 1 mg/kg via intraperitoneal injection using an injection volume of 1 mL/kg. For our vehicle control, 0.9% sterile saline solution was utilized.

3.4.6 Animals

Male and female Sprague Dawley rats were obtained from Charles River Laboratories (Wilmington, MA, USA), were housed two animals of the same sex per cage, and were given *ad libitum* access to food and water. The experiments began when the rats were 8 weeks of age. Lights in the vivarium were turned on at 07:00 hours and turned off at 19:00 hours. Behavioral

experiments were performed during the light-on phase, with experiments taking place between 08:00 and 18:00 hours unless otherwise noted. Treatment groups were randomly assigned, but each cage housed one animal from the DMT- and one from the VEH-treated groups. When appropriate, behavioral tests were counterbalanced to avoid systematic errors. All experimental procedures involving animals were approved by the UC Davis Institutional Animal Care and Use Committee (IACUC) and adhered to principles described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Over the course of one of the microdosing experiments, one animal began to exhibit signs of poor health, and was euthanized according to our IACUC protocol. The University of California, Davis is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

3.4.7 Tissue Collection

After completion of the behavioral and metabolic experiments, the animals were sacrificed via decapitation. Blood and brain tissue were collected for metabolomics, Golgi-Cox staining, and gene expression studies as described below.

3.4.8 Novelty-Induced Locomotion (NIL)

Rats were allowed to acclimate to the test room for 10 min prior to being gently placed into the center of an AccuScan Instruments (Columbus, OH) open field chamber (Digiscan Animal Activity Monitor model #RXYZCM(16)CCD) and allowed to freely explore the chamber for 45 min. At the conclusion of the test, animals were returned to their home cages and the test chambers cleaned with 10% Nolvasan. Horizontal motion, rotations, and stereotypies (repetitive beam breaks) were recorded in 1 min intervals for the duration of the test and analyzed using the program

Integra. The margin of the arena was defined as being 10 cm from the wall. The open field chamber measured 41.9 cm L × 41.9 cm W × 28.6 cm H and was illuminated to between 25 and 30 lx.

3.4.9 Novel Object Recognition (NOR)

The NOR chamber measured 53.3 cm L x 53.3 cm W 34.3 cm H, was illuminated to between 25 and 30 lx, and contained a pair of identical objects (either a 4 inch cone or a 3–4 inch jar filled with cement). On the first day, rats were allowed to freely explore the objects for 10 min before being returned to their home cages. After 24 h, rats were reintroduced to the NOR chamber, but one of the objects was replaced with a new, distinctly different object. EthoVision XT (version 9) software was used to assess the subject rat's preference for the novel vs the familiar object by quantifying the amount of time the nose point spent within the "sniff zone"—defined as a circle surrounding the object with a radius of 2 cm. Additionally, a discrimination index (DI) was calculated as $\text{Time Exploring}^{\text{Novel Object}} - \text{Time Exploring}^{\text{Familiar Object}} / \text{Total Time Exploring}^{\text{Both Objects}}$. The entire apparatus was cleaned with 10% Nolvasan between trials.

3.4.10 Spontaneous Alternation Behavior (SAB)

Rats were placed on one arm of a T-maze facing the central node and allowed to freely explore for 10 min. Animal movement was recorded and analyzed by an experimenter blinded to treatment after the completion of the behavioral task. Percent alternation was calculated by dividing the number of times the rodent sequentially accessed the 3 distinct arms of the maze by the total number of arm entries minus one. The entire apparatus was cleaned with 10% Nolvasan between trials.

3.4.11 Elevated Plus Maze (EPM)

The EPM apparatus consisted of a black plus-shaped plastic platform positioned 50 cm above the ground and illuminated to between 20 and 25 lux. Two opposite arms of the maze possessed vertical walls measuring 31.75 cm high, with the other two arms being open with unprotected edges. Rats were placed in the center of the maze facing an open arm and allowed to explore freely for 5 min. At the conclusion of the test, rats were returned to their home cages and the apparatus was cleaned with 10% Nolvasan. Animal movement was recorded and analyzed during the trial using EthoVision XT (version 9) software.

3.4.12 Forced Swim Test (FST)

The FST apparatus consisted of a clear Plexiglas cylinder measuring 40 cm tall, 20 cm in diameter and filled with 30 cm of 24 ± 1ºC water. Fresh water was used for every rat. Animals were subjected to a pre-test phase in which they were placed in the cylinder for 15 mins before being dried and returned to their home cage. Twenty-four hours later, rats were again placed in the FST apparatus for 5 mins and their activity was video recorded. Each video was scored for immobility, swimming, and climbing behavior by a trained observer blinded to treatment condition. The dominant behavior of the animal (i.e., immobility, swimming, or climbing) was determined every 5 sec and quantified as a "count." As the experiment lasted for 5 mins, the sum of the counts for all four behaviors equals 60.

3.4.13 3-Chambered Social Approach

The social approach apparatus consisted of three identical chambers separated by removable gates, with each chamber measuring 101.6 cm L x 33.3 cm W 33.7 cm H. Each chamber was illuminated

to between 25-30 lux and housed a cage for holding "social animals" or objects. The cages restrained the movement of the social animals while still enabling interaction with the test animals. One day prior to the experiment, social animals were acclimated to the cages of the social approach chamber in two 15-min sessions. On the test day, subject rats were habituated to the apparatus with no social animal or object present for 10 mins, before being gently corralled back into the center chamber and the gates closed. A novel object was then placed in one of the adjacent chambers, and a social animal placed in the other chamber. The gates were then opened and the subject rat was allowed to freely interact with the social animal or object, and their activity was video recorded. EthoVision XT (version 9) software was used to assess the subject rat's preference for social interaction. Time spent in the "sniff zone" was quantified using the nose point of the animal. The sniff zone was defined as 2 cm from the edge of the cage holding either the social animal or the object. Social animals were the same sex and approximate age as subject rats. The entire apparatus was cleaned with 10% Nolvasan between trials.

3.4.14 Fear Conditioning

Rats were placed in a fear conditioning apparatus (Med Associates model # MED-VFC2-SCT-R) for 3.5 min prior to three presentations of auditory cues (80 dB white noise, 30 s), each coterminating with a foot shock (0.8 mA, 2 s.) and spaced 90 s apart. The fear conditioning apparatus consisted of a 30.5 cm × 24.1 cm × 21 cm internal soundproof chamber, with metal grated floors, an infrared camera, a sound generator, and a light source. After the last shock, the animals remained in the chambers for an additional 2 min before being returned to their home cages. During fear conditioning, the apparatus was illuminated to 100 lx and did not contain any additional odor cues. The apparatus was cleaned with 70% EtOH in between trials. All fear

conditioning and extinction experiments were performed between the hours of 08:00−11:00. Freezing behavior was scored using Med Associates Video Freeze software v2.25 (motion threshold = 18 au, detection method = linear, minimum freeze duration = 30 frames, which is equal to a 1 s freeze). Pre-training was defined as the 3.5 min prior to receiving footshocks, while post-training refers to the 2 min that the animal remained in the fear conditioning chamber after the last foot shock.

3.4.15 Contextual Fear Memory

On the first day after fear conditioning, contextual fear memory was assessed by exposing the animals to the conditioning context for 10 min and measuring their freezing behavior over this time. The apparatus was cleaned with 70% EtOH in between trials.

3.4.16 Cued Fear Memory and Extinction Training

On the second day after fear conditioning, the animals were administered DMT and then placed back in their home cages. On the third day after fear conditioning, cued fear memory was assessed by exposing the animals to a novel context (lights off, A-frame insert, smooth plastic floor insert, additional vanilla odor) for 2 min prior to 8 presentations of auditory cues (80 dB white noise, 30 s) spaced 30 s apart. Freezing responses for cue testing are presented as the percentage of time spent freezing during the last 4 auditory presentations. Cued fear memory testing also served as cued fear extinction training. The apparatus was cleaned with 70% EtOH in between trials.

3.4.17 Cued Fear Extinction Memory

On the fourth day after fear conditioning, the cued fear memory procedure was repeated.

3.4.18 Comprehensive Laboratory Animal Monitoring System (CLAMS)

Rats were singly housed in CLAMS chambers (Columbus Instruments) for ~48 h and fed powdered chow diet (2018 Teklad Global 18% Protein Rodent Diet) *ad libitum*. Cage sensors monitored food consumption as well as horizontal and vertical activity. Oxygen consumption and carbon dioxide production were measured and used to calculate energy expenditure (i.e., heat dissipated) and respiratory exchange ratio (RER = VCO_2/VO_2).

3.4.19 Tissue Collection Following Microdosing

After CLAMS data were collected, the animals administered chronic, intermittent low doses of DMT as well as the vehicle controls were sacrificed via decapitation. Blood and brain tissue were collected for metabolomics, Golgi-Cox staining, and gene expression studies as described below.

3.4.20 Golgi-Cox Staining

Brain tissue was prepared following the protocol outlined in the FD Neurotechnologies Rapid GolgiStain Kit (FD Neurotechnologies) with slight modifications. Brains were stored in solution C for 2 weeks prior to slicing into 130 μm sections using a vibratome. These slices were placed onto microscope slides that were pre-coated with (3-aminopropyl)triethoxysilane. Slices were air dried for a week before staining. Slides were immersed in water twice for 2 minutes, DE solution for 10 minutes, and then water for 2 minutes. After this, slides were immersed sequentially in 25% ethanol for 1 minute, 50% ethanol for 4 minutes, 75% ethanol for 4 minutes, 95% ethanol for 4 minutes, and 100% ethanol for 4 minutes. Slides were then briefly dipped into xylenes before being mounted using DPX Mountant For Histology (Sigma), air-dried, and imaged on a Zeiss AxioScope. Spines were traced using Neurolucida software (version 10) at 100x magnification.

Data acquisition and analysis was performed by an experimenter blinded to treatment conditions. Data represent individual neurons taken from 2 different animals per treatment. Representative images were acquired with a 60x oil immersion objective (1.42 NA) using the 488 nm laser and transmission detector on an Olympus FV1000 confocal microscope.

3.4.21 ddPCR

Tissue from the PFC of rats subjected to chronic, intermittent, low doses of DMT or vehicle was removed and lysed using QIAzol Lysis Reagent (QIAGEN). Extraction of RNA was accomplished using the RNeasy isolation kit (QIAGEN) following the instructions of the manufacturer. The resulting RNA was converted to cDNA using the iScript cDNA Synthesis Kit (BioRad). The cDNA was diluted 1:100 prior to droplet digital PCR (ddPCR). Droplets containing cDNA, ddPCR master mix (BioRad), and TaqMan probes (ThermoFisher) were generated using the QX200 Droplet Digital PCR System (BioRad). Following PCR amplification, the signal of the gene of interest (FAM-labeled) was quantified and normalized to the housekeeping gene ESD (VIC-labeled). Taqman probes included those for FOS (ThermoFisher, Rn02396759_m1), EGR1 (ThermoFisher, Rn00561138_m1), EGR2 (ThermoFisher, Rn00586224_m1), ARC (ThermoFisher, Rn00571208_g1), BDNF (ThermoFisher, Rn02531967_s1), HTR2A (ThermoFisher, Rn00568473_m1), and ESD (ThermoFisher, Rn01468295_g1).

3.4.22 Metabolomics

Blood was allowed to clot for 15 min at room temperature, centrifuged at 1,000 x g for 10 min at 0ºC, and the resulting serum was transferred to a new tube and stored at -80ºC until analysis via

liquid chromatography-mass spectrometry. Samples were extracted by the *Shake and Shoot* protocol[44] with minor modification. Briefly, samples were allowed to thaw on wet ice and then inverted five times to homogenize the serum. To a 1 mL 96-well plate (Eppendorf, Hamburg, Ger.) was added 50 µL of sample serum followed sequentially by 25 µL anti-oxidant solution, 25 µL of surrogate standards in methanol, 25 µL of CUDA and PHAU standards in methanol, and 125 µL acetonitrile/methanol (1:1). The plate was then vortexed for 30 s and centrifuged for 5 min to pellet precipitated proteins in a Genevac EZ-2 centrifugal evaporator (Ipswich, UK). Supernatant was filtered through an Agilent PVDC 0.2 µm filter-plate (Santa Clara, CA). The plate was then sealed and placed in the autosampler and maintained at 6ºC. Extracted samples were analyzed for steroids by liquid chromatography-mass spectrometry (LC-MS/MS). A Waters ACQUITY i-Class LC system (Milford, MA) was coupled to a Sciex 6500+ QTRAP (Redwood City, CA) operated in multiple reaction monitoring (MRM) mode. An Agilent InfinityLab Poroshell 120 EC-C18 (2.1 x 100 mm, 1.9 µm) column was used with an acetonitrile/water gradient. Formic acid (0.1% v/v) was added to both mobile phases. A full list of steroid analytes, MRM transitions, retention times, declustering potentials, collision energies, entrance potentials, and cell exit potentials can be found in Supplementary Figure 4a.

3.4.23 Fat Pad Analysis

Male rats were treated with DMT (12 rats) or vehicle (12 rats) every third day for 4 weeks, and weights were recorded at regular intervals. At the completion of the experiment, the animals were sacrificed via decapitation and their fat pads dissected and weighed. Fat pad weights are presented as a percentage of total body weight.

3.4.24 Statistical Analysis

Statistical analyses were performed using GraphPad Prism (version 7.0a). Descriptive statistics were used to describe the human survey data. Populations were compared using a Pearson's χ^2 test (Fig. 1). For rodent studies, appropriate samples sizes were estimated based on our previous experiences performing similar experiments in conjunction with a power analysis. The data for males and females were analyzed together unless there were obvious sex differences. Data analyses were performed by experimenters blinded to treatment conditions. For most comparisons between DMT-treated and VEH-treated groups, a two-tailed Student's t-test was utilized (Fig 3, Fig. 5, Fig. S2, Fig. S3, Fig. S4, Fig. S5, Fig. S7). In the case of fear conditioning and fear extinction experiments (Fig. 4), a Mann-Whitney Test was used as a bimodal distribution was observed. Similar results in rat fear extinction experiments have been observed by others[45] and suggest that there are responders and non-responders to extinction training. For Supplementary Figures S6a and S6b, a two-way repeated measures analysis of variance (ANOVA) and a two-way ANOVA with Sidak's post hoc test were used, respectively. A Grubbs' test was used to verify obvious outliers. For the social interaction test, a computer error caused us to lose data for one animal. For the metabolomics studies, the ggplots2 package in R studio was used to create boxplots. Principal components analysis was performed using MetaboAnalyst 4.0. Microsoft Excel was used to perform two-tailed Student's t-tests.

3.5 Contributions & Collaborations

I couldn't have done this alone. Thank you to all my collaborators who helped me get this far. Contributions are outlined below.

Angela Nazarian helped with the statistical analysis in **Section 3.1: Human experiences with psychedelic microdosing**, with guidance and input from LPC and DEO.

Janata Lab

University of California, Davis

Charles J. Benson ran the rat behavioural experiments, which were designed by DEO. LPC performed the Golgi-Cox staining, gene expression studies, and performed all analyses on behavioural data.

Olson Lab

University of California, Davis

Brian C. DeFelice and Oliver Fiehn performed the metabolomics study.

Fiehn Lab

University of California, Davis

Jon Wong, Eden Barragan, LPC and John A. Gray performed the electrophysiology studies with input from LPC and DEO.

Gray Lab

University of California, Davis

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3.7 References

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Chapter 4

Non-Hallucinogenic Psychedelic Analogs with Therapeutic Potential

Are hallucinations necessary for psychedelics to produce therapeutic effects? In other words, does a person need to experience hallucinations to have a therapeutic effect, or are hallucinations phenomena that happen in parallel to therapeutic effects? As promising as psychedelics might be for treating neuropsychiatric disorders, their notorious perceptual effects have greatly hindered their potential as therapeutics.

A foray into researching psychedelic microdosing provided some insight into the necessity of hallucinations (see Chapter 3), however this practice still uses hallucinogenic compounds, which can be subject to recreational use or impairments with incorrect dosing. As such, our lab initiated a structure-activity relationship (SAR) study which examines the various components of psychedelic compounds to determine moieties of the molecule that are necessary for the therapeutic effects, and which parts of the molecule contribute to the hallucinogenic effects. The goal was to find the optimal structure that could produce therapeutic effects without hallucinations.

4.1 Structure-Activity Relationships (SAR) of psychedelic compounds

There have only been several studies investigating the basic pharmacophore of hallucinogenic compounds, however there is only one study to our knowledge (described below) which investigates the optimal pharmacophore for psychoplastogenicity.

4.1.1 Structure of the 5HT2A receptor binding pocket

The indole ring core of tryptamine-based psychedelic compounds fits securely into the 5HT2A receptor via pi-stacking with amino acids in the receptor-binding region. The indole nitrogen forms a hydrogen bond with the serine on helix 6 ($S242^{5.46}$).[1] In tryptamine-based compounds, like serotonin, an aspartic acid ($D155^{3.32}$) on helix 3 forms a salt bridge with the tertiary amine. Mutation of $D155^{3.32}$ reveals that agonists can no longer interact with the 5HT2A receptor, deeming that the interaction here is critical for activation of the receptor.[1]

From this basic indole scaffold, Lee Dunlap from the Olson lab explored how changes in structure can change hallucinogenic and psychoplastogenic effects. Dunlap created a library of analogs with changes in methylation of the terminal amine, as well as looking at addition of electron withdrawing and donating groups at various positions around the indole.

4.1.2 Role of the terminal amine group

First, he explored increasing the methylation of the terminal amine group. He found that increasing methylation on the tertiary amine increased psychoplastogenic potential, as measured by neuronal growth and Sholl analysis (**Figure 4.1.2-1**).[2] *N,N*-dimethyltryptamine (DMT) was found to be optimal, as methylation increases lipophilicity and allows the drug to cross the membrane and access a higher number of receptors. A trimethyl group on the terminal amine introduces a

permanent positive charge at the nitrogen such that it is not able to pass the membrane. This positively-charged salt version is discussed further in Chapter 5.

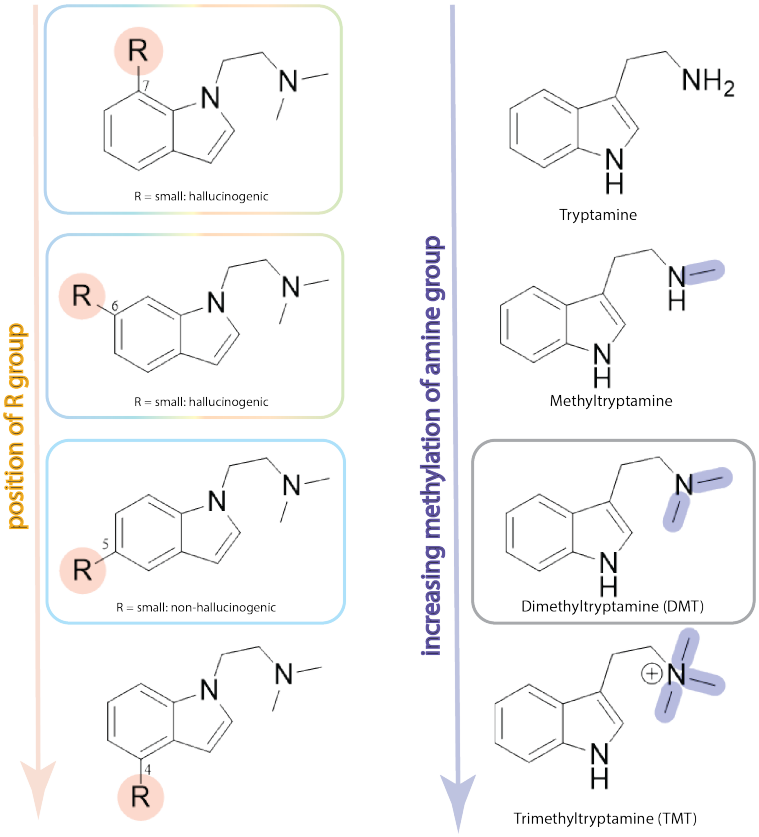

Figure 4.1.2-1. *Structural analysis of therapeutic vs. hallucinogenic properties.*

4.1.3 Role of substitution on the indole ring

Finally, Dunlap investigated the substitution pattern along the isoDMT skeleton (**Figure 4.1.2-1**), which has similar properties for promoting plasticity to DMT. To investigate this, he created molecules with either a fluoride (small and electron withdrawing), methoxy (small and electron donating) or a benzyl ether (large and electron donating) substituent at either the 4-, 5-, 6-, or 7-position on the indole ring. This is comparable to the 7-, 6-, 5-, or 4- position of DMT, respectively.

He found that small, strongly electron donating groups, like methoxy-substitutions at the 4-, 5-, or 6- position on the isoDMT scaffold were able to best potentiate growth in neuronal cultures. This may explain the psychoplastogenic effects of compounds 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT), psilocin (the active drug from the prodrug psilocybin) and bufotenin, as they all have small electron donating groups within their structures. When considering pharmacokinetics, the use of a methoxy-substituted compound instead of a hydroxy-substituted compound was clever, as it creates a more brain-penetrant version.

In summary, an indole core (preferably with a 4-, 5- or 6-substituted small electron donating group) with a dimethylated free amine is critical for psychoplastogenic effects, as measured by Sholl analysis. Please refer to **Chapter 5** for an in-depth discussion on mechanism of action.

4.2 Development of a non-hallucinogenic psychedelic analog

4.2.1 Structurally similar compounds with different behavioural effects

Since the discovery of psychedelic compounds in the mid-20th century, chemists have tweaked chemical structures to create hundreds of new versions of these compounds.[3,4] Interestingly, not all these psychedelic analogs are hallucinogenic. In particular, a compound called 6-methoxy-*N,N*-dimethyltryptamine (6-MeO-DMT) poorly substitutes for the psychedelic compound DOM in a drug discrimination assay.[5] This compound is of particular interest given its structural similarity

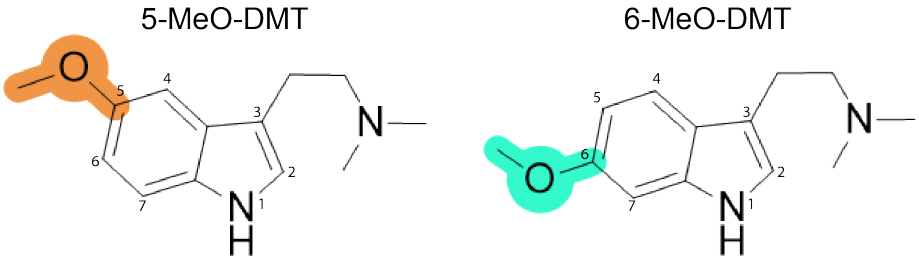

Figure 4.2.1-1. *Comparison of the structures of 5-MeO-DMT and 6-MeO-DMT. Structural differences are highlighted.*

to the highly hallucinogenic counterpart, 5-MeO-DMT. A comparison between structures of these two compounds can be seen in **Figure 4.2.1-1**.

To further investigate this phenomenon, we assessed the potential of 5-MeO-DMT and 6-MeO-DMT using the head-twitch response (HTR) assay. When animals are administered a serotonergic psychedelic, they exhibit a very stereotyped response known as a head-twitch (a quick head motion from side to side). The potency for the number of head-twitches is highly correlative with the human hallucinogenic potency.[6]

Animals administered 5-MeO-DMT exhibit a dose-dependent increase in the number of head-twitches exhibited in this assay (**Figure 4.2.1-2**). On the other hand, 6-MeO-DMT did not produce any head-twitches at any of the doses tested (1 mg/kg, 10 mg/kg, 50 mg/kg).

This data, in combination with previous studies,[5, 7] suggests that the 6-substituted position of indole alkylamines may render a compound non-hallucinogenic.

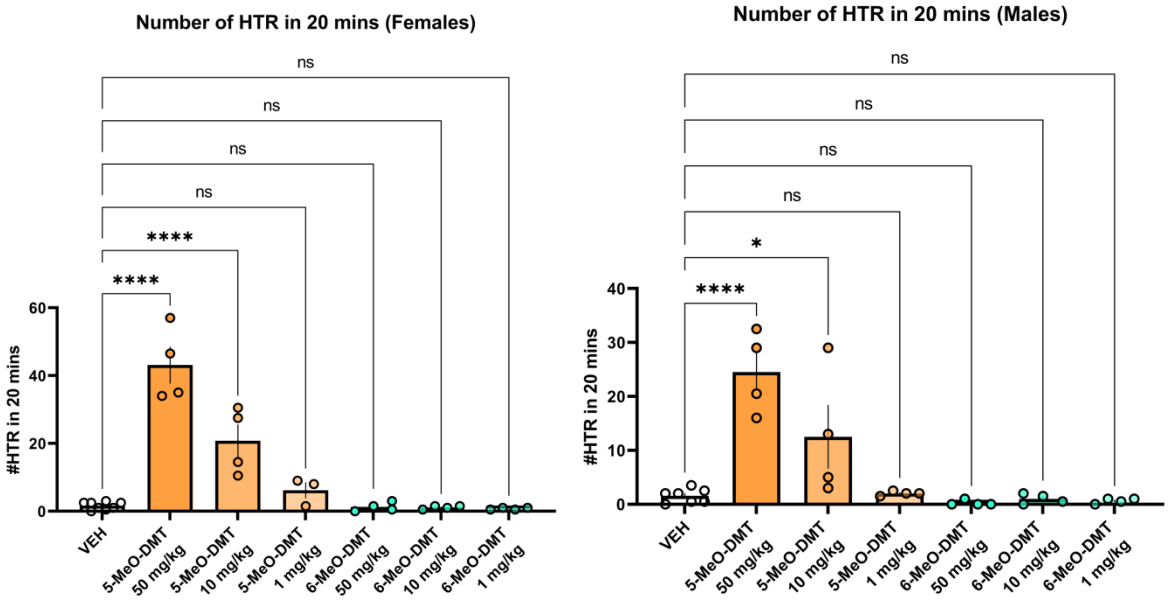

Figure 4.2.1-2. *5-MeO-DMT elicits a robust head-twitch response, whereas 6-MeO-DMT does not. There is a more robust head-twitch response in females.*

*N = 3−8 mice per condition. Data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, as compared to the vehicle control following a one-way ANOVA with Dunnett's post hoc test. VEH = vehicle (saline), 5-MeO-DMT = 5-methoxy-N,N-dimethyltryptamine, 6-MeO-DMT = 6-methoxy-N,N-dimethyltryptamine.*

When these two compounds are tested side-by-side in an FST assay, we find that both compounds reduce the amount of time animals spent immobile when administered acutely, and these effects last for up to 1 week, the longest timepoint tested (**Figure 4.2.1-3**). In fact, the non-hallucinogenic analog appears to provide a more robust response than the hallucinogenic compound. It is possible that this effect is due to a lack of anxiogenic response associated with psychedelic use.[8]

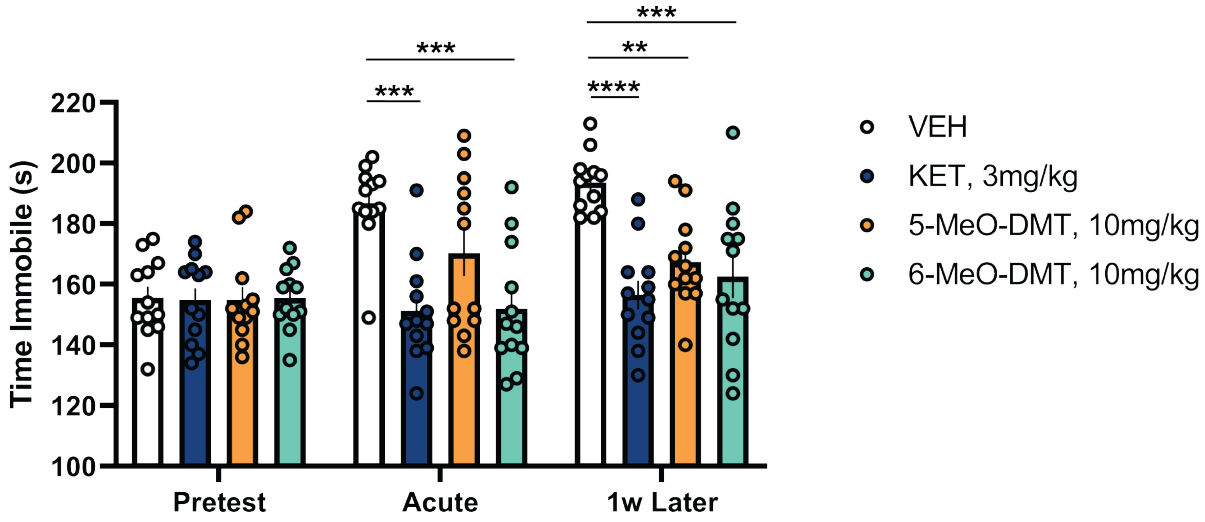

Figure 4.2.1-3. *Animals treated with 5-MeO-DMT and 6-MeO-DMT display therapeutic effects comparable to ketamine.*

*N = 12 mice per condition. Data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, as compared to the vehicle control following a one-way ANOVA with Dunnett's post hoc test. VEH = vehicle (saline), KET = ketamine, 5-MeO-DMT = 5-methoxy-N,N-dimethyltryptamine, 6-MeO-DMT = 6-methoxy-N,N-dimethyltryptamine.*

4.3 Development of Tabernanthalog

4.3.1 A brief history of ibogaine

Using this knowledge, we sought to develop other psychedelic analogs. We started with the psychedelic ibogaine—an alkaloid found in the root from the West African plant *tabernanthe iboga*—which has purported anti-addictive effects. The root has been used for centuries for coming of age ceremonies and typically leaves individuals feeling more mature and spiritually enlightened.[9] In the 1930s, ibogaine was sold under the name Lamberene in France as an antidepressant.[10, 11] Unfortunately, ibogaine has a high affinity for hERG (human ether-a-go-go) channels in the heart which are responsible for the cardiac repolarizing current.[12, 13] The blockage of this current results in heart attacks and ibogaine was subsequently pulled off the market in 1966. Later in the 1960s, the World Health Assembly classified ibogaine as a "substance likely to cause dependency or endanger human health." The drug was never legalized in the US and was deemed a Schedule I compound by the FDA in 1970.[14]

Howard Lotsof pioneered many of the original studies on ibogaine. In 1962, Lotsof was 19 years old and a heroin addict. By accident, he came across ibogaine, tried it, and no longer had a desire to use heroin for years afterwards.[15] This launched a series of academic and medical studies investigating the antiaddictive properties of ibogaine. Scientists verified ibogaine's ability to attenuate opioid withdrawal,[16] and diminish morphine[17] and cocaine[18] self-administration in rats. Subsequent studies demonstrated ibogaines efficacy in attenuating opioid withdrawal followed, in both humans and rodents.[19, 20, 21, 22]

Synthetic routes to make ibogaine have been difficult. There are only three published synthetic routes for synthesizing racemic ibogaine. These routes have 9–16 steps with overall yields ranging from 0.1–4.8%.[23] In fact, it is more efficient to extract ibogaine from raw materials

(the *Tabernanthe iboga* plant root), which requires enormous amounts of raw material, and associated extraction resources. Finally, ibogaine is lipophilic and drug formulation is quite challenging. Lipophilic drugs tend to accumulate in adipose tissue and release slowly over time, greatly extending the half-life of the compound,[24, 25] which may contribute to the long 'trips' associated with this molecule. Given these practical hurdles, the development of a safer, more rapid acting, and synthetically more efficient molecule is warranted.

Here, we deconstruct the skeleton of ibogaine to determine the therapeutic pharmacophore and modify the structure to increase safety.

4.3.2 Development of ibogaine analogs

We first began by deconstructing the ibogaine molecule into its component parts: the indole ring, the tetrahydroazepine ring and the isoquinuclidine.[26] We kept the indole core and systematically modified the tetrahydroazepine and isoquinuclidine rings, deleting various bonds and adding

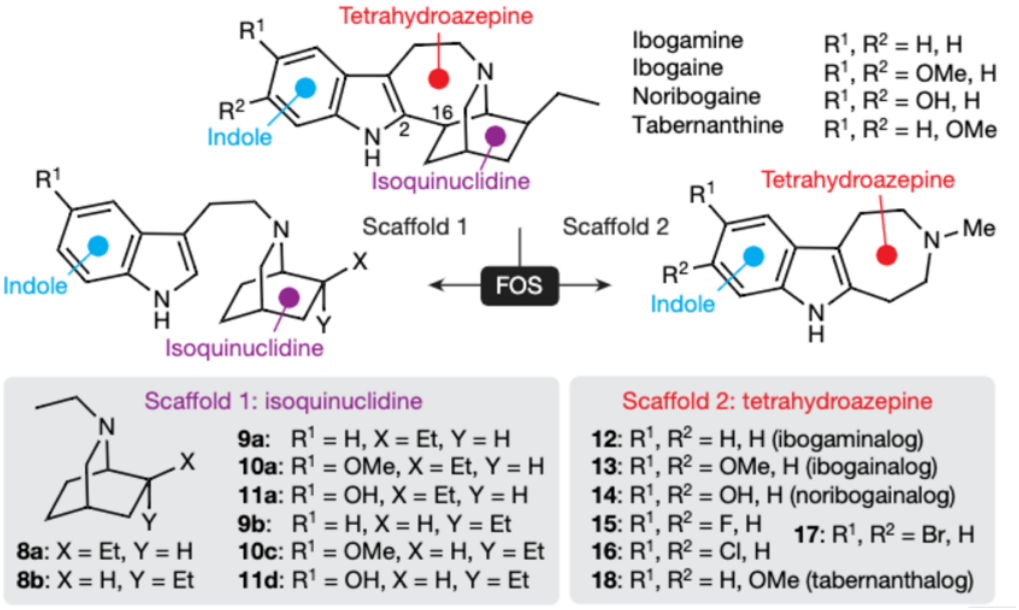

Figure 4.3.2-1. *Two sets of ibogaine analogs were created, incorporating either the isoquinuclidine or azepine moiety.*

substituents (bigger vs. smaller, electron donating vs. electron withdrawing) until we came up with a library of 18 analogs to screen in Sholl analysis (**Figure 4.3.2-1**).

We found that compound #13 increased growth of cortical neurons to a greater extent than other compounds tested (**Figure 4.3.2-2**). Perhaps unsurprisingly, it is structurally very similar to 5-MeO-DMT. We named this structure ibogainalog (IBG).

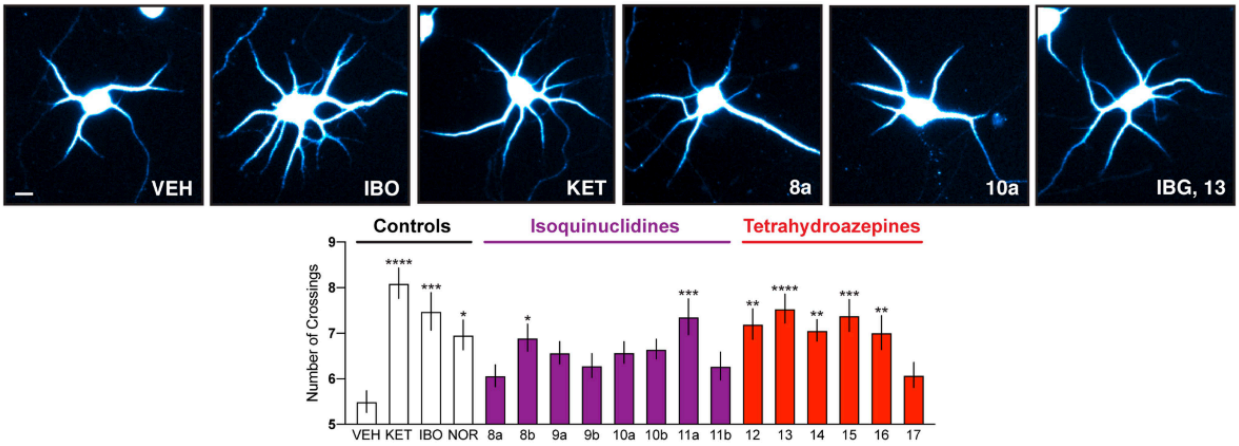

Figure 4.3.2-2. *Ibogaine analogs containing an azepine ring increase cortical neuron growth.*

*N = 31−83 neurons per condition over three separate experiments. Data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, as compared to the vehicle control following a one-way ANOVA with Dunnett's post hoc test. VEH = vehicle (saline), KET = ketamine, IBO = ibogaine, NOR = noribogaine. Compound numbers correspond to structures in* ***Figure 4.3.2-1****.*

4.3.3 Development of Tabernanthalog (TBG)

Looking at the structure of IBG, it was apparent that it shared structural similarities to 5-MeO-DMT. We previously reported that 6-MeO-DMT was non-hallucinogenic (as tested in the HTR assay), so we created a new molecule with the structure of IBG, but with the methoxy group at the 6-position on the indole (**Figure 4.3.3-1**). We named this compound tabernanthalog (TBG) based on its similarity to the natural product tabernanthine. Notably, by "tying down" the terminal amine group back to the indole, this conformationally restricts TBG in comparison to 6-MeO-DMT. Conformationally restricting molecules in such a way is known to increase potency and activity.[27]

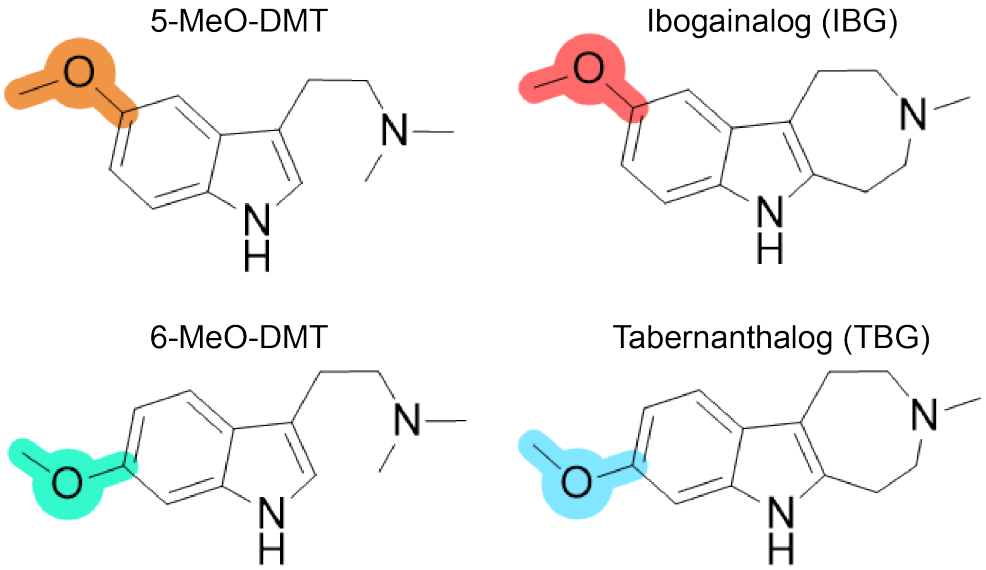

Figure 4.3.3-1. *Structural similarities between tabernanthalog (TBG), ibogainalog (IBG), 5-MeO-DMT and 6-MeO-DMT. Structural differences are highlighted.*

We next tested to see if this compound increased structural plasticity as well, or if this structural tweak compromised its ability to increase neuronal growth. We found that TBG was indeed capable of increasing growth of cortical neurons (**Figure 4.3.3-2a**), an effect which was blocked by pretreatment with the serotonin 2 antagonist ketanserin (**Figure 4.3.3-2b**). In addition, treatment with TBG was able to increase growth of spines on cortical neurons in vitro (**Figure**

4.3.3-2c) and in vivo using 2-photon imaging before and after drug treatment in a Thy-1 GFP mouse line (**Figure 4.3.3-2d**). Specifically, this was due to an increase in spine formation, with no changes to spine elimination.

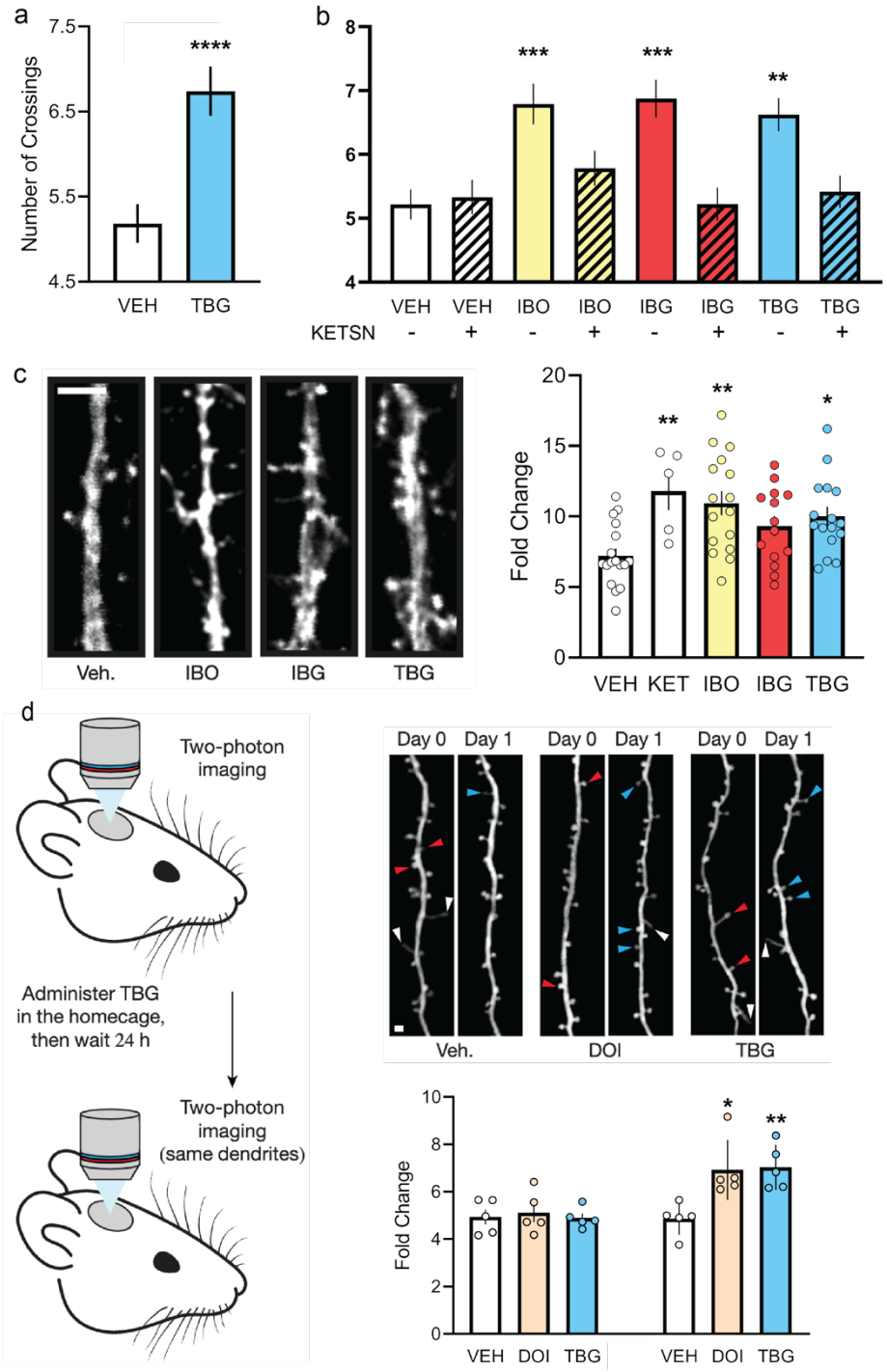

Figure 4.3.3-2. *TBG promotes neural plasticity. (a) Maximum numbers of crossings (Nmax) of Sholl plots obtained from rat embryonic cortical neurons (DIV6). N = 31−38 cells. (b) The effects of TBG (1 uM) on dendritic growth are blocked by the 5-HT2A antagonist ketanserin (KETSN, 10 uM). N = 23−28 cells. (c) Representative images of secondary branches of rat embryonic cortical neurons (DIV20) after treatment with ibogaine, IBG and TBG for 24 h. Scale bar, 2 µm. TBG increases dendritic spine density on rat embryonic cortical neurons (DIV20) after treatment for 24 h. N = 5−17 cells. (d) Left: Schematic of the design of transcranial two-photon imaging experiments. Right top: Representative images of the same dendritic segments from the mouse primary sensory cortex before (day 0) and after (day 1) treatment. Blue, red and white arrowheads represent newly formed spines, eliminated spines and filopodia, respectively. Scale bar, 2 µm. Right bottom: DOI and TBG increase spine formation but have no effect on spine elimination. N = 100−200 spines per treatment group.*

*Data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, as compared to the vehicle control following a one-way ANOVA with Dunnett's post hoc test. VEH = vehicle (saline), KET = ketamine, DOI = 2, 5-Dimethoxy-4-iodoamphetamine, KETSN = ketanserin, IBO = ibogaine, NOR = noribogaine.*

4.3.4 Hallucinogenic potential of tabernanthalog

To determine if our SAR hypothesis about the hallucinogenicity was correct, we injected animals with either 10 mg/kg or 50 mg/kg of IBG or TBG. Indeed, we found that IBG produced head-twitches, whereas all doses tested of TBG did not (**Figure 4.3.4-1a**).

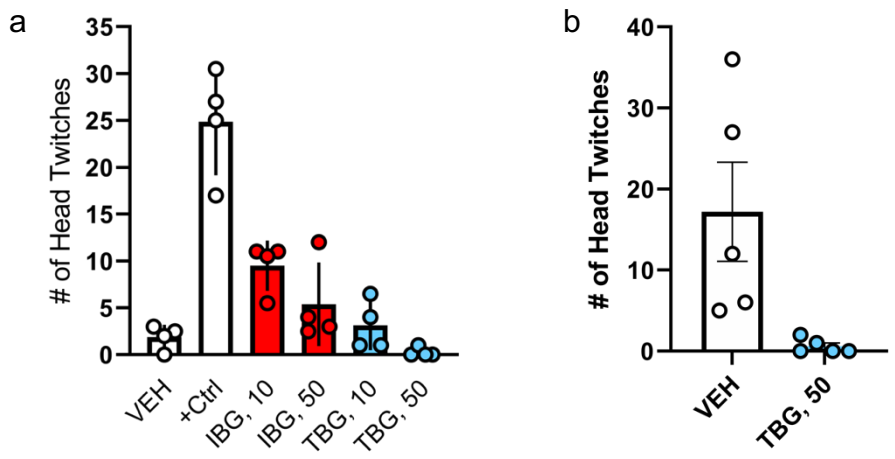

***Figure 4.3.4-1**. (a) TBG does not produce any head-twitches in the HTR assay. (b) Pretreatment of animals with TBG (50 mg/kg) but not VEH, is able to abolish 5-MeO-DMT (10 mg/kg)-induced head twitches, demonstrating engagement of the 5HT2A receptor. The numbers 10 and 50 indicate 10 mg/kg and 50 mg/kg respectively.*

N = 4 mice per treatment group. VEH = vehicle (saline), +Ctrl = 5-MeO-DMT (10 mg/kg). IBG = ibogainalog, TBG = tabernanthalog

In addition, I ran an HTR assay where I pretreated animals with vehicle (VEH), or TBG (50 mg/kg) for 15 mins, followed by an injection of 5-MeO-DMT (10 mg/kg). TBG is able to block HTRs elicited by this dose of 5-MeO-DMT (**Figure 4.3.4-1b**), demonstrating engagement of the 5HT2A receptor in vivo.

4.3.5 Solubility of tabernanthalog

Solutions of ibogaine are notoriously difficult to prepare, as it is exceptionally lipophilic. **Figure 4.3.5-1b** illustrates the difficulty of working with this compound and the extreme parameters needed to create an optimal drug formulation.

We endeavoured to test the solubility of our novel compound in comparison to ibogaine and found that both IBG and TBG were much more soluble than ibogaine (**Figure 4.3.5-1a**). This is likely due to the exclusion of the lipophilic isoquinuclidine moiety that is present within the ibogaine structure.

a

Solubility at [Cmpd] (mg/mL)			
Cmpd	40	8	4
IBO	no ✗	no ✗	no ✗
IBG	yes ✓	yes ✓	yes ✓
TBG	yes ✓	yes ✓	yes ✓

b

Vehicle Conditions	Concentration	Soluble?
Saline + 10% EtOH + 10% DMSO	40 mg/ml	NO ✗
Saline + 10% EtOH	8 mg/ml	NO ✗
Saline + 10% DMSO	8 mg/ml	NO ✗
Saline + 10% EtOH + 10% DMSO	8 mg/ml	NO ✗
Saline + 10% EtOH + 10% Kolliphor	8 mg/ml	NO ✗
Saline + 10% EtOH + 20% Kolliphor	8 mg/ml	NO ✗
Saline + 10% EtOH + 25% Kolliphor	8 mg/ml	NO ✗
Saline + 10% EtOH + 30% Kolliphor	8 mg/ml	NO ✗
Saline + 10% EtOH + 40% Kolliphor	8 mg/ml	NO ✗
Saline + 10% DMSO + 10% Kolliphor	8 mg/ml	NO ✗
Saline + 10% DMSO + 25% Kolliphor	8 mg/ml	NO ✗
Saline + 10% DMSO + 15% Glycerol	8 mg/ml	NO ✗
Saline + 10 mM ATP	8 mg/ml	NO ✗
Saline + 10% DMSO	4 mg/ml	NO ✗
Saline + 10% DMSO + 25% Kolliphor	4 mg/ml	NO ✗
Saline + 10% DMSO	1 mg/ml	YES ●

Figure 4.3.5-1. *TBG and IBG are much more soluble than ibogaine in a variety of solvents. (a) The fumarate salts of TBG and IBG are readily soluble in saline (0.9%), whereas ibogaine hydrochloride is not. (b) Ibogaine hydrochloride exhibits limited solubility in various saline-based vehicles. Solutions of saline (0.9%) containing various percentages of co-solvents/additives were added to finely crushed ibogaine hydrochloride. All of our attempts to improve its solubility through pulverizing, sonication, and mild heating (<50 °C) were unsuccessful. Moreover, the addition of co-solvents (ethanol, dimethyl sulfoxide, glycerol), surfactants (Kolliphor), or hydrotropes (ATP) to the vehicle did not substantially improve its solubility. We confirmed the purity and identity of the ibogaine hydrochloride used in these studies through a combination of NMR, LC–MS, and X-ray crystallography experiments.*

4.3.6 Pharmacokinetics of tabernanthalog

To probe the kinetics of TBG, animals were injected IP with either 1 mg/kg, 10 mg/kg or 50 mg/kg of the compound and sacrificed 15 mins or 3 h later. Whole brains and livers were collected, dried, homogenized and extracted with methyl *tert*-butyl ether (MTBE). Quantification was accomplished using LC–MS and concentrations of TBG in the two organs were calculated. Several samples for the 10 and 1 mg kg−1 doses at the 3 h time point had TBG at levels below the limit of quantification (~5 nmol g−1). In those cases, the values were recorded as 0.

We found that TBG was minimally detected at 10 mg/kg, with low quantifications in liver and brain at even the early timepoints (**Figure 4.3.6-1**). In contrast, 50 mg/kg TBG was detectable in high concentrations in both brain and liver at 15 minutes after dosing. These high concentrations were cleared rapidly and undetectable 3 h later. This suggests that the structural and behavioural changes measured 24 h (or later) after dosing are a result of structural changes to brain circuitry, not because the drug is still present in the animals' systems.

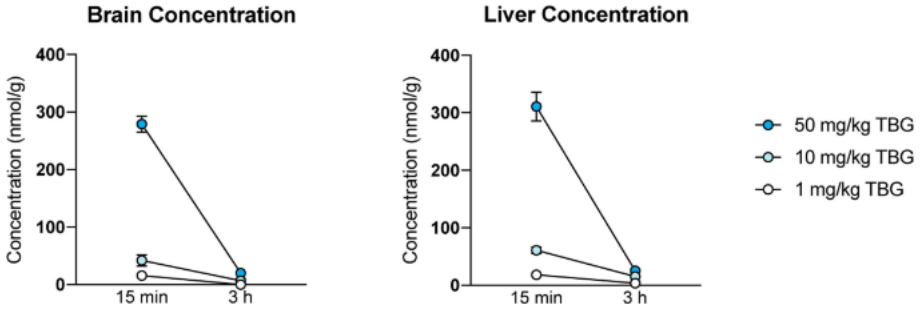

Figure 4.3.6-1. *TBG (50 mg/kg) reaches high brain concentrations and is rapidly eliminated from the body.*

4.3.7 Safety profile of tabernanthalog

As indicated previously, ibogaine has therapeutic potential but has several safety concerns associated with its use. Here we systematically test TBG to assess the safety of this novel compound.

4.3.7.1 Ibogaine blocks hERG channels significantly more than tabernanthalog does

Ibogaine is known to potently inhibit hERG channels.[12, 13] hERG channels are also known as Kv11.1 and are potassium channels responsible for the repolarizing current of the cardiac action potential.

To test the effect of TBG on hERG channels, we patch clamped HEK293 cells stabling expressing hKv11.1 and washed on drug while measuring current changes. We found ibogaine potently inhibited hERG channels, while IBG and TBG did not (**Figure 4.3.7-1a**). In fact, TBG was almost 150x safer than ibogaine with respect to hERG channel inhibition.

To corroborate these data, we treated zebrafish with our compounds and looked for bradycardia and arrhythmias before and after treatment. Zebrafish express Zerg, an orthologue of hERG. We found that ibogaine significantly reduced heart rate, but IBG and TBG did not (**Figure 4.3.7-1b**).

4.3.7.2 Tabernanthalog does not have seizurogenic potential

There are conflicting reports that ibogaine may have seizurogenic properties.[28, 29] We tested this using a larval zebrafish expressing GCaMP5 to assess the seizurogenic potential of TBG. We found that treatment with ibogaine, IBG and TBG did not induce excessive neural activity (**Figure 4.3.7-1c**).

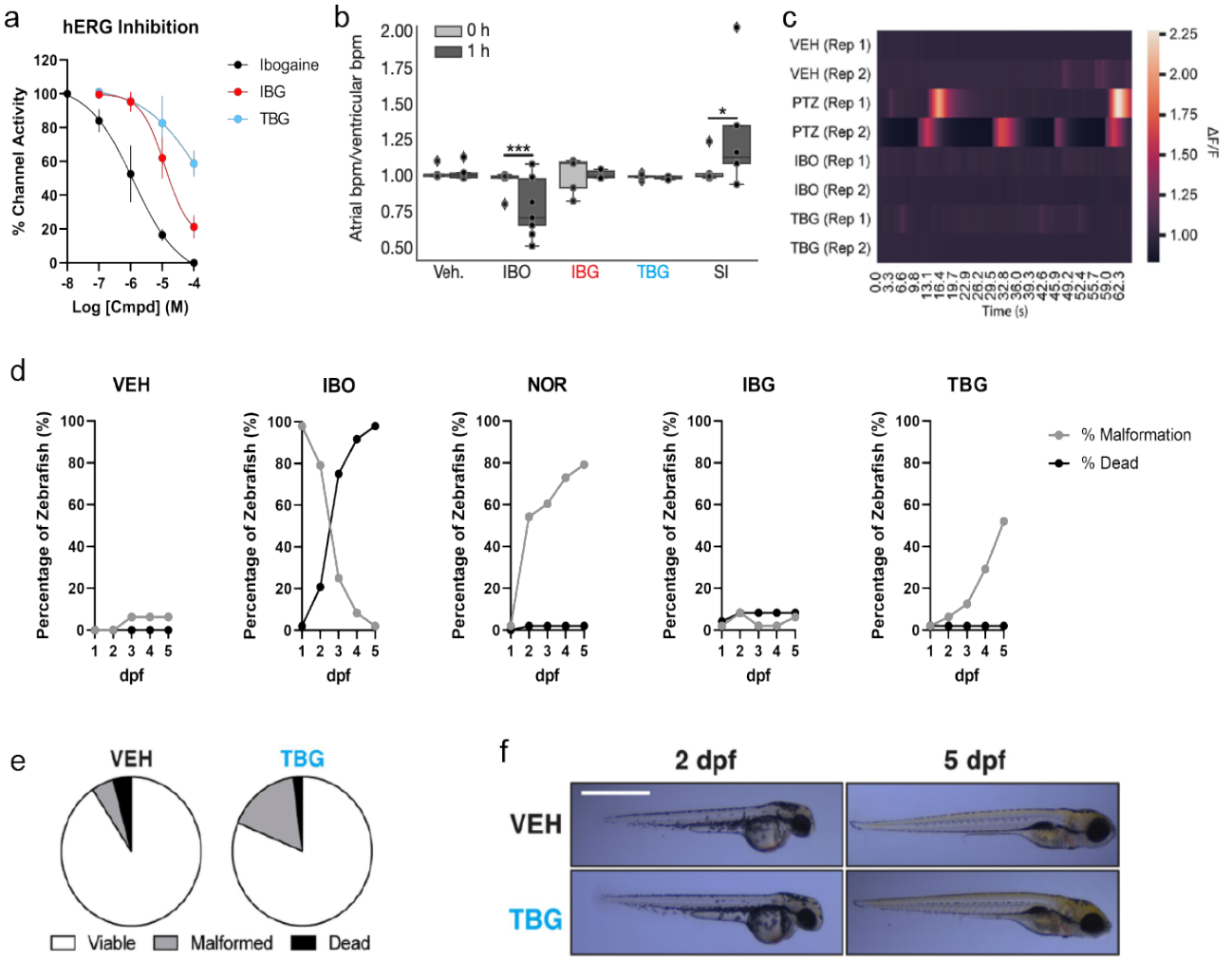

Figure 4.3.7-1. *TBG is a safer iboga alkaloid. (a) Inhibition of hERG channels expressed in HEK293 cells. Data are mean ± s.d. IBO, ibogaine. (b) Unlike ibogaine, IBG and TBG do not increase the risk of arrhythmias in larval zebrafish. Sertindole (SI) was used as a positive control. bpm, beats per minute. (c) Transgenic larval zebrafish expressing GCaMP5G were immobilized in agarose, treated with compounds, and imaged over time. The known seizure-inducing compound PTZ was used as a positive control. IBO and TBG were treated at 50 μM . N = 3−6 per condition, paired T-test between time 0 h and 1 h. (d) Compound-induced malformations and death over time N = 48 zebrafish for all treatment groups. (e) Proportion of viable and non-viable (malformed + dead) zebrafish following treatment with vehicle and TBG (66 μM) for 5 dpf (Fisher's exact test: P = 0.3864). (f) Representative images of zebrafish treated with vehicle and TBG (66 μM) for 2 and 5 dpf are shown. Scale bar, 2 mm.*

**p < 0.05, ***p < 0.001. VEH = vehicle (saline), IBO = ibogaine, NOR = noribogaine, IBG = ibogainalog, TBG = tabernanthalog.*

Data generated in panels b-c were generated by Robert J. Tombari (Olson Lab, UC Davis) and Matthew N. McCarroll (Kokel Lab, UCSF). Panels d-f were generated by RJ Tombari and Bianca Yaghoobi (Lein lab, UC Davis).

4.3.7.3 Tabernanthalog causes minimal teratogenicity.

Larval zebrafish were treated with ibogaine, IBG or TBG and monitored for viability and malformations over time (**Figure 4.3.7-1d**). At high doses (100 uM), ibogaine causes significant

malformations and increased mortality at 2- and 5-days post fertilization (dpf). At both these time points, the proportion of viable fish was significantly different than vehicle-treated fish. Noribogaine resulted in greater survival, but extreme malformations. In contrast, IBG and TBG resulted in significant viability compared to ibogaine. Importantly, at a dose of 66 uM, TBG-treated animals are indistinguishable from vehicle-treated animals (**Figure 4.3.7-1e,f**).

4.3.7.4 Tabernanthalog's effects on target receptors

We screened IBG and TBG against a series of serotonin and opioid receptor targets using functional assays in HEK cells to determine other potential side effects. Unlike ibogaine, IBG and TBG showed little or no opioid activity.[26] IBG and TBG work as partial agonists at 5HT2A with good potency (**Figure 4.3.7-2**). Many 5HT2A agonists also hit 5HT2B receptors, which can lead to cardiac valvulopathy, however, IBG and TBG appear to act more like antagonists at 5HT2B (**Figure 4.3.7-2**). As predicted, IBG and TBG interact with more selective receptoromes than the less-conformationally restricted 5-MeO-DMT and 6-MeO-DMT (**Figure 4.3.7-3**).

A full screen against 81 potential targets reveals that TBG exhibits high selectivity for 5HT2 receptors (**Figure 4.3.7-4**).

The safety data presented here is corroborated with a phenotypic assay performed in zebrafish. IBO- or NOR-treated animals produced behavioural responses similar to the lethal control, whereas IBG- and TBG- treated animals produced behavioural profiles more similar to the vehicle control (data not shown in this dissertation, please refer to original manuscript).[26]

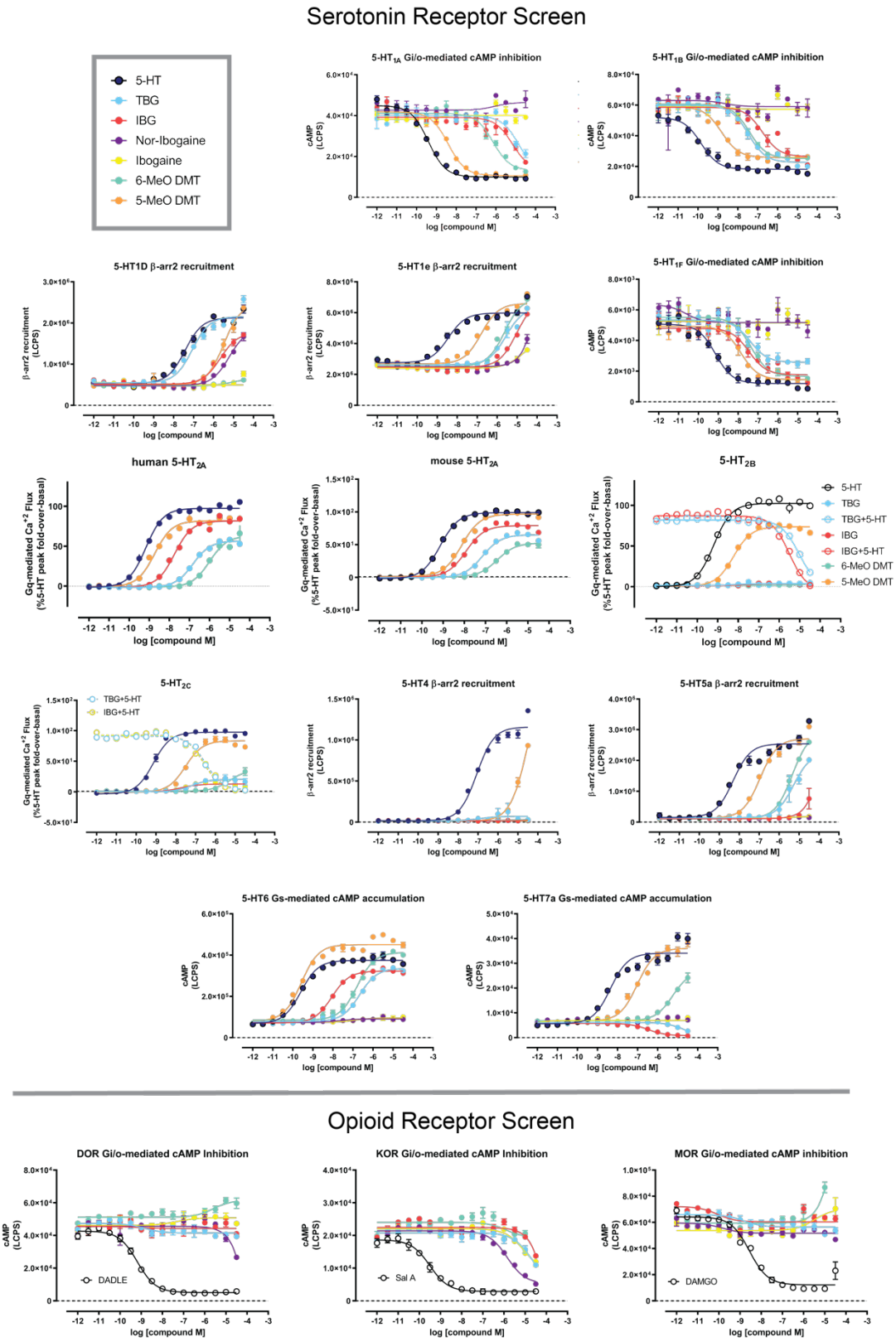

Figure 4.3.7-2. *Concentration–response curves demonstrating the abilities of ibogalogs and related compounds to activate 5-HT and opioid receptors. All compounds were assayed in parallel using the same drug dilutions. Graphs reflect representative concentration–response curves plotting mean and SEM of data points performed in duplicate or triplicate.*

	5-MeO-DMT			6-MeO-DMT			IBG			TBG			IBO			NOR		
Target Receptor	EC_{50}, nM	E_{max} % Control	$\log(E_{max}/EC_{50})$	EC_{50}, nM	E_{max} % Control	$\log(E_{max}/EC_{50})$	EC_{50}, nM	E_{max} % Control	$\log(E_{max}/EC_{50})$	EC_{50}, nM	E_{max} % Control	$\log(E_{max}/EC_{50})$	EC_{50}, nM	E_{max} % Control	$\log(E_{max}/EC_{50})$	EC_{50}, nM	E_{max} % Control	$\log(E_{max}/EC_{50})$
5-HT1A	3.92	98	8.40	725.40	91	6.10	6911.00	91	5.12	14600.0	95	4.81	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT1B	1.53	78	8.71	37.06	85	7.36	170.40	76	6.65	33.66	87	7.41	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT1D	37.01	98	7.42	3246.00	120	5.57	6043.00	82	5.13	2180.00	76	5.54	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT1e	160.20	119	6.87	2363.00	131	5.74	9309.00	126	5.13	2784.00	117	5.62	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT1F	14.00	93	7.82	44.50	88	7.30	35.10	85	7.38	40.40	64	7.20	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT2A	1.85	82	8.65	1003.00	63	5.80	18.10	82	7.66	147.00	57	6.59	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT2B	5.87	73	8.09	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT2C	30.70	84	7.44	5358.00	36	4.83	19.00	13	6.84	68.50	21	6.49	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT4	>10,000	N.D.	<5.00	>10,000	N.D.	<5.00	>10,000	N.D.	<5.00	>10,000	N.D.	<5.00	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT5a	110.00	107	6.99	4543.00	117	5.41	>10,000	N.D.	<5.00	>10,000	N.D.	<5.00	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT6	0.24	125	9.72	162.00	113	6.84	8.80	83	7.97	214.00	87.5	6.61	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
5-HT7a	65.70	107	7.21	5653.00	75	5.12	335.00	-17	N.D.	>10,000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
MOR	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
KOR	Inactive	Inactive	Inactive	>10,000	N.D.	<5.00	>10,000	N.D.	<5.00	>10,000	N.D.	<5.00	>10,000	N.D.	<5.00	1400	85	5.78
DOR	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive

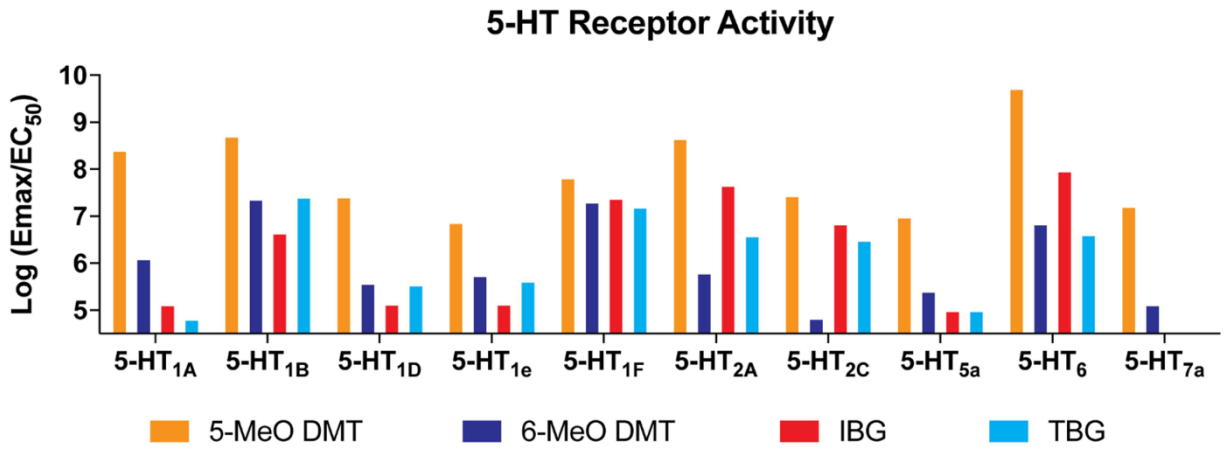

Figure 4.3.7-3. *Pharmacological profiles of ibogalogs and related compounds. EC50 and Emax estimates from at least two independent concentration-response curves performed in duplicate or triplicate. log(Emax/EC50) activity relative to the system Emax. Inactive, inactive in agonist mode; N.D., not determined; blue boxes indicate that the compound exhibits antagonist activity; dark grey boxes indicate that the compound is inactive in agonist mode but not tested in antagonist mode; orange boxes indicate that the compound is an inverse agonist. Ibogalogs are more selective 5-HT2Aagonists than is 5-MeO-DMT.*

Eurofins Catalog #	Assay Name	Species	% Inhibition
107710	ATPase, Na+/K+, Heart, Pig	pig	-9
104010	Cholinesterase, Acetyl, ACES	human	13
116030	Cyclooxygenase COX-1	human	16
118030	Cyclooxygenase COX-2	human	7
140010	Monoamine Oxidase MAO-A	human	66
140120	Monoamine Oxidase MAO-B	human	16
107300	Peptidase, Angiotensin Converting Enzyme	rabbit	2
112510	Peptidase, CTSG (Cathepsin G)	human	-2
152300	Phosphodiesterase PDE3A	human	6
154420	Phosphodiesterase PDE4D2	human	1
200510	Adenosine A1	human	-4
200610	Adenosine A2A	human	-8
203110	Adrenergic α1A	human	15
203210	Adrenergic α1B	human	20
203400	Adrenergic α1D	human	15
203630	Adrenergic α2A	human	81
203710	Adrenergic α2B	human	27
204010	Adrenergic β1	human	9
204110	Adrenergic β2	human	9
206000	Androgen (Testosterone)	human	6
210030	Angiotensin AT1	human	3
212620	Bradykinin B2	human	9
214510	Calcium Channel L-Type, Benzothiazepine	rat	18
214600	Calcium Channel L-Type, Dihydropyridine	rat	6
215000	Calcium Channel L-Type, Phenylalkylamine	rat	42
216000	Calcium Channel N-Type	rat	-3
217050	Cannabinoid CB1	human	1
217100	Cannabinoid CB2	human	-21
217510	Chemokine CCR1	human	11
244500	Chemokine CXCR2 (IL-8RB)	human	-2
219500	Dopamine D1	human	3
219600	Dopamine D2L	human	18
219700	Dopamine D2S	human	0
224010	Endothelin ETA	human	8
226010	Estrogen ERα	human	1
226810	GABAA, Chloride Channel, TBOB	rat	-1
226600	GABAA, Flunitrazepam, Central	rat	-2
226630	GABAA, Ro-15-1788, Hippocampus	rat	10
228610	GABAB1A	human	0
232030	Glucocorticoid	human	12
232600	Glutamate, AMPA	rat	8
232710	Glutamate, Kainate	rat	8
237000	Glutamate, Metabotropic, mGlu5	human	1
232810	Glutamate, NMDA, Agonism	rat	0
232910	Glutamate, NMDA, Glycine	rat	2
233000	Glutamate, NMDA, Phencyclidine	rat	3
234000	Glutamate, NMDA, Polyamine	rat	-2
239000	Glycine, Strychnine-Sensitive	rat	19
239610	Histamine H1	human	35
239710	Histamine H2	human	-12
250460	Leukotriene, Cysteinyl CysLT1	human	3
251100	Melanocortin MC1	human	-3
251350	Melanocortin MC4	human	-8
252610	Muscarinic M1	human	2
252710	Muscarinic M2	human	13
252810	Muscarinic M3	human	18
252910	Muscarinic M4	human	7
257010	Neuropeptide Y Y1	human	16
258700	Nicotinic Acetylcholine α1, Bungarotoxin	human	19
258730	Nicotinic Acetylcholine α3β4	human	16
260130	Opiate δ1 (OP1, DOP)	human	14
260210	Opiate κ (OP2, KOP)	human	7
260410	Opiate μ (OP3, MOP)	human	17
299037	Platelet Activating Factor (PAF)	human	7
265600	Potassium Channel [KATP]	hamster	6
265900	Potassium Channel hERG	human	28
267500	PPARγ	human	-6
299005	Progesterone PR-B	human	4
271110	Serotonin (5-Hydroxytryptamine) 5-HT1A	human	39
271230	Serotonin (5-Hydroxytryptamine) 5-HT1B	human	66
271650	Serotonin (5-Hydroxytryptamine) 5-HT2A	human	57
271700	Serotonin (5-Hydroxytryptamine) 5-HT2B	human	86
271800	Serotonin (5-Hydroxytryptamine) 5-HT2C	human	99
271910	Serotonin (5-Hydroxytryptamine) 5-HT3	human	14
279510	Sodium Channel, Site 2	rat	24
255520	Tachykinin NK1	human	18
202000	Transporter, Adenosine	guinea pig	-9
226400	Transporter, GABA	rat	1
274030	Transporter, SERT	human	88
287530	Vasopressin V1A	human	-6
252030	Transporter, Vesicular Monoamine (Non-Selective)	human	10

Figure 4.3.7-4. *Safety pharmacology Screen. TBG (10 uM) was tested on a wide range of targets by Eurofins Discovery. Assays were conducted in duplicate and results were averaged. Targes with >50% inhibition are highlighted in blue.*

4.3.8 The effects of Tabernanthalog on behaviours relevant to depression

Thus far, we have demonstrated that TBG is a safer iboga analog compared to ibogaine, is more water soluble and increases the growth of cortical neurons through a 5HT2A-mediated mechanism. We now sought to understand if therapeutic properties of ibogaine were retained in this novel analog. We started by testing antidepressant efficacy using the forced swim test (FST), as several 5HT2A agonists and ketamine display robust decreases in immobility in this test.[30,31]

We subjected animals to 7 days of unpredictable mild stress (UMS), which is known to induce behavioural depressive-like phenotypes in animals (**Figure 4.3.8-1a**). Indeed there are increases in immobility in the FST after UMS (**Figure 4.3.6-1b)**. We then subjected animals to treatment with either VEH, TBG 10 mg/kg or TBG 50 mg/kg. We found only a dose of 50 mg/kg was able to rectify the behavioural deficits in the FST assay (**Figure 4.3.8-1b**). Therefore, we did all subsequent assays at 50 mg/kg. This data agrees with the pharmacokinetic data presented in Section 4.3.6, which shows that TBG minimally enters the brain at 10 mg/kg but is detectable at appreciable quantities when animals are treated at 50 mg/kg.

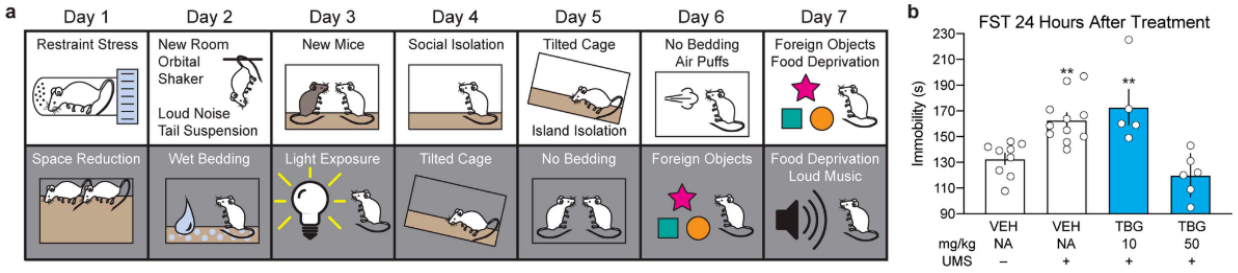

Figure 4.3.8-1. *Deficits in the forced swim test are rectified after a 50 mg/kg dose of TBG. (a) Schematic illustrating the stressors used as part of the 7-day UMS protocol. White and grey boxes represent the light and dark phases of the light cycle, respectively. (b) TBG rescues the effects of UMS on immobility.*

*N = 6–11 mice per treatment group. Data are represented as mean ± SEM. **p < 0.01, as compared to the vehicle control following a one-way ANOVA with Dunnett's post hoc test. VEH = vehicle (saline), TBG = tabernanthalog.*

We next decided to determine the length of TBG's effects, as well as its mechanism of action. We ran a simplified FST (**Figure 4.3.8-2a**), where animals were subjected to a pretest, 24

h later dosed with drug, and 24 h after that tested in the FST. We found that 24 h later, TBG reduced immobility in this test, indicating this compound has antidepressant efficacy. This effect is comparable to ketamine (3 mg/kg)-treated animals. Interestingly, if animals were administered 4 mg/kg ketanserin 10 mins before the TBG, the therapeutic effect was abolished (**Figure 4.3.8-2b**). This suggests that engagement of the 5HT2A receptor is necessary for TBG to elicit therapeutic behavioural changes.

When retested in the FST 1 week later, animals with TBG returned to baseline levels of immobility, unlike ketamine (**Figure 4.3.8-2c**).

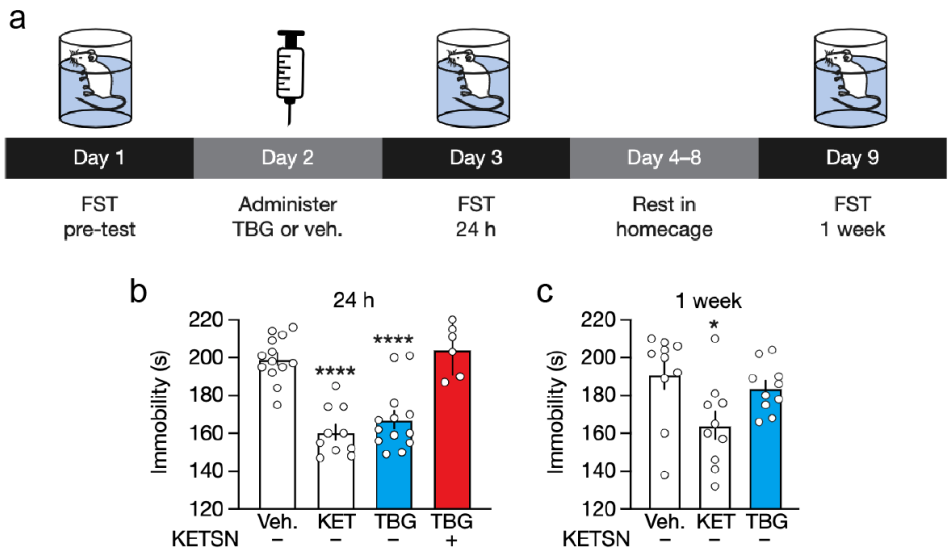

Figure 4.3.8-2. *Animals treated with TBG display rapid-acting antidepressant properties compared to ketamine in the FST. (a) Schematic illustrating the experimental design of FST. (b) 24 h after treatment, animals treated with TBG display reduced immobility, compared to ketamine. This is blocked with pretreatment of 4 mg/kg ketanserin (KETSN). (c) The reduced immobility effects of TBG do not last up to the one-week timepoint, whereas the ketamine-treated animals do.*

*N = 6−13 mice per condition. Data are represented as mean ± SEM. *p < 0.05, ****p < 0.0001, as compared to the vehicle control following a one-way ANOVA with Dunnett's post hoc test. VEH = vehicle (saline), KET = ketamine, TBG = tabernanthalog.*

4.3.9 The effects of tabernanthalog on behaviours relevant to addiction and drug-seeking behaviour

Ibogaine has gained a reputation as being able to help those with substance use disorders (SUD). Therefore, we decided to test TBG on alcohol- and heroin-seeking paradigms.

4.3.9.1 The effects of tabernanthalog on alcohol seeking behaviour

To assess the effects of TBG on alcohol-seeking behaviour, we used an intermittent-access, two-bottle choice experiment in which animals were given intermittent access to both water and alcohol (20% ethanol (v/v) in water). Mice underwent repeated cycles of intermittent access resulting in binge drinking and withdrawal for 7 weeks, which resulted in high alcohol consumption (11.44 ± 0.76 g per kg per 24 h) and binge-drinking-like behaviour (3.89 ± 0.33 g per kg per 4 h). This generated high blood alcohol content equivalent to that of humans with alcohol use disorder (AUD). After establishment of this alcohol-preference, animals were injected with TBG 3 h before the next drinking session (**Figure 4.3.9-1a**). The amount of alcohol consumed was measured at a 4 h, 24 h, 48 h and 120 h.

During the initial binge, there was a drastic reduction in alcohol intake, but not water intake, indicating a change in preference for water over alcohol (**Figure 4.3.9-1b**). This effect was significant for the entire first and second days but was no longer present by day 5 (**Figure 4.3.9-1c**).

A possible interpretation of these data is that there is a disruption of pleasure and this is not just blocking hedonic behaviour. To address this question, we preformed the same paradigm with a sucrose two-bottle choice paradigm (sucrose preference test; SPT). Similar to the alcohol two-bottle choice assay, animals were exposed to the apparatus 3 h after injection, and sucrose preference was measured at a 4 h and 24 h timepoint. There was no difference in sucrose preference

between vehicle-treated and TBG-treated animals (**Figure 4.3.9-1d**). Importantly, there was also no difference in the total liquid consumed (**Figure 4.3.9-1e**).

Together, these data suggest that TBG administration transiently blocks hedonic alcohol-seeking behaviour, without impairing basic their ability to perceive natural rewards.

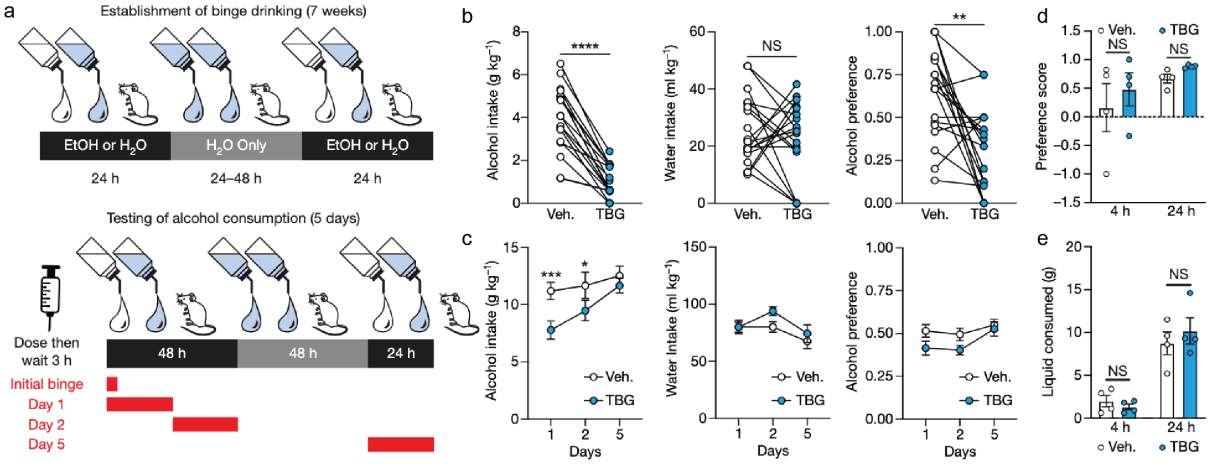

Figure 4.3.9-1. *TBG administration blocks alcohol self-administration. (a) Timeline of alcohol binge-drinking experiment. White and blue droplets represent 20% ethanol (EtOH) and H2O, respectively. (b) TBG acutely reduced ethanol consumption and preference during a binge-drinking session without affecting H2O intake. N = 19 animals per condition, two-tailed paired T-test. (c) Acute TBG administration decreased ethanol consumption for at least 48 h. N = 19 animals per condition, two-way ANOVA with Sidak's post-hoc test. (d-e) TBG did not decrease sucrose preference (d) or reduce total liquid consumption (e) in a two-bottle choice experiment. N = 4 animals per condition, two-way ANOVA with Sidak's post-hoc test.*

*Data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, as compared to the vehicle control following a one-way ANOVA with Dunnett's post hoc test. VEH = vehicle (saline), TBG = tabernanthalog.*

4.3.9.2 The effects of tabernanthalog on heroin seeking behaviour

We next sought to determine the effects of TBG treatment on heroin-seeking behaviour. We used adult male and female Wistar rats, which were implanted with an intravenous jugular catheter. Animals began training on a fixed ratio 1 (FR1) schedule of reinforcement for 1 week (**Figure 4.3.9-2b**). Operant chambers had an inactive lever and active lever. When the active lever was pressed, light cue and a tone (3.5 kHz for 5 sec) was played, and animals would receive 0.04 mg of heroin (50 ul over 2.85 sec; **Figure 4.3.9-2a**). Both levers would retract upon initiation of a

heroin infusion and would remain retracted until the tone and light cues terminated. After 6 sessions on an FR1 schedule, animals progressed to a variable ratio 5 schedule (VR5) for 3 sessions. The last stage was a variable ratio 15 (VR15) for five sessions. Each session lasted 2.5 h. For each of these paradigms, TBG (40 mg/kg) was administered 30 mins prior to being placed in the operant chambers.

When rats were administered TBG during the VR15 session, there was a significant decrease in the amount of lever-responding (**Figure 4.3.9-2c**) and heroin self-administered (**Figure 4.3.9-2d**). This effect did not last, as animals returned to the same number of lever presses by the next testing day (**Figure 4.3.9-2b**).

Rats then began extinction training (EXT, 1 h sessions) in which animals were placed in the same operant chambers (context) however no heroin was administered and no cues (light, tone, retraction of levers) occurred. Lever pressing resulted in no outcome for the rat, but all lever presses were recorded by the experimenter. Rats underwent 7 extinction sessions. A second cohort of rats (drug-naïve) were administered TBG on the first day of extinction training. There was also a significant decrease in the amount of lever pressing for heroin (**Figure 4.3.9-2e**), but again, these animals had the same number of lever presses as drug-naïve animals by testing the next day (**Figure 4.3.9-2b**).

Finally, rats underwent a cue extinction test. During the cue extinction (CUE) test (1 h) the animals are placed in the operant boxes, and cues (light, tone, and lever retraction) were available each time the active lever was pressed on a VR5 schedule, however no heroin was administered. This test is critical because studies show that cue induced reinstatement is a correlate of relapse.[32] Animals administered TBG during the CUE portion of the experiment significantly decreased the lever pressing. Importantly, animals that had previously been administered TBG during the VR15

or EXT paradigm also demonstrated significantly less lever responding (**Figure 4.3.9-2f**), which was up to 2 weeks after their initial drug dose.

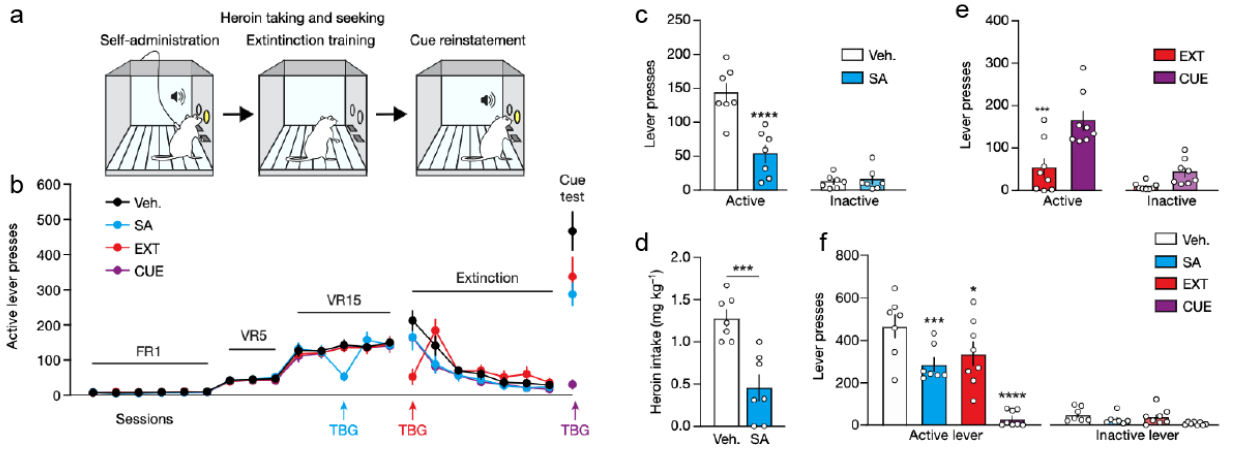

Figure 4.3.9-2. *TBG reduced heroin-seeking behaviour. (a) Schematic of the design of the heroin self-administration experiments. (b) Heroin seeking over time. Coloured arrows indicate when each group received TBG. Vehicle was administered at all other time points to each group. SA, self-administration; EXT, extinction; CUE, cued reinstatement; FR1, fixed ratio 1; VR5, variable ratio 5; VR15, variable ratio 15. (c, d) TBG acutely reduced heroin self-administration—both lever pressing (c) and heroin intake (d). (e) TBG acutely reduced heroin-seeking when administered immediately before the first extinction session. The cued reinstatement (injection 1, vehicle; injection 2, vehicle) and extinction (injection 1, vehicle; injection 2, TBG) groups were compared, as they were matched for the number of withdrawal days between the last self-administration and first extinction session. (f) Acute TBG administration completely blocked cued reinstatement (purple bar). A single previous (12–14 d) administration of TBG during heroin self-administration or on the first day of extinction (blue and red bars, respectively) inhibited cued reinstatement.*

*N = 7−8 rats per condition. Data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001,using a two-way repeated measures ANOVA with Sidak's post-hoc test. VEH = vehicle (saline), TBG = tabernanthalog, SA = self-administration, EXT = extinction training, CUE = cue reinstatement.*

Importantly, when animals are injected with TBG during an identical study assessing sucrose preference instead of heroin, animals have transient decreases in responding, but during CUE testing, there is no changes to self-administration for natural rewards (please refer to manuscript ED Fig 8).[26] This suggests that while there may be transient decreases in pleasurable experiences, TBG administration may have a more long lasting-effect on hedonic drug seeking without impairing pleasure of natural rewards.

4.3.10 Abuse potential of tabernanthalog

It could be argued that TBG merely "overrides" or "replaces" desire for hedonic substances (such as alcohol or heroin), and that TBG itself may be addictive. To test this, we ran a conditioned-place preference assay (CPP).

On day 1, the amount of time the mice spent in each distinct side of a two-chamber apparatus was recorded. Next, vehicle and TBG were administered to mice on alternating days while they were confined to chamber A (**Figure 4.3.10-1a**, white box) or chamber B (**Figure 4.3.10-1a**, grey parallel lines), respectively. Conditioning lasted for a total of 6 days. On day 8, preference for each distinct side of the two-chamber apparatus was assessed.

Animals administered 1 mg/kg of TBG did not display a preference for either the VEH- or TBG-paired side. In contrast, animals administered 10 mg/kg or 50 mg/kg spent reduced time in

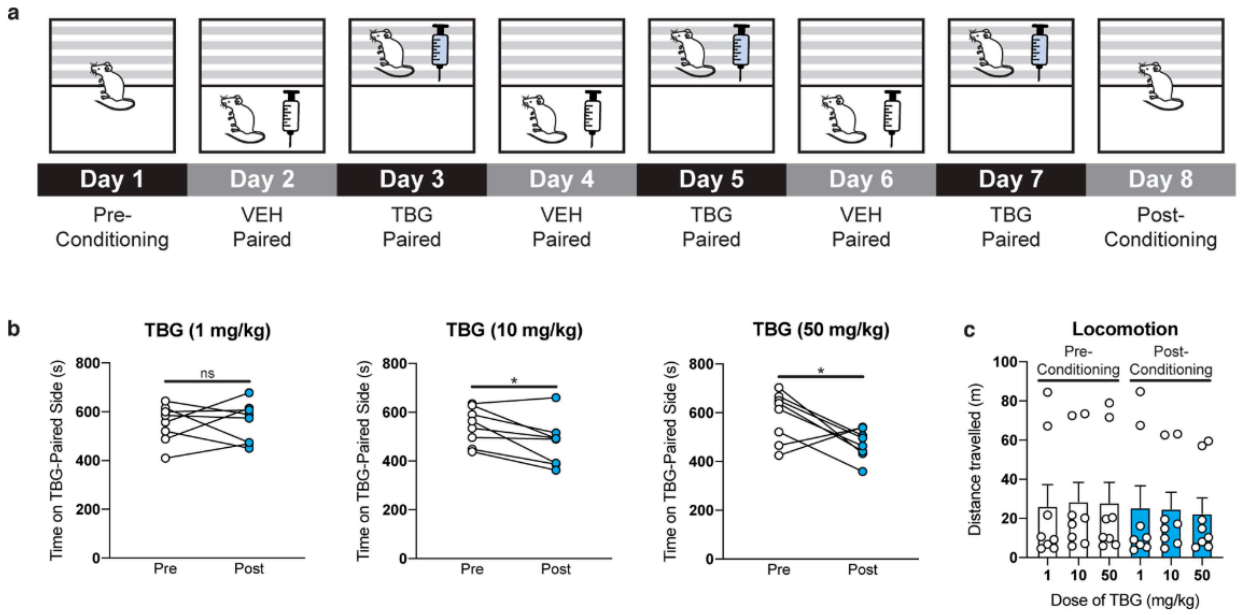

Figure 4.3.10-1. *High doses of TBG do not produce a conditioned place preference. (a) Schematic of the design of the conditioned place preference experiments. (b) A low dose of TBG (1 mg/kg) did not produce conditioned place preference or conditioned place aversion. Higher doses (10 and 50 mg/kg) produce a modest conditioned place aversion. (c) TBG does not produce any long-lasting (>24 h) effects on locomotion. There is no statistical difference in locomotion between any pre- or post-conditioning groups (P = 0.9985, one-way ANOVA). White bars indicate groups before receiving TBG (that is, pre-conditioning), and blue bars indicate groups 24 h after the last TBG administration (that is, post-conditioning).*

*N = 8 per condition. Data are represented as mean ± SEM. *p < 0.05, as compared to the vehicle control using a two-tailed paired T-test. VEH = vehicle (saline), TBG = tabernanthalog, ns = not significant.*

that the TBG-paired side (**Figure 4.3.10-1b**). This indicates that TBG may have a mild aversive effect at high doses, and therefore has a minimal recreational abuse potential.

This repeated dosing regimen had no effect on locomotion, as measured in the post-conditioning test (24 h after the last dose; **Figure 4.3.10-1c**).

4.3.11 The therapeutic effects of tabernanthalog are unlikely due to performance deficits

Importantly, these behavioural results are unlikely to occur from a performance deficit, as there are no changes in locomotion (**Figure 4.3.11-1**).

Rats were subjected to novelty-induced locomotion (baseline) for 30 min. At that time, cocaine was administered and psychostimulant-induced locomotion (+ cocaine) was assessed for 60 min. There were no differences between the vehicle- and TBG-treated groups with respect to total distance travelled or average velocity. Furthermore, there was no difference in thigmotaxis measured during the baseline period (that is, the percentage of time in the centre of the open field).

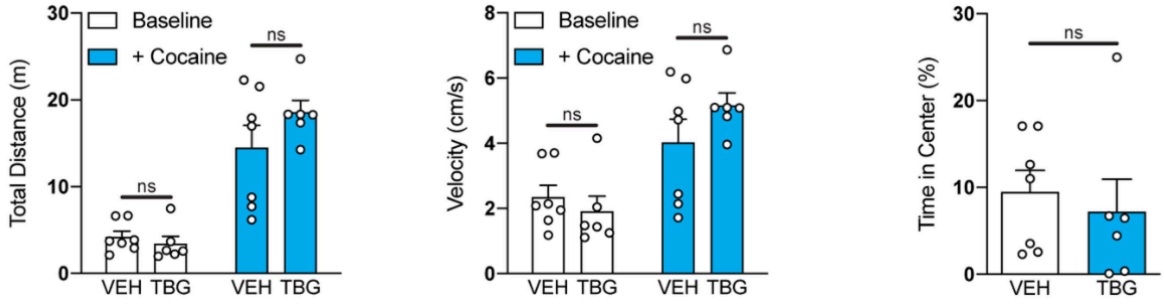

Figure 4.3.11-1. *Acute TBG administration does not cause changes in locomotion in the open field.*

*N = 6−7 per condition. Data are represented as mean ± SEM. *p < 0.05, as compared to the vehicle control following a two-way ANOVA with Sidak's post-hoc test. VEH = vehicle (saline), TBG = tabernanthalog, ns = not significant.*

4.4 Conclusion and Discussion

In this chapter, I discuss the development and testing of a non-hallucinogenic psychedelic analog, TBG. This compound increases the growth of cortical neurons (dendrites and spines), appears safer than ibogaine and has therapeutic effects in rodent models of depression and SUD.

4.4.1 Top-down control over circuits involved in depression and drug-seeking

The PFC is a critical region of the brain responsible for goal-oriented behaviour and motivation by exerting a top-down control of subcortical regions involved in emotion. By increasing the growth and stimulating the neurons involved in these circuits, it may be possible to override depressive or drug-seeking behaviour.

Ketamine,[33] psychedelics[8,34] and non-hallucinogenic analogs[26] increase the function of neurons in the prefrontal cortex via increases in amplitude and frequency of sEPSCs. Increases in frequency may be attributed to increases in synapse number or changes in the probability of release; our data suggests at minimum there is an increase in the number of spines/synapses if not changes in the probability of release as well. Increases in amplitude of sEPSCs reveal there is a postsynaptic strengthening of signal after drug administration. Ketamine and psychedelics are both known in cause mTOR activation,[33,30] and though it was not explicitly tested here, it is likely that non-hallucinogenic analogs are working via a similar mechanism.

Sub-anesthetic/therapeutic doses of ketamine increase spine density after administration.[33] A recent paper from the Liston lab demonstrates the necessity of spines in the therapeutic effects of ketamine by ablating newly-formed spines in the PFC after treatment. Indeed, abolishment of these spines fully blunted the long-lasting therapeutic effects of ketamine.[35] Other fast-acting

antidepressants, such as psychedelics[8,34] and their non-hallucinogenic counterparts, capable of increasing spine growth are hypothesized to function in a similar manner.

There are ample studies demonstrating that stimulation of the PFC is necessary for achieving antidepressant-like effects in the FST, both through optogenetic manipulations[36, 37] and administration of ketamine (which is known to increase cortical activity).[35] In addition, blocking activity of neurons in the PFC pharmacologically[37] or through use of a BDNF-sequestering antibody[38] all block behavioural antidepressant-like effects of ketamine. These data suggest that rapid-acting antidepressants indeed work through structural growth and functional strengthening of neurons in the PFC. Here, I present data that demonstrates that both psychedelic and non-hallucinogenic compounds increase the growth and function of neurons in the frontal cortex, suggesting a convergent circuit-level mechanism of action for different types of psychoplastogens.

Drug addiction is the transition from initial hedonic drug use to habitual and compulsive drug use and seeking, resulting from changes to specific neural circuits.[39, 40] It has been demonstrated that the PFC plays an integral role in drug seeking behaviour. Human imaging studies describe hypofunction of the PFC contributes to loss of control over drug intake in humans with SUD.[41, 42, 43] Conversely, humans who exhibit more self-control show a greater amount of activity in the dlPFC.[44]

Seeking a less correlational and more causal relationship between the PFC and drug seeking behaviour, Chen et al. utilized a compulsive drug seeking model in which a subset of rats persists to seek cocaine despite noxious foot shocks.[45] The authors illustrate how ex vivo intrinsic excitability of layer 5 pyramidal neurons is decreased after chronic cocaine use. Further, stimulation of these neurons in prelimbic cortex (restoration of activity) is sufficient to prevent compulsive drug seeking, whereas inhibition of this area significantly increased compulsive drug

seeking. Other groups have demonstrated that increasing neural activity in infralimbic cortex (via infusing of AMPA) is sufficient to extinguish animals cocaine seeking.[46] Together, this illustrates the integral role of PFC in modulating drug-seeking behaviour.

Therefore, increasing the growth and function of layer 5 pyramidal neurons in the PFC may have therapeutic effects over circuits involved in habitual drug seeking.

4.4.2 Role of the 5HT2A receptor

The 5HT2A receptor is the target of psychedelic compounds, and happens to be densely expressed in layer 5 pyramidal cells in the PFC.[47, 48] Psychedelic compounds are active at 5HT2A receptors and are known to exert their hallucinogenic properties via this receptor.[49, 5051] Interestingly, this receptor also appears to be necessary for the growth effects of cortical neurons.[30]

TBG displays reasonable activation of 5HT2A receptors. In both cellular and behavioural assays, the effects of TBG are blocked with pretreatment of ketanserin, a 5HT2A antagonist. And the fact that TBG is efficacious in the FST is consistent with the fact that other 5HT2A agonists have demonstrated potential for the treatment of depression.[8, 34] A discussion on the mechanism of action and role of the 5HT2A receptor can be found in this dissertation in **Chapter 5**.

Establishment of cortical populations containing the 5HT2A receptor and which subcortical circuits they modulate are warranted.

4.4.3 Potential role of the 5HT1B receptor

A recent paper came out suggesting that activation of 5HT1B receptors on PFC terminals in the NAc reduces compulsive drug seeking.[52] Given that TBG is a full agonist at 5HT1B (albeit less potent than serotonin itself, **Figure 4.3.7-2**), this stimulation may contribute to TBG's effects on

drug-seeking behaviour. It is likely that this behaviour results from a combination of 5HT2A and 5HT1B stimulation.

4.4.4 Advances in the field of non-hallucinogenic psychedelic analogs

Here I will comment on advancements in the field of non-hallucinogenic psychedelic analogs since the completion of my studies.

4.4.4.1 Further studies with TBG

Studies following the work in Section 4.3 assessed the deficits in elevated plus maze (EPM), whisker-dependent texture discrimination (WTD) and 4-choice odor discrimination and reversal task after UMS. Lu and colleagues found that UMS produced anxiogenic behaviours, as assessed by the EPM, and deficits in the WTD and 4-choice paradigms.[53] These deficits could be rectified with a 10 mg/kg dose of TBG (**Figure 4.4.4-1b-d, f-h, j-l**), but the rescue was blocked when KETSN (4 mg/kg) was pretreated 10 mins prior to TBG (**Figure 4.4.4-1e,i,m**). This further points to the fact that TBG may be used to treat affective disorders, and this effect is likely mediated via 5HT2A receptor engagement.

Using in vivo imaging of cortical neurons (somatosensory cortex, barrel field, S1BF), Lu et al. also described a reduction of spines after UMS (**Figure 4.4.4-2a**), specifically via an increase in spine elimination with no changes to spine formation (**Figure 4.4.4-2d,e**). Administration of TBG increases spine formation with spine elimination rates comparable to control, resulting in an overall increase in spine density (**Figure 4.4.4-2f,g**). Interestingly, it enhances regrowth of previously-eliminated spines (**Figure 4.4.4-2h**). Current studies via a collaboration with our lab are looking at KETSN's ability to block the effects on spine dynamics.

Together, this suggests demonstrates that TBG is capable of rectifying circuits damaged by UMS anatomically and behaviourally.

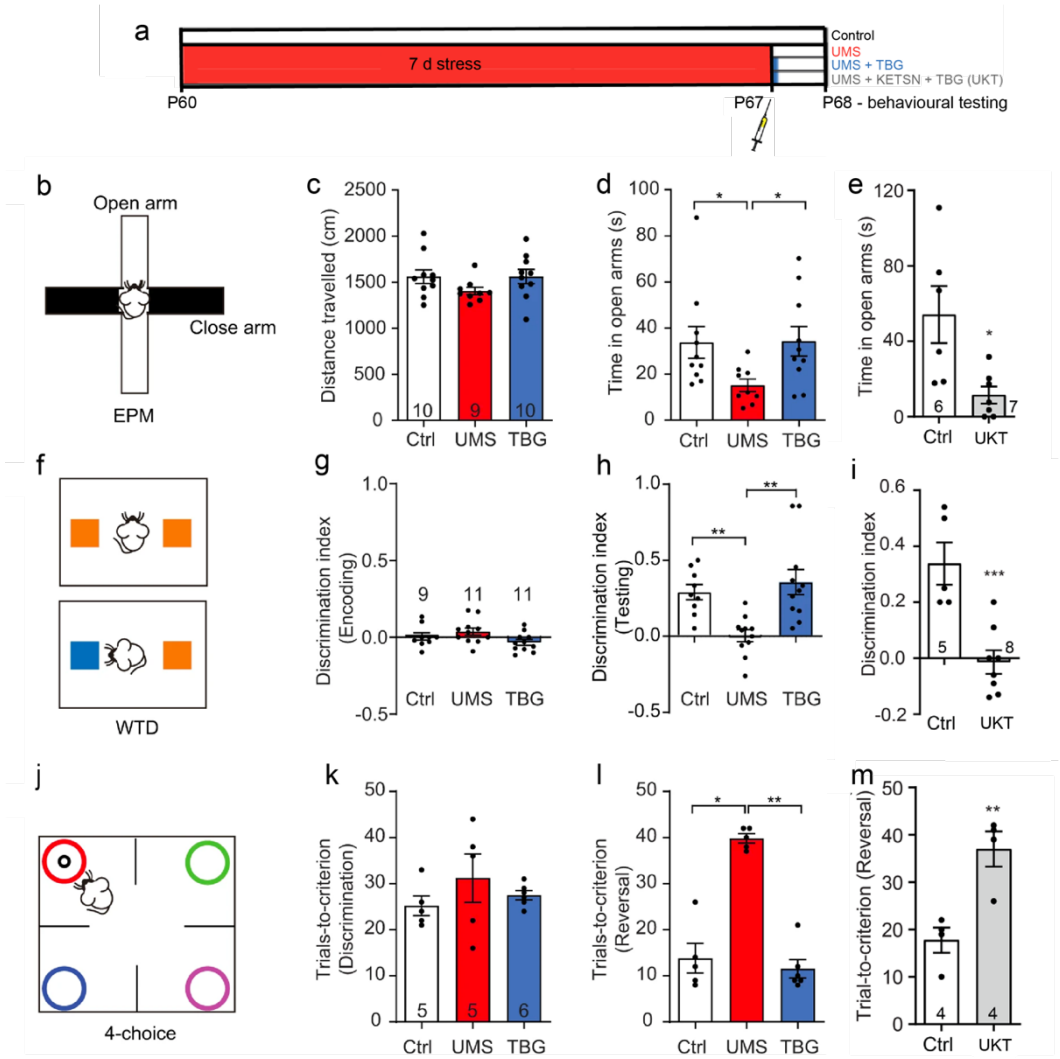

Figure 4.4.4-1. *TBG rescues UMS-induced behavioral deficits in mice and is dependent on 5HT2A receptor activation. (a) Timeline of UMS, drug injection, and behavioral tests. (b) Schematic of the elevated plus maze (EPM) test. (c) Total distance traveled in EPM. (d) Time spent in the open arms. (e) TBG-mediated rescue of time in open arms is blocked by preadministration of ketanserin (KETSN), 4 mg/kg, 10 mins before TBG injection. (f) Schematic of the whisker-dependent texture discrimination (WTD) task. The two colors represent distinct textures. (g, h) Texture preference during encoding and testing. Discrimination index is defined as the number of approaches to one texture (e.g., novel) minus the number of approaches to the other texture (e.g., familiar), divided by the total number of approaches. (i) TBG-mediated restoration of discrimination index is blocked by pretreatment with KETSN. (j) Schematic of the 4-choice odor discrimination and reversal task. Each color symbolizes a distinct odor; only one is associated with the food reward (black circle). (k, l) Number of trials taken to reach the performance criterion in the initial discrimination and reversal phase. The performance criterion is 8 correct choices out of 10 consecutive trials. (m) pretreatment with KETSN blocks TBG-mediated trials to criterion after chronic stress.*

*N = 4−5 per condition. Data are represented as mean ± SEM. *p < 0.05, **p < 0.01. Ctrl = unstressed control group, TBG = tabernanthalog, UMS = unpredictable mild stress, UKT = unpredictable mild stress + ketanserin + TBG treatment.*

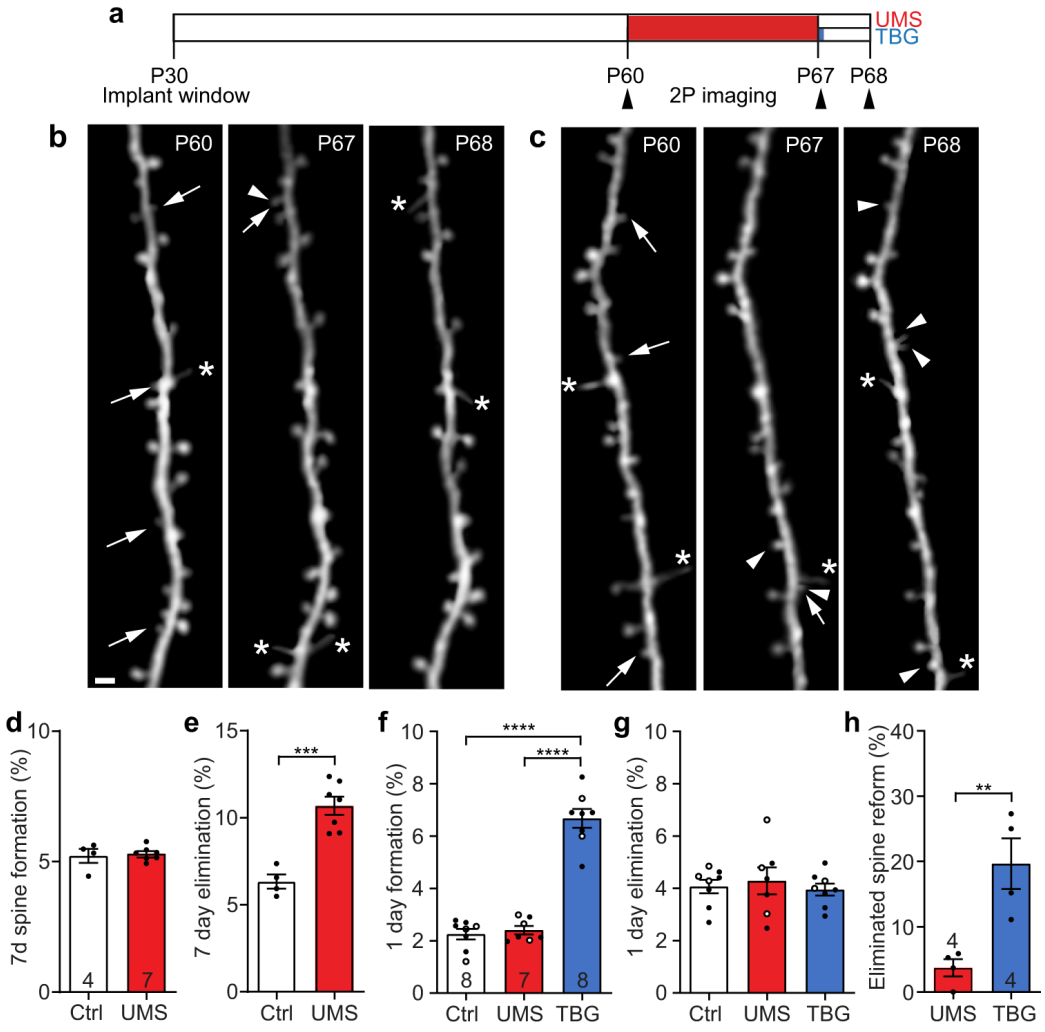

Figure 4.4.4-2. *TBG promotes spine formation that partially compensates for UMS-induced spine loss in the mouse cortex. (a) Timeline of dendritic spine imaging experiments. (b) Example of the same set of S1BF spines imaged before UMS, immediately after UMS, and after 1-day recovery. (c) Example spine imaging over the same time course but with post-stress TBG treatment. Arrow: eliminated spine; arrowhead: new spine; asterisk: filopodium. Scale bar: 2 μM. (d, e) Spine formation and elimination over 7 days. Herein after filled circles represent data from S1BF and empty circles represent data from frontal cortex. (f, g) Spine formation and elimination over 1 day in control and during post-UMS recovery. (h) Percentage of spines eliminated during UMS that re-emerged during recovery.*

*N = 4–5 per condition. Data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Ctrl = unstressed control group, TBG = tabernanthalog, UMS = unpredictable mild stress.*

A recent study by Peters and Olson investigated the anti-addictive properties of TBG further, with the attempt to lengthen the therapeutic properties of the compound.[54] Reports demonstrate that in humans, sometimes up to 3 doses are needed to have long lasting anti-addictive effects,[55] so it is perhaps unsurprising that TBG may also need a multiple-dosing regimen.

First, Peters and Olson used treated rats with either TBG (30 mg/kg) or VEH and exposed them to a progressive-ratio test to seek food or heroin. Typically, animals have much greater motivation for heroin-seeking, however this was diminished after TBG treatment and was comparable to food-seeking (**Figure 4.4.4-3a**).

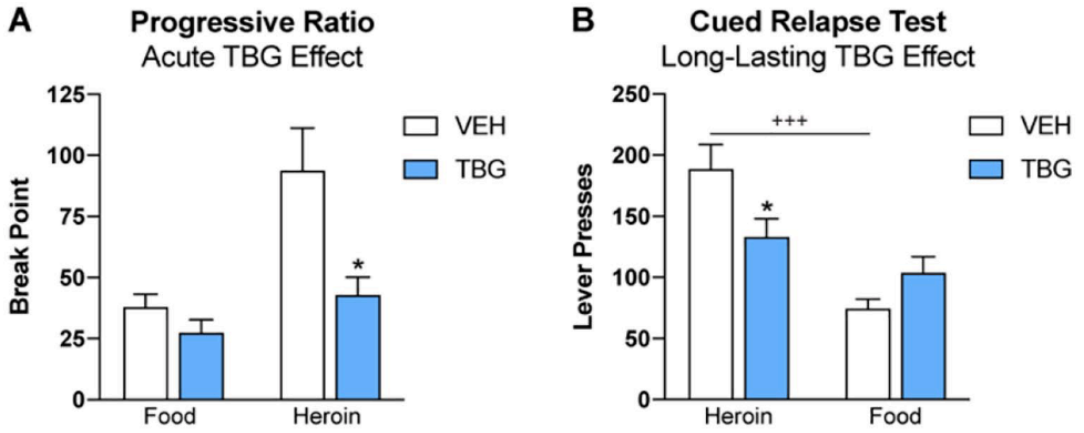

Figure 4.4.4-3. *TBG reduces motivation for heroin-seeking without impairing food seeking. (a) Two groups of rats were used to administer either TBG (30 mg/kg) or VEH prior to a progressive ratio test to seek food or heroin. (b) TBG significantly inhibits cued-relapse for heroin but not for food.*
Image adapted from Peters & Olson (2021).[54]

In the Section 4.3.9.2, I outlined how 40 mg/kg TBG had a transient effect on immediate heroin-seeking, and a mild long-lasting effect. Peters and Olson attempted a repeated dosing regimen of TBG from 2.5 mg/kg, then 10 mg/kg, then 30 mg/kg spaced a few days apart (for a cumulative dose of 42.5 mg/kg). Using this regimen, TBG provided a long-lasting effect in reducing heroin use with no effect on food seeking for up to a week after administration (the longest timepoint tested; **Figure 4.4.4-3b**).

4.4.4.2 Discovery of AAZ

Since the initial description of TBG,[26] other non-hallucinogenic psychoplastogens have been described, including AAZ-A-154 (AAZ). AAZ produces antidepressant-like effects in the FST and causes a transient increase in sucrose preference after treatment.[56] It has yet to be determined if AAZ is efficacious in reducing drug-seeking behaviour. It is currently unclear if all psychoplastogens may treat these disorders, or if there is something unique about the ibogaine/5-MeO-DMT backbone that lends TBG to treating these SUDs effectively.

In summary, TBG appears to work across affective disorders, including animal behavioural tasks for antidepressant efficacy, anxiety, working memory, as well as both alcohol- and heroin-use disorders. Future work in addiction may explore the effects of TBG for treating nicotine, cocaine and methamphetamine use disorders.

Therapeutic strategies using circuit-based approaches to treating mental illness through enhancing PFC function may be beneficial for treating more than one SUD.

4.5 Methods

4.5.1 Data Analysis and Statistics

Treatments were randomized, and data were analyzed by experimenters blinded to treatment conditions. Statistical analyses were performed using GraphPad Prism (version 8.1.2) unless noted otherwise. All comparisons were planned prior to performing each experiment. Data are represented as mean ± SEM, unless otherwise noted, with asterisks indicating *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. Boxplots depict the three quartile values of the distribution with whiskers extending to points that lie within 1.5 IQRs (interquartile range) of the lower and upper quartile. Observations falling outside this range are displayed independently.

4.5.2 Drugs

The NIDA Drug Supply Program provided ibogaine hydrochloride (IBO), noribogaine (NOR), heroin (diamorphine hydrochloride), and cocaine hydrochloride. Other chemicals were purchased from commercial sources such as ketamine hydrochloride (KET, Fagron), ketanserin (KETSN, ApexBio), eugenol (Tokyo Chemical Industries), and 5-hydroxytryptamine (Sigma-Aldrich). The fumarate salt of 5-methoxy-*N*,*N*-dimethyltryptamine (2:1, 5-MeO-DMT:fumaric acid) was synthesized in house as described previously[2] and judged to be analytically pure based on NMR and LC-MS data. For cell culture experiments, VEH = 0.1% (agonist studies) or 0.2% (antagonist studies) molecular biology grade dimethyl sulfoxide (Sigma-Aldrich). For in vivo experiments, VEH = USP grade saline (0.9%). Free bases were used for all cellular experiments while the fumarate salts of ibogainalog and tabernanthalog were used for the in vivo studies.

4.5.3 Animals

All experimental procedures involving animals were approved by either the UCD, UCSF, UCSC or CU Anschutz Institutional Animal Care and Use Committee (IACUC) and adhered to principles described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The University of California, Davis (UCD), the University of California, San Francisco (UCSF), the University of California, Santa Cruz (UCSC), and the University of Colorado Denver, Anschutz Medical Campus (CU Anschutz) are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

4.5.4 Calculation of CNS MPO Score

CNS MPO scores were calculated using a previously published method.[57] Predicted pK_a values were determined using Marvin Sketch (19.25.0). LogP and total polar surface area were predicted using Molinspiration (https://www.molinspiration.com/). LogD was calculated using the following equation $LogD = LogP - LOG_{10}(1+10^{(pka-7.4)})$.

4.5.5 Dendritogenesis Experiments

For the dendritogenesis experiments conducted using cultured cortical neurons, timed pregnant Sprague Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). Full culturing, staining, and analysis details were performed as previously described.[2]

4.5.6 Head-Twitch Response (HTR)

The head-twitch response assay was performed as described previously[2] using both male and female C57BL/6J mice (2 per treatment). The mice were obtained from Jackson Laboratory

(Sacramento, C.A.) and were approximately 8 weeks old at the time of the experiments. Compounds were administered via intraperitoneal injection (5 mL/kg) using 0.9% saline as the vehicle. As a positive control, we utilized 5-MeO-DMT fumarate (2:1 amine/acid), which was synthesized as described previously.[2] Behavior was videotaped, later scored by two blinded observers, and the results were averaged (Pearson correlation coefficient = 0.93).

4.5.7 hERG Inhibition Studies

All experiments were conducted manually using an EPC-10 amplifier (HEKA, Lambrecht/Pfalz, Germany) at room temperature in the whole-cell mode of the patch-clamp technique. HEK293 cells stably expressing hKv11.1 (hERG) under G418 selection were a generous gift from Craig January (University of Wisconsin, Madison). Cells were cultured in DMEM containing 10% fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin, 100 µg/mL streptomycin, and 500 mg/ml G418. Before experiments, cells were cultured to 60–80% confluency and lifted using TrypLE and plated onto poly-L-lysine-coated coverslips. Patch pipettes were pulled from soda lime glass (micro-hematocrit tubes) and had resistances of 2–4 MΩ. For the external solution, normal sodium Ringer was used (160 mM NaCl, 4.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.4 and 290–310 mOsm). The internal solution used was potassium fluoride with ATP (160 mM KF, 2 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES, 4 mM NaATP, pH = 7.2 and 300–320 mOsm). A 2-step pulse (applied every 10 sec) from -80 mV first to 40 mV for 2 sec and then to -60 mV for 4 sec, was used to elicit hERG currents. The percent reduction of tail current amplitude by the drugs was determined and data are shown as mean +/- SD. For all experiments, solutions of the drugs were prepared fresh from 10 mM stock solutions in DMSO. The final DMSO concentration never exceeded 1%.

4.5.8 Larval Zebrafish Heart Rate Experiments

Zebrafish express Zerg, an orthologue of hERG, and many hERG inhibitors induce bradycardia an arrhythmia in zebrafish.[58] Heart rate was recorded and calculated as reported previously[59] with slight modifications (n = 3–9). Briefly, 7 dpf zebrafish larvae were anesthetized with tricaine (Acros Organics) and immobilized in a lateral orientation using 1% low melt agarose (LMA, Gene Mate) dissolved in egg water.[60] Tricaine was washed out and drug was added to 4 mL embryo media in a 6-well plate (final concentration = 50 μM). Videos were collected at 30 frames per second (fps) using a Leica M80 scope with an ACHRO 1x nosepiece attachment and a Leica IC80 HD camera. Regions of interest (ROIs) were drawn around the atrium and ventricle of individual zebrafish and average pixel dynamics were calculated using the ImageJ plugin Time Series Analyzer V3. This pixel change oscillation was graphically smoothed using the Savgol filter in SciPy. Peaks were detected using the SciPy package "find_peaks". Peak time interval and BPM were calculated using custom code. The arrythmia score was calculated as the ratio of atrium BPM to ventricle BPM (n = 6–18).

4.5.9 Larval Zebrafish Seizure Experiments

At 6 dpf, transgenic zebrafish larvae (*Tg(elavl3:GCaMP5G)a4598*)[61] were anesthetized with tricaine and immobilized in a dorsal orientation using 1% LMA dissolved in egg water. Tricaine was washed out and zebrafish were treated for 1 h with compounds (50 μM for IBO and TBG; 15 mM for PTZ). Videos were acquired using a Zeiss Axiozoom.V16, and GCaMP5G fluorescence was induced using a Lumencor sola light engine. Zen software V2 blue edition controlled an Axiocam 506 mono camera set to 33 fps. Short videos (1–3 min) were acquired per condition.

Change in fluorescence intensity was calculated using ImageJ from an ROI drawn in the cerebellar region, and ΔF/F was calculated and visualized using custom functions.

4.5.10 Larval Zebrafish Toxicity

Tropical 5D wild-type larval zebrafish were obtained from the Sinnhuber Aquatic Research Laboratory (SARL) at Oregon State University (Corvallis, Oregon), and subsequent generations were raised at UC Davis. Zebrafish husbandry, spawning, dechorionation of embryos, and exposures were performed as described previously.[62] Chemical stocks were prepared at 100 mM in DMSO and diluted to 200 μM with embryo media. This solution was diluted 2-fold into individual wells of 96-well plates housing larval zebrafish. The final compound and DMSO concentrations were 100 μM and 0.1% (v/v), respectively. Wells were covered with Parafilm M (Bemis, North America, Neenah, Wisconsin) then covered with the plate lid. Plates were maintained in an incubator at 28.5 °C with a 14 h light (~300 lux)/10 h dark cycle. Fish were statically exposed to compounds 6 h post-fertilization (hpf) through 5 dpf. All compounds were tested for mortality/teratology in triplicate experiments (three experiments conducted on independent days using fish from independent spawns). For each experiment, 16 fish were tested per concentration per compound (n = 48 fish/condition). At 1, 2, 3, 4, and 5 dpf, fish were examined for mortality and developmental malformations using a Leica Stereo Microscope Model S6D (Leica, Germany) up to 4.5x magnification.

4.5.11 Serotonin and Opioid Receptor Functional Assays

Functional assay screens at 5-HT and opioid receptors were performed in parallel using the same compound dilutions and 384-well format high-throughput assay platforms. Assays assessed

activity at all human isoforms of the receptors, except where noted for the mouse 5-HT2A receptor. Receptor constructs in pcDNA vectors were generated from the Presto-Tango GPCR library[63] with minor modifications. All compounds were serially diluted in drug buffer (HBSS, 20 mM HEPES, pH 7.4 supplemented with 0.1% bovine serum albumin and 0.01% ascorbic acid) and dispensed into 384-well assay plates using a FLIPR^{TETRA} (Molecular Devices). Every plate included a positive control such as 5-HT (for all 5-HT receptors), DADLE (DOR), salvinorin A (KOR), and DAMGO (MOR). For measurements of 5-HT2A, 5-HT2B, and 5-HT2C Gq-mediated calcium flux function, HEK Flp-In 293 T-Rex stable cell lines (Invitrogen) were loaded with Fluo-4 dye for one hour, stimulated with compounds and read for baseline (0–10 seconds) and peak fold-over-basal fluorescence (5 min) at 25°C on the FLIPR^{TETRA}. For measurement of 5-HT6 and 5-HT7a functional assays, Gs-mediated cAMP accumulation was detected using the split-luciferase GloSensor assay in HEKT cells measuring luminescence on a Microbeta Trilux (Perkin Elmer) with a 15 min drug incubation at 25°C. For 5-HT1A, 5-HT1B, 5-HT1F, MOR, KOR, and DOR functional assays, Gi/o-mediated cAMP inhibition was measured using the split-luciferase GloSensor assay in HEKT cells, conducted similarly as above, but in combination with either 0.3 μM isoproterenol (5-HT1A, 5-HT1B, 5-HT1F) or 1 μM forskolin (MOR, KOR, and DOR) to stimulate endogenous cAMP accumulation. For measurement of 5-HT1D, 5-HT1E, 5-HT4, and 5-HT5A functional assays, β-arrestin2 recruitment was measured by the Tango assay utilizing HTLA cells expressing TEV fused-β-arrestin2, as described previously[63] with minor modifications. Data for all assays were plotted and non-linear regression was performed using "log(agonist) vs. response" in Graphpad Prism to yield Emax and EC_{50} parameter estimates.

4.5.12 Safety Pharmacology Profiling Panel

Eurofins Discovery (Taipei, Taiwan) screened TBG (10 μM) against their SafetyScreen87™ Panel and in their VMAT (Non-Selective) Human Vesicular Monoamine Transporter Binding Assay.

4.5.13 Conditioned Place Preference (CPP)

The CPP apparatus consisted of two chambers (18 cm L x 20 cm W x 35 cm H) connected by a corridor (10 cm L x 20 cm W x 35 cm H). One chamber had a smooth floor and black walls while the second chamber had a mesh floor and patterned walls. A block was placed in the corridor to restrict mice to a particular chamber. On Day 1 (pre-conditioning), male C57/BL6J mice (9-10 weeks old) were allowed to explore the entire apparatus for 30 min. Mice were randomly sorted into treatment groups (TBG at 50 mg/kg, 10 mg/kg and 1 mg/kg), ensuring that their initial preferences for what would become the TBG-paired side were approximately equal. Next, the mice were administered an intraperitoneal injection of either VEH (saline) or TBG (counterbalanced) immediately before being confined to one of the two chambers for 30 min. The following day, the other treatment was administered, and the mice were confined to the opposite chamber for 30 min. This sequence was repeated twice, such that all mice received 3 VEH-side pairings and 3 TBG-side pairings. The mice were returned to their home cages in between treatment-side pairings. On Day 8 (post conditioning), the mice were allowed to explore the entire apparatus for 30 min, and the time spent on the VEH- and TBG-paired sides was quantified using ANYmaze software (version 6.2). The apparatus was cleaned with 70% ethanol between trials. Drug solutions were prepared fresh daily.

4.5.14 Pharmacokinetic Studies

Male and female C57/BL6J mice (12 weeks old) were administered TBG via intraperitoneal injection at doses of either 50 mg/kg, 10 mg/kg or 1 mg/kg. Mice were sacrificed 15 min or 3 h post-injection via cervical dislocation. Two males and two females were used per dose/timepoint. Brain and liver were harvested, flash frozen in liquid nitrogen, and stored at -80°C until metabolomic processing. Metabolites were extracted from tissue as described previously.[64] Briefly, whole brain and liver sections were lyophilized overnight to complete dryness, then homogenized with 3.2 mm diameter stainless steel beads using a GenoGrinder for 50 seconds at 1500 rpm. Ground tissue was then extracted using 225 µL cold methanol, 190 µL water, 750 µL methyl tert-butyl ether (MTBE). Seven method blanks and seven quality control (QC) samples (pooled human serum, BioIVT) were extracted at the same time as the samples. The nonpolar fraction of MTBE was dried under vacuum and reconstituted in 60 µL of 90:10 (v/v) methanol: toluene containing 1-cyclohexyl-dodecanoic acid urea (CUDA) as an internal standard. Samples were then vortexed, sonicated and centrifuged prior to analysis. For analysis of TBG in liver and brain, samples were randomized prior to injection with method blanks and QC samples analyzed between every ten study samples. A six-point calibration curve was analyzed after column equilibration using blank injections, and then after all study samples. Blanks were injected following the calibration curve to ensure no tabernanthalog was retained on the column and carried over to samples. Reconstituted sample (5 µL) was injected onto a Waters Acquity UPLC CSH C18 column (100 mm x 2.1 mm, 1.7 µm particle size) with an Acquity UPLC CSH C18 VanGuard precolumn (Waters, Milford, MA) using a Vanquish UHPLC coupled to a TSQ Altis triple quadrupole mass spectrometer (ThermoFisher Scientific, San Jose, CA). Mobile phase A consisted of 60:40 v/v acetonitrile/water with 10 mM ammonium formate and 0.1% formic acid.

Mobile phase B was 90:10 v/v isopropanol/acetonitrile with 10 mM ammonium formate and 0.1% formic acid. Gradients were run from 0–2 minutes at 15% B; 2–2.5 minutes 30% B; 2.5–4.5 minutes 48% B; 4.5–7.3 minutes 99% B; 7.3–10 minutes 15% B. The flow rate was 0.600 mL/min and the column was heated to 65°C. Mass spectrometer conditions were optimized for TBG by direct infusion. Selected reaction monitoring for the top five ions, with collision energy, source fragmentation, and radio frequency optimized for TBG. Data were processed with TraceFinder 4.1 (ThermoFisher Scientific, San Jose, CA). Organ weights were recorded. The concentration in the brain was calculated using the experimentally determined number of mols of TBG in the whole organ divided by the weight of the organ.

4.5.15 Spinogenesis Experiments

Spinogenesis experiments were performed as previously described[30] with the exception that cells were treated on DIV19 and fixed 24 h after treatment on DIV20. The images were taken on a Nikon HCA Confocal microscope a with a 100x/NA 1.45 oil objective. DMSO and ketamine (10 μM) were used as vehicle and positive controls, respectively.

4.5.16 In Vivo Spine Dynamics

Male and female *Thy1*-GFP-M line mice[65] (n = 5 per condition) were purchased from The Jackson Laboratory (JAX #007788) and maintained in UCSC animal facilities according to an IACUC approved protocol. In vivo transcranial two-photon imaging and data analysis were performed as previously described.[66] Briefly, mice were anesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine (87 mg/kg) and xylazine (8.7 mg/kg). A small region of the exposed skull was manually thinned down to 20–30 μm for optical access. Spines on apical dendrites in mouse

primary sensory cortices were imaged using a Bruker Ultima IV two-photon microscope equipped with an Olympus water-immersion objective (40x, NA = 0.8) and a Ti:Sapphire laser (Spectra-Physics Mai Tai, excitation wavelength 920 nm). Images were taken at a zoom of 4.0 (pixel size 0.143 × 0.143 μm) and Z-step size of 0.7 μm. The mice received an i.p. injection of DOI (10 mg/kg) or TBG (50 mg/kg) immediately after they recovered from the anesthesia of the first imaging session. The mice were re-imaged 24 h after drug administration. Dendritic spine dynamics were analyzed using ImageJ. Spine formation and elimination were quantified as percentages of spine numbers on day 0.

4.5.17 Antidepressant-Like Response Following Unpredictable Mild Stress (UMS)

Male and female mice (8 weeks old) were subjected to 7 d of UMS, as described previously.[67] Briefly, the following stressors were utilized: Day 1: Light Phase = 30 min of restraint stress x 2; Dark Phase = home cage space reduction. Day 2: Light Phase = exposure to a new room + 30 min on the orbital shaker, sudden loud noise x 5, tail suspension for 6 min; Dark Phase = wet bedding. Day 3: Light Phase = exposure to new mice; Dark Phase = exposure to light. Day 4: Light Phase = social isolation; Dark Phase = tilted cage. Day 5: Light Phase = tilted cage, island isolation; Dark Phase = no bedding. Day 6: Light Phase = no bedding, random puff of air x 5–10; Dark Phase = foreign objects. Day 7: Light Phase = foreign objects, food deprivation; Dark Phase = food deprivation, continual exposure to loud music. Immediately following UMS, TBG or VEH were administered via intraperitoneal injection, and 24 h later the mice were subjected to a FST using the same procedure as described below.

4.5.18 Forced Swim Test (FST) in the Absence of UMS

Male C57/BL6J mice (9-10 weeks old at time of experiment) were obtained from the Jackson Lab and housed 4–5 mice/cage in a UCD vivarium following an IACUC approved protocol. After 1 week in the vivarium each mouse was handled for approximately 1 minute by a male experimenter for 3 consecutive days leading up to the first FST. All experiments were carried out by the same male experimenter who performed handling. During the FST, mice underwent a 6 min swim session in a clear Plexiglas cylinder 40 cm tall, 20 cm in diameter, and filled with 30 cm of 24 ± 1ºC water. Fresh water was used for every mouse. After handling and habituation to the experimenter, drug-naïve mice first underwent a pretest swim to more reliably induce a depressive phenotype in the subsequent FST sessions. Immobility scores for all mice were determined after the pre-test and mice were randomly assigned to treatment groups to generate groups with similar average immobility scores to be used for the following two FST sessions. The next day, the mice received intraperitoneal injections of TBG (50 mg/kg), a positive control (ketamine, 3 mg/kg), or vehicle (saline). One additional group received ketanserin (4 mg/kg IP) 10 min prior to intraperitoneal administration of TBG (50 mg/kg). The following day, the mice were subjected to the FST and then returned to their home cages. One week later, the FST was performed again to assess the sustained effects of the drugs. All FSTs were performed between the hours of 8 am and 1 pm. Experiments were video-recorded and manually scored offline. Immobility time—defined as passive floating or remaining motionless with no activity other than that needed to keep the mouse's head above water—was scored for the last 4 min of the 6 min trial.

4.5.19 Alcohol Consumption

Male C57/BL6J mice (6-8 weeks old) were obtained from The Jackson Laboratory (Bar Harbor, ME) and were individually housed in a reverse light/dark cycle room (lights on 10:00pm–10:00am). Temperature was kept constant at 22 ± 2°C, and relative humidity was maintained at 50 ± 5%. Mice were given access to food and tap water *ad libitum*. After one week of habituation to the vivarium, the two-bottle choice alcohol-drinking experiment was conducted as described previously.[68] For 7 weeks, mice were given intermittent access in their home cage to alcohol. On Mondays, Wednesdays, and Fridays, two bottles were made available for 24 h—one containing 20% ethanol and another containing only water. On Tuesdays, Thursdays, Saturdays, and Sundays, the mice were only given access to water. After 7 weeks, mice were administered TBG (50 mg/kg) or vehicle (saline) via intraperitoneal injection 3 h before the beginning of a drinking session. Ethanol (g/kg) and water (ml/kg) intake were monitored during the first 4 h (initial binge), the first 24 h, and the second 24 h. Next, the mice were only given water for 48 h before the start of another drinking session when ethanol and water consumption was monitored. The placement (right or left) of the bottles was altered in each session to control for side preference. Spillage was monitored using an additional bottle in a nearby unused cage. Alcohol preference was calculated as the ratio between alcohol/(water + alcohol). Mice were tested using a counterbalanced, within subject design with one week of drug-free alcohol drinking regimen between treatments. One mouse was excluded because the bottle was leaking.

4.5.20 Sucrose Preference

Male C57/BL6J mice were individually housed and subjected to a two-bottle choice experiment. First, mice were administered TBG (50 mg/kg) or vehicle (saline) via intraperitoneal injection 3 h

before the beginning of a two-bottle choice session. During this 3 h period, mice were not given access to water in an attempt to increase their thirst. At the start of the experiment, mice were given one bottle of water and one bottle of water containing 5% sucrose. Sucrose solution and water intake were monitored during the first 4 h and the first 24 h. Sucrose preference was calculated was calculated as the amount of sucrose solution consumed minus the amount of water consumed, divided by the total amount of liquid consumed.

4.5.21 Heroin Self-Administration Behavior

Subjects were age-matched male (n = 16) and female (n = 16) Wistar rats (Charles River). Rats were single housed in a temperature and humidity-controlled room with a 12 h light/dark cycle (7:00 A.M. lights on) with free access to standard laboratory chow and water. Two rats (one male and one female) were excluded from the final dataset due to defective catheters for a final n = 30 rats. Rats were surgically implanted with an intravenous catheter as previously described.[69] Heroin self-administration training began at least one week after surgery on a fixed ratio 1 (FR1) schedule of reinforcement. Operant chambers were equipped with both an active (heroin-delivering) and inactive lever, and each heroin infusion (0.04 mg, 50 µl, 2.85 s) was coupled with delivery of a light cue located above the active lever and a 3.5 kHz tone (5 s). Both levers retracted upon initiation of a heroin infusion and remained retracted during the tone + light heroin cue presentation. After six self-administration sessions (2.5 h) on FR1, rats progressed to a variable ratio 5 (VR5) for three sessions and continued to the final variable ratio 15 (VR15) for five sessions. Rats then began extinction training. Extinction training sessions (1 h) were conducted in the same operant chambers (context) where rats previously self-administered heroin, but in the absence of heroin and its tone + light cue. Both levers remained extended throughout the session,

and responding was recorded, but produced no consequence. After completing a total of 7 extinction sessions, rats underwent a cued reinstatement test (1 h, withdrawal day 10–12). During the cue test, the heroin tone + light cues were available, but heroin was not. The first active lever press resulted in presentation of the heroin cues, and then cues were available on a VR5 schedule (active lever only) for the remainder of the test. Lever retraction occurred during cue presentation (as during self-administration). Injections of TBG (40 mg/kg i.p.) or vehicle (VEH) were administered on the third VR15 session, the first extinction session, and the cued reinstatement test. For each of these timepoints, TBG or VEH was injected 30 min prior to placement in the chamber. Treatment groups were balanced based on response rates, heroin intake, and sex. Behavioral sessions were conducted daily (weekdays only). Catheters were flushed after each self-administration session with cefazolin and taurolidine citrate solution to prevent infection and/or catheter occlusion. Statistical tests were performed in Prism (GraphPad Prism, RRID:SCR_002798; V8.0) software.

4.5.22 Sucrose Self-Administration Behavior

Sucrose self-administration procedures were designed to mimic heroin self-administration conditions. Subjects were age-matched male (n = 24) and female (n = 24) Wistar rats (Charles River). Rats were single housed and had free access to standard laboratory chow and water throughout the experiment. Eight rats (seven males and one female) were excluded from the final dataset due to failure to acquire sucrose self-administration for a final n = 40 rats. The final groups consisted of VEH, SA (2.5), SA (10), SA (40), Ext (40), and CUE (40). The number of animals in each group was 7, 6, 7, 7, 7, 6, respectively (40 animals total). At least one week after arrival and acclimation to the animal facility, sucrose self-administration training began on a fixed ratio

1 (FR1) schedule of reinforcement. Operant chambers were equipped with an active (sucrose-delivering) and inactive lever, and each sucrose reward (45 mg pellet; Bio-Serv F0023) was coupled with the same tone + light cues used for the heroin study. Levers retracted upon pellet delivery and remained retracted during cue presentation (5 s). After six self-administration sessions (2 h) on FR1, rats progressed to a variable ratio 5 (VR5) for three sessions and continued to the final variable ratio 15 (VR15) for five sessions. Rats then began extinction training. Extinction training sessions (1 h) were conducted in the same operant chambers (context) where animals previously self-administered sucrose, but neither sucrose nor the sucrose cues were available. Responding on both levers was recorded during each session, but produced no consequence. After completing 7 extinction sessions, rats underwent a cued reinstatement test (1h). During the cue test, the sucrose tone + light cues were available, but sucrose was not. The first active lever press resulted in presentation of the sucrose cues, and then cues were available on a VR5 schedule (active lever only) for the remainder of the test. Lever retraction occurred during cue presentation (as during self-administration). Injections were administered on the third VR15 session, the first extinction session, and the cued reinstatement test. For each of these tests, TBG (2.5 mg/kg, 10 mg/kg, or 40 mg/kg IP) or vehicle (VEH) was injected 30 min prior to placement in the chamber. The low (2.5 mg/kg) and intermediate (10 mg/kg) doses of TBG were tested only on the third VR15 sucrose self-administration session. The high dose (40 mg/kg) was tested at all three test time points. Statistical tests were performed in Prism (GraphPad Prism, RRID:SCR_002798; V8.0) software.

4.5.23 Open Field Test

Naïve male (n = 7) and female (n = 6) Wistar rats (Charles River) were allowed to acclimate to the animal facility for at least one week after arrival. Spontaneous locomotion in response to a novel open field (44 cm long x 36 cm wide x 43 cm tall) was assessed 30 min after injection of vehicle or TBG (40 mg/kg, IP). Videos were recorded with an overhead camera connected to the tracking software EthoVision XT (Noldus, The Netherlands) for subsequent offline analysis. Rats were allowed to move freely in the open field for 30 min, then they were briefly removed from the apparatus to receive an injection of cocaine (15 mg/kg, IP). The rats were immediately returned to the open field for an additional hour to assess cocaine-induced locomotion. Each open field chamber was cleaned with Clidox-S in between sessions. Locomotion was tracked using EthoVision XT to assess the velocity (cm/s) and total distance traveled (m) during the baseline (first 30 min) and cocaine (last 60 min) phases separately. Thigmotaxis was assessed as the percentage of time spent in the center of the apparatus (26 cm long x 18 cm wide; i.e., 9 cm perimeters) was also analyzed during the baseline period to determine if TBG alters anxiety in the open field.

Data is available at the following DOI: 10.6084/m9.figshare.11634795.

4.6 Contributions & Collaborations

I couldn't have done this alone. Thank you to all my collaborators who helped me get this far. Contributions are outlined below.

Alexander Pell and Zefan Q. Hurley synthesized all the ibogalog compounds. Maxemiliano V. Vargas ran the simplified FST protocol to assess 5HT2A involvement and the length of TBG's effects. LPC and DEO helped design all experiments.

Olson Lab

University of California, Davis

Michelle Tija, Ju Lu and Yi Zuo performed the UMS and FST studies. LPC, Ju Lu and Yi Zuo also completed the in vivo spine imaging. All experiments were designed with LPC and DEO.

Zuo Lab

University of California, Santa Cruz

LPC, Brandon M. Brown and Heike Wulff performed hERG channel patch clamp studies with guidance from LPC and DEO.

Wulff Lab

University of California, Davis

LPC sacrificed animals; Zachary Rabow and Oliver Fiehn ran tissue samples for the metabolomics data with guidance from LPC and DEO.

Fiehn Lab

University of California, Davis

Robert J. (RJ) Tombari, Matthew N. McCarroll, and David Kokel designed and ran the zebrafish heart rate and seizure activity experiments with input from LPC and DEO.

Kokel Lab

University of California, San Francisco

Robert J. (RJ) Tombari, Bianca Yaghoobi, Pamela Lein designed and ran the zebrafish toxicity assays with input from LPC and DEO.

Lein Lab

University of California, Davis

Lauren J. Laskowski, Emilie I. Anderson, and John D. McCorvy helped with the receptor screening of TBG and related analogs with input from LPC and DEO.

McCorvy Lab

Medical College of Wisconsin

Yann Ehinger and Dorit Ron ran the intermittent two-bottle choice alcohol assay within input from LPC and DEO.

Ron Lab

University of California, San Francisco

Jamie Peters designed and executed the heroin self-administration assays with input from LPC and DEO.

Peters Lab

University of Colorado, Boulder

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Chapter 5

MECHANISM OF ACTION OF PSYCHOPLASTOGENS

Psychoplastogens such as psychedelics and non-hallucinogenic analogs are capable of increasing cortical neuron growth, strengthening synapses, and rescuing behavioural deficits. But how?

5.1 The Serotonin 2A Receptor

The 5HT2A receptor is densely expressed on apical dendrites of layer 5 pyramidal cells in the mPFC. Psychedelics and their non-hallucinogenic analogs require engagement with the 5HT2A receptor to increase the growth cortical neurons (**Figure 2.3.1-2**, **Figure 4.3.3-2**).[1, 2]

5.1.1 Necessity of 5HT2A for psychoplastogen-mediated cortical neuron growth

I chose to screen a set of hallucinogenic and non-hallucinogenic pairs of compounds that have significant structural similarities (**Figure 5.1.1-1a**): namely 5-MeO-DMT vs TBG, LSD vs lisuride (LIS), and DMT vs 6-fluoro-diethyltryptamine (6-F-DET). 5-MeO-DMT, LSD and DMT are all classic psychedelic compounds. I demonstrated the non-hallucinogenic potential of TBG in

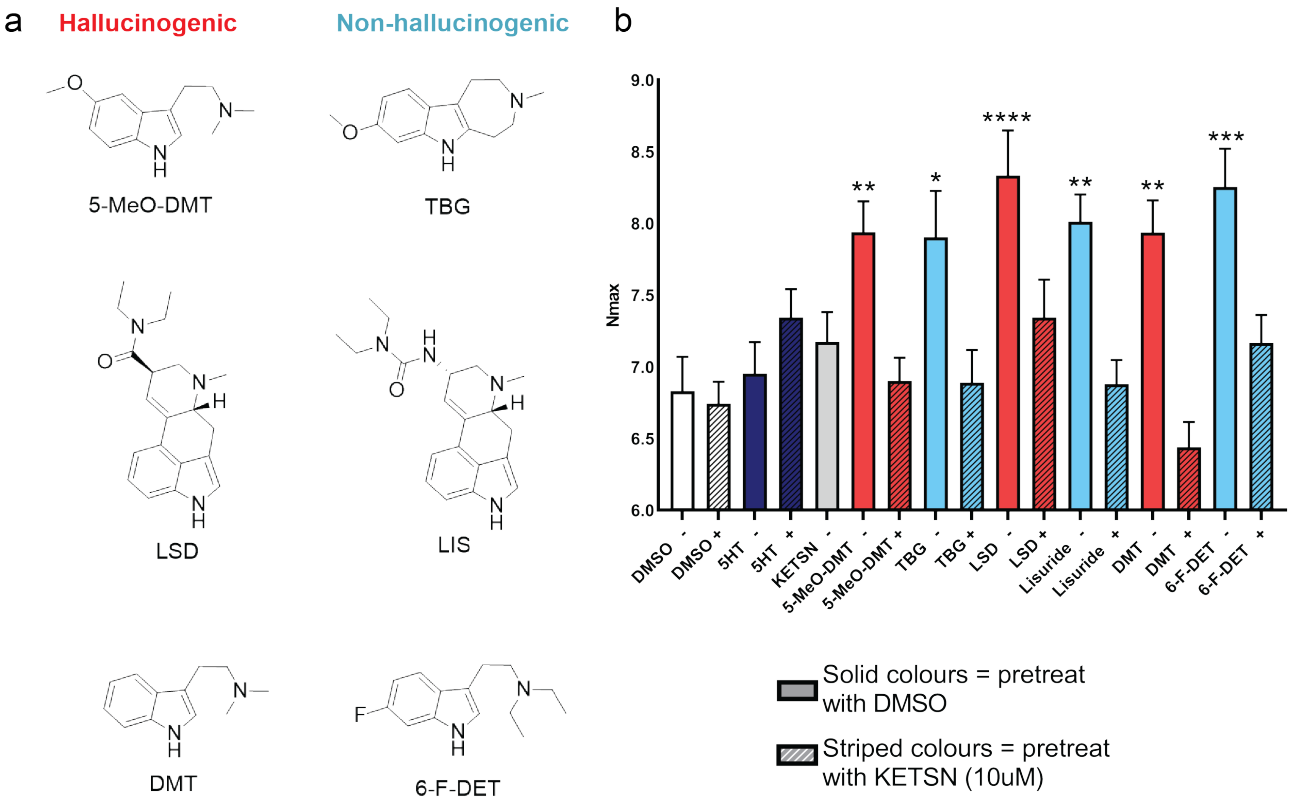

Figure 5.1.1-1. *Both hallucinogenic and non-hallucinogenic compounds can increase growth of cortical neurons. (a) Panel of hallucinogenic and non-hallucinogenic pairs that were screened in this assay. (b) Pretreatment of cortical cultures with KETSN blocks psychoplastogen-induced growth. Red represents hallucinogenic compounds, light blue represents non-hallucinogenic compounds. White, gray and navy represent controls.*

Chapter 4. Lisuride is non-hallucinogenic,[3] and is approved to treat Parkinson's disease in Europe and several other countries.[4] Finally, 6-F-DET is non-hallucinogenic, as validated by a lack of hallucinogenic response in humans following an intramuscular injection.[5] Importantly, 6-F-DET elicits positive changes in mood without hallucinations.

Using Sholl analysis in rat cortical cultures, I screened all these compounds and found that psychoplastogens (1 uM) from both classes increased cortical growth, and this growth was blocked by a 15 min pretreatment with the 5HT2A antagonist, ketanserin at 10 uM (KETSN; **Figure 5.1.1-1b**).

KETSN is an antagonist and can hit other receptors when used in high concentrations,[6] particularly 5HT2C. To test the necessity of 5HT2A specifically, we used cortical cultures

generated from 5HT2A WT and KO lines. Cultures generated from WT mouse embryos displayed similar growth patterns to those generated using our normal rat cortical cultures (**Figure 5.1.1-2a**). However, when mouse cultures were generated using 5HT2A KO mouse embryos, no psychoplastogens were able to promote growth (**Figure 5.1.1-2b**). Administration of psychoplastogens to hippocampal cultures—which contain low expression levels of 5HT2A[7,8]—demonstrate no changes to neuronal growth (**Figure 5.1.1-2c**).

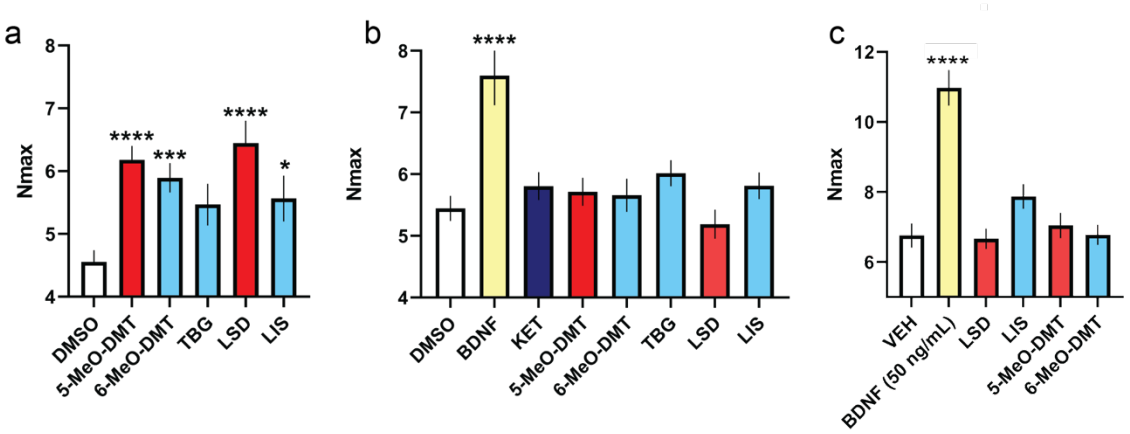

Figure 5.1.1-2*. Sholls with both hallucinogenic and non-hallucinogenic compounds in (a) WT mouse cortical cultures, (b) 5HT2A KO mouse cortical cultures and (c) rat hippocampal cultures.*

Importantly, serotonin itself (5HT) does not promote plasticity (**Figure 5.1.1-1**), and KETSN does not decrease it. Together, this suggests that 5HT2A is necessary for psychoplastogen-induced growth.

5.1.2 Necessity of 5HT2A receptor for behavioural effects of psychoplastogens

We next sought to determine if the 5HT2A receptor was necessary for the therapeutic effects of psychoplastogens in behavioural paradigms. First, we ran a forced swim test with the psychedelic 5-MeO-DMT, and either pretreated them with KETSN (4 mg/kg) or VEH 10 mins before drug. We found that animals treated with VEH + 5-MeO-DMT displayed decreased immobility,

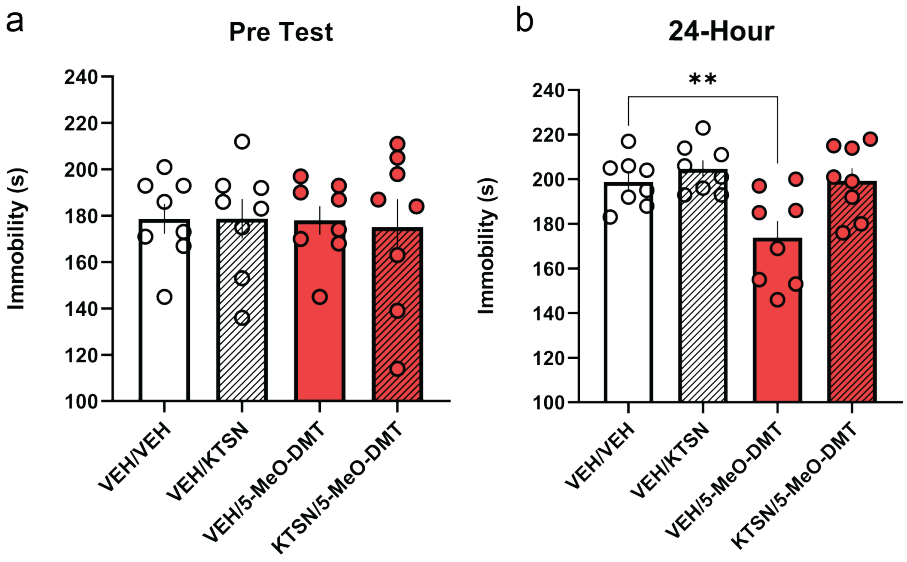

Figure 5.1.2-1*. Animals treated with 5-MeO-DMT (50 mg/kg) display reduced immobility in the FST. This effect is blocked with pretreatment of ketanserin (4 mg/kg). (a) Levels of immobility in the pretest display equal amount of immobility across treatment groups. (b) treatment with 5-MeO-DMT deceases immobility in the FST, and this effect is blocked with pretreatment of ketanserin. One way ANOVA with multiple comparisons with VEH/VEH group.*

however animals that were pretreated with KETSN before 5-MeO-DMT fialed to display the same decrease in immobility (**Figure 5.1.2-1**). This demonstrates that engagement with the 5HT2A receptor is necessary for the therapeutic behavioural effects of psychedelic compounds.

Unfortunately, the 5HT2A KO and WT animals (on an S129 background) displayed excessive immobility at baseline in the FST, and consequently effects of any treatements were unable to be detected (data not shown). It is common that with each subsequent test immobility increases (and antidepressants tend to block this increase). Typically, we see ~150-180 seconds of immobility for a 240 second period; however with this mouse line, the average time immobile was 227 seconds immobile. These mice are immobile for nearly the whole assay. Therefore, when we do subsequent tests and see increases in immobility in untreated/VEH-treated animals, we are unable to see this change since they are already immobile. In this vein, even ketamine was unable to produce a response in this task. For this reason, FST is unsuitable to use with this mouse line.

We next sought to understand the role of the 5HT2A receptor for ameliorating anhedonia-like symptoms using a chronic corticosterone (CORT) model followed by a sucrose preference test (SPT; **Figure 5.1.2-2**). For this study, we chose to evaluate the therapeutic effects of psilocybin due to its prevalence of use in clinics. We evaluated this in 5HT2A WT vs KO mice.

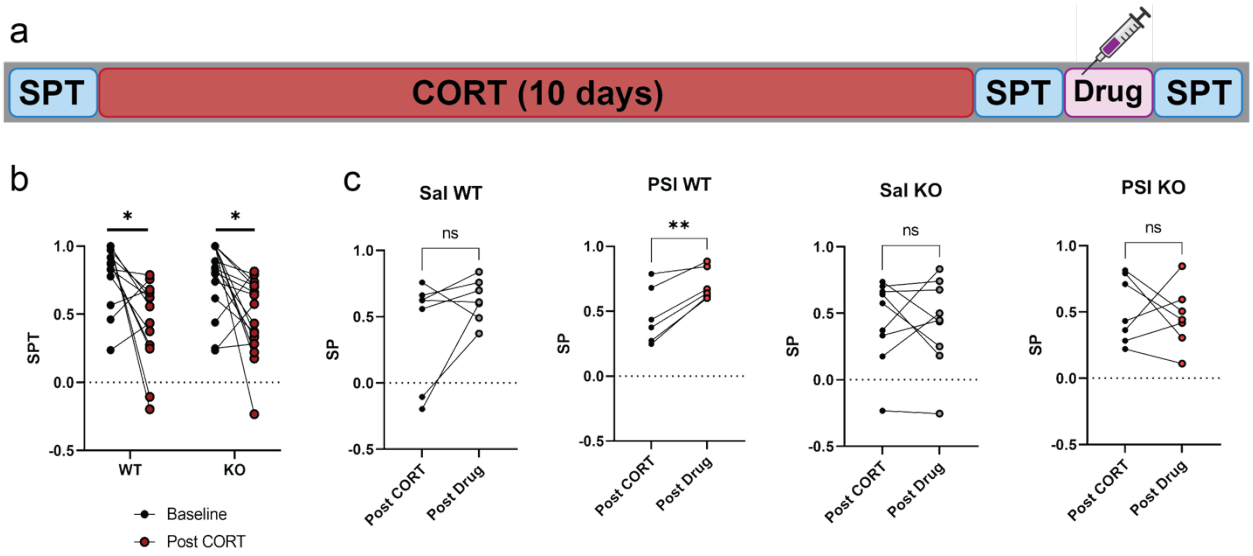

Figure 5.1.2-2*. Treatment with psilocybin increases sucrose preference in WT but not KO animals. (a) Timeline of experiment. (b) Both WT and KO animals had comparable levels of SPT at baseline, and responded similarly to CORT treatment. (c) Only WT animals that were treated with psilocybin displayed an increased preference for sucrose after drug treatment. A Students T-Test was performed between post-CORT and post-drug for each treatment group. SPT = sucrose preference test, CORT = corticosterone (20 mg/kg) treatment, PSI = psilocybin, Sal = saline.*

We found an equal amount of sucrose preference between groups at baseline, and nearly all animals demonstrated a decrease in sucrose preference after CORT treatment (**Figure 5.1.2-2b**). WT and KO animals responded similarly to CORT treatment. Next, animals were administered either psilocybin or VEH. WT animals that were administered psilocybin had a recovery of the sucrose preference, comparable to the pre-CORT baseline test. 5HT2A KO animals displayed no such recovery of sucrose preference after administration of psilocybin; an effect which was comparable to both WT and KO animals being treated with VEH (**Figure 5.1.2-2**). This demonstrates that the 5HT2A receptor is necessary for psilocybin-mediated return of sucrose preference.

Current studies in or lab are now using this exact SPT paradigm with 5HT2A WT and KO animals using TBG to assess the necessity of 5HT2A engagement in the therapeutic effects of non-hallucinogenic analogs.

5.2 5HT2A is required for changes in neuronal function

To see if TBG has similar effects on cortical neuron function as traditional psychedelics, we injected 129S6/SvEv male mice with either vehicle (VEH), 5-MeO-DMT (50 mg/kg), or TBG (50 mg/kg). Animals were sacrificed 24 h later and neurons in the PFC were patched to assess sustained effects after drug administration. Administration of both 5-MeO-DMT (50 mg/kg) and

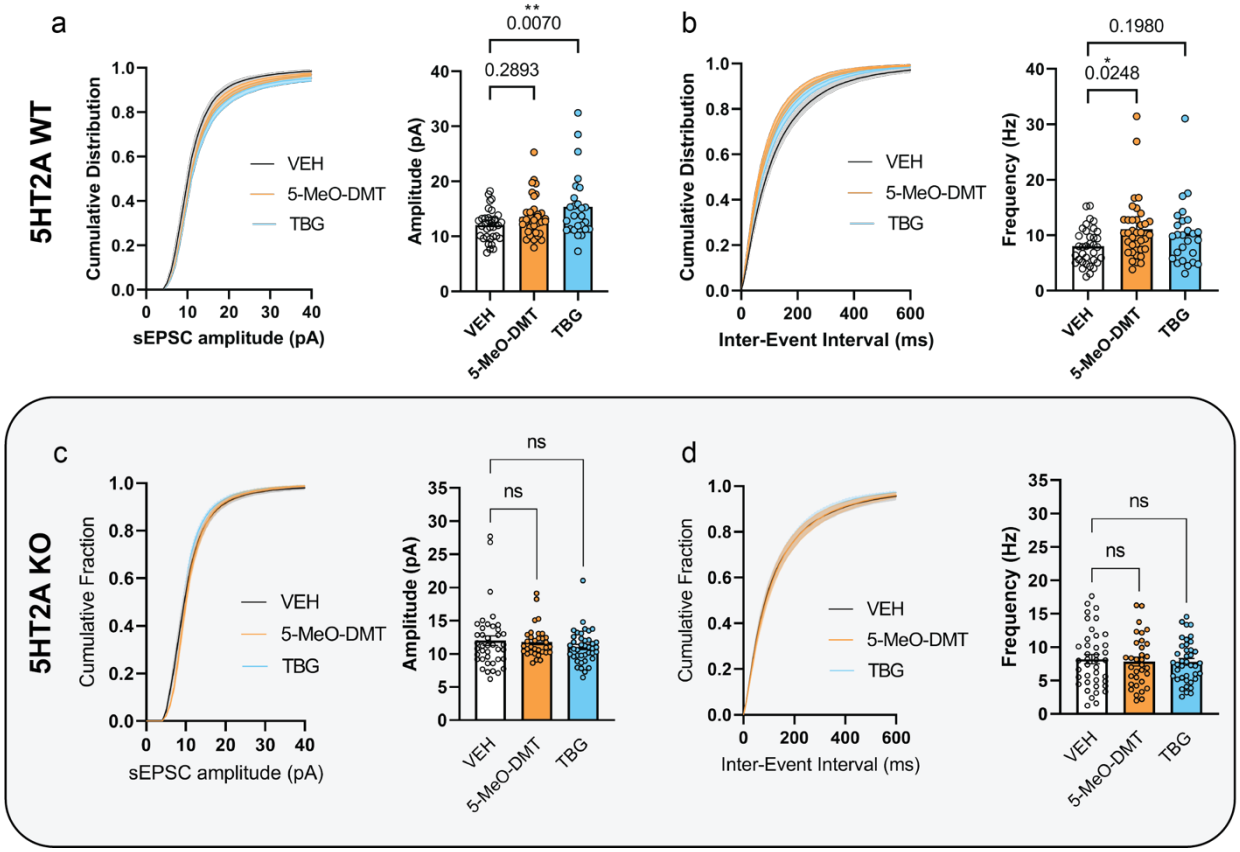

Figure 5.1.2-1. *5HT2A receptors are necessary for increases in frequency and amplitude in mPFC neurons 24 h after treatment with psychedelics (5-MeO-DMT, 50 mg/kg) or non-hallucinogenic analogs (TBG, 50 mg/kg). (a) sEPSC amplitude and (b) frequency in 5HT2A WT animals 24 h after treatment. This effect on amplitude (c) and frequency (d) is blocked in 5HT2A KO animals. Error bars represent SEM.*

TBG (50 mg/kg) caused increases in both the frequency and amplitude of sEPSCs in pyramidal cortical neurons (**Figure 5.1.2-1a**). When we repeat this experiment in 2A KO animals, this effect is abolished ((**Figure 5.1.2-1b**). This experiment highlights that both hallucinogen and non-hallucinogen psychoplastogens can cause increased activity of neurons on the prefrontal cortex and this effect is dependent on 5HT2A.

5.3 Conclusion and Discussion

In this chapter, I demonstrate using pharmacological and genetic approaches the necessity of 5HT2A receptor engagement for both growth of cortical neurons, changes in mPFC function as well as therapeutic effects. Both ketanserin and 5HT2A KO animals show fully blunted effects when treated with psychedelics compared to their VEH-treated or WT counterparts.

A recent study came out suggesting that 5HT2A is not responsible for the therapeutic effects of psilocybin using the SPT,[9] however this study had three major methodological flaws:

1. A low dose of ketanserin (1 mg/kg) was used. Ketanserin has poor brain penetrance (only about 30% receptor occupancy at this dose in mice),[10] likely due to limited transport past the blood brain barrier in rodents.[11] For this reason, a higher dose of ketanserin is advised. Furthermore, our lab has found that there is limited membrane permability, as determined by a PAMPA assay with quantification by LCMS (data not shown) and the clogP value for ketanserin is 2.81, which furthermore suggests a hindered ability to cross membranes. Since many of the 5HT2A receptors are intracellular,[12,13] a higher dose of ketanserin is needed to reach the brain and fully block 5HT2A receptors. The fact that the dose of ketanserin employed in this study does not block psychedelic-mediated HTR demonstrates this fact.
2. Ketanserin is metabolized faster than psilocybin, and is therefore not able to block its effects. Ketanserin's halflife is 2-5 h,[14,15] whereas psilocybin first must be converted to the active drug psilocin, which has effects for up to 8 h in rats and significant quantities of metabolites are found in the urine up to a week later.[16] This is why using a KO animal line or more long-lasting antagonist when testing the necessity of 2A with psilocybin. Alternatively, ketanserin is fine to use if the halflife outlasts that of another psychedelic

being tested. In addition to this, ketanserin was pretreated for 1 h prior to drug dosing, meaning that the drug would have been mostly metabolized prior to psilocybin administration.

3. Ketanserin is a non-selective 5HT2A antagonist. Ketanserin is about 10x more selective for 5HT2A over 5HT2C.[6] Although ketanserin is an important starting point for cellular and behavioural studies, it is important to follow up these studies with genetic knockout lines.

For these reason, we performed the SPT using 5HT2A KO animals. Using this genetic model, we found that 5HT2A was necessary for the therapeutic effects of psilocybin.

Other data by the Kwan group suggests that although ketanserin may block psilocybin-induced changes to spine width, protrusion length and formation rate, *ketaneserin is unable to block psilocybin-induced changes in spine density*.[17] Furthermore, though they blocked HTR with ketanersin pretreatment, they never blocked motivated behaviours in a mood-related paradigm.

Recent data by Gonzalez-Maeso's lab also demonstrates that 5HT2A receptors may be necessary for facilitated fear extinction learning,[18] which utilizes similar circuits to those involved in depression. Finally, a key study by Kometer and colleagues demonstrated that mood-enhancing effects of psilocybin are blocked by 5HT2A antagonism in human volunteers.[19] Together, these demonstrate that circuit-level approaches for understanding the link between psychedelics, the 5HT2A receptor and neuropsychiatric disorders may be fruitful.

5.3.1 Intracellular signaling with the 5HT2A receptor

How do psychedelics signal to create a therapeutic effect? As I mentioned in the introduction, simply increasing the amount of serotonin in the brain doesn't have rapid-acting antidepressant

effects, and while 5HT itself is a full agonist at 5HT2A, it is not capable of promoting the same robust increases in dendritic growth seen with psychoplastogens (**Figure 5.1.1-1**). If we look at downstream signaling events, we find that most psychedelics are actually partial agonists of the 5HT2A receptor for both G-protein engagement and β-Arrestin signaling (see **Appendix 2**). In terms of G-protein signalling, data from Bryan Roth's group suggests that psychedelics appear to selectively signal through Gq pathways, and do not engage across a spectrum of G-proteins,[20] which suggests that it is not simply a shift in G-protein activation that is responsible for the therapeutic actions of psychedelics.

The 5HT2A receptor is the most highly expressed of all serotonin receptors,[21] and it is densely expressed in layer 5 pyramidal neurons in the PFC.[12,13] Integral to the discussion of 5HT2A necessity is the receptor distribution. Experiments that classify psychedelics as partial agonists are typically performed in HEK cells, where receptors are uniformly expressed on the cell surface.[22] However, 90% of 5HT2A receptors are located *intracellularly* on neurons in the PFC.[12] This is crucial, because psychoplastogens tend to be highly membrane permeable and have high MPO scores (data not shown). Although 5HT can fully stimulate receptors, it cannot pass cell membranes and thus can only stimulate 10% of receptors. On the other hand, while psychoplastogens may be partial agonists, their membrane permeability allows them to stimulate the surface *and* intracellular receptors, thereby resulting in an overall larger response than just 5HT alone (for graphical schematic, please see **Figure 5.3.1-1**).

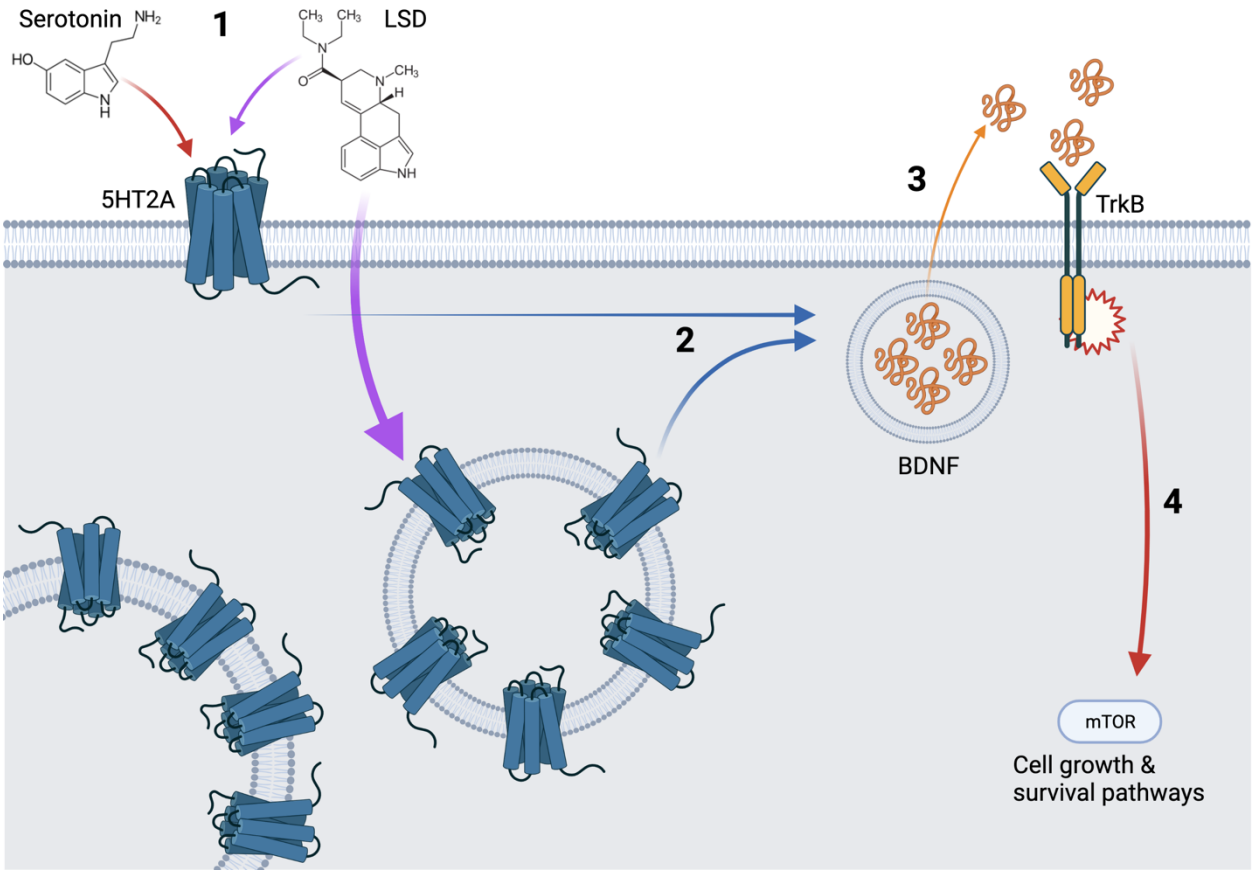

Figure 5.3.1-1. *Schematic of hypothesized mechanism for 5HT2A-mediated cell growth. (1) Compounds stimulate 5HT2A. Molecules like serotonin cannot pass cell membranes and thus can only stimulate receptors on the cell surface. On the other hand, lipophilic molecules like LSD are able to pass the membrane and stimulate many more receptors internally. Each of these in turn will stimulate BDNF release (2), which will bind to TrkB in an autocrine fashion (3) transactivate these receptors, leading to downstream signalling events including mTOR activation (4), resulting inactivation of cell growth and survival pathways.*

To test this hypothesis, I ran an assay with psychedelics (psilocin and DMT) and charged versions of these compounds that are membrane impermeable (psilocybin and TMT). I found that both psilocin and DMT were able to stimulate cell growth in cortical neurons—as measured via Sholl analysis—whereas the membrane impermeable compounds were unable to stimulate growth (data not shown). However, when I electroporated these membrane impermeable compounds into cells, they were now able to stimulate growth, demonstrating that the target of these compounds is intracellular.

Maxemiliano Vargas has since taken this project further. He demonstrated that ketanserin (membrane permeable) can block this effect, but a membrane impermeable analog of ketanserin cannot.

He demonstrated that serotonin cannot increase Sholls (same result as I show in **Figure 5.1.1-1**). He reasoned that if this hypothesis were true, then getting serotonin into the cortical neurons should be sufficient to increase cortical neuron growth. To do this, he expressed the serotonin transporter (SERT)—a transporter that is typically found in serotonergic presynaptic terminals to reuptake serotonin at the synapses—on cortical neurons where it is not normally expressed. Now that SERT was expressed on these cortical neurons and serotonin could be transported inside the cell, serotonin was now able to cause an increase in growth in neurons compared to vehicle. In a final experiment, Max showed that if you virally express SERT on cortical neurons in vivo, a burst of serotonin release can decrease immobility time in the FST, comparable to psychedelic-treated animals. This data suggests that the target is inside cortical neurons can modulate neuronal growth and rapid-acting antidepressant effects. My data demonstrates that this target is the 5HT2A receptor. Psychedelics are lipophilic, can easily cross membranes, and engaging their target. On the other hand, membrane impermeable compounds such as serotonin are unable to cause a growth and therapeutic response due to the limited number of targets it can access. This may explain the lack of rapid-acting effects of current antidepressant agents, as simply increasing serotonin alone is not sufficient.[i]

Future work will investigate the role of calcium signalling in these cells by using a membrane permeable calcium chelator to see if it is able to disrupt growth of cortical neurons. If

[i] Max Vargas is preparing this manuscript for publication now. This data will be available in his manuscript and his dissertation.

this theory is true, it is possible that artificial transport of all 5HT2A receptors to the surface or overexpression of other Gq coupled receptors on cortical neurons along with stimulation may be able to replicate these growth patterns.

It is also possible that there are differences in the intracellular environment that allows growth when internal 5HT2A receptors are stimulated versus when extracellular receptors are stimulated.

5.4 Methods

5.4.1 Drugs

The NIDA Drug Supply Program provided lysergic acid diethylamide (LSD). Other chemicals were purchased from commercial sources such as ketamine hydrochloride (KET, Fagron), ketanserin (KETSN, ApexBio), 5-hydroxytryptamine (Sigma-Aldrich), Lisuride (Tocris). The following drugs were synthesized in house, as described previously:

- 5-methoxy-*N*,*N*-dimethyltryptamine (2:1, 5-MeO-DMT:fumaric acid)[23]
- 6-methoxy-*N*,*N*-dimethyltryptamine (2:1, 6-MeO-DMT:fumaric acid)[23]
- *N*,*N*-dimethyltryptamine (2:1, DMT:fumaric acid)[24]
- 6-fluoro-diethyltryptamine (6-F-DET)[25]
- Psilocybin (PSI)

and judged to be analytically pure based on NMR and LC-MS data.

For cell culture experiments, VEH = 0.1% (agonist studies) or 0.2% (antagonist studies) molecular biology grade dimethyl sulfoxide (Sigma-Aldrich). For in vivo experiments, VEH = USP grade saline (0.9%). For all cellular experiments, tabernanthalog freebase was used, while the fumarate salt of tabernanthalog was used for the in vivo studies.

5.4.2 Animals

All experimental procedures involving animals were approved by UCD Institutional Animal Care and Use Committee (IACUC) and adhered to principles described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The University of California, Davis (UCD) are accredited by the Association for Assessment and Accreditation of Laboratory Animal

Care International (AAALAC). Animals were housed 2-5 animals of the same sex per cage, and were given *ad libitum* access to food and water. The experiments began when the mice were 8-24 weeks of age. Lights in the vivarium were turned on at 07:00 hours and turned off at 19:00 hours.

5.4.3 Dendritogenesis Experiments using Cultured Rat Neurons

For the dendritogenesis experiments conducted using cultured cortical neurons, timed pregnant Sprague Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). Full culturing methods were previously described,[1] as were staining and analysis details.[23] For blocking studies, KETSN or DMSO (VEH) was treated 15 mins before drug application.

5.4.4 Dendritogenesis Experiments using Cultured Mouse Neurons

Animals were generated on IACUC approved breeding protocols. Pups born P0-P2 were sacrificed, and dissection was carried out as previously described.[1] Cells were plated in mouse plating media, which consisted of 10% Horse Serum (Gemini Bioproducts), 1% sodium pyruvate (Thermo Fisher), 1% 1M HEPES (ThermoFisher), and 0.4% glucose in Minimum Essential Medium (ThermoFisher). After 3 hours, a media was fully replaced with mouse maintenance media 2% B27 plus (ThermoFisher), 1% sodium pyruvate (ThermoFisher), 1% 1M HEPES (ThermoFisher) and 0.25% Glutamax (ThermoFisher) in Neurobasal (ThermoFisher). Cells treatments and fixation processes were previously described,[1] as were staining and analysis details.[23]

5.4.5 Forced Swim Test (FST)

Male C57/BL6J mice (9-10 weeks old at time of experiment) were obtained from the Jackson Lab and housed 4–5 mice/cage in a UCD vivarium following an IACUC approved protocol. After 1 week in the vivarium each mouse was handled for approximately 1 minute by a male experimenter for 3 consecutive days leading up to the first FST. All experiments were carried out by the same male experimenter who performed handling. During the FST, mice underwent a 6 min swim session in a clear Plexiglas cylinder 40 cm tall, 20 cm in diameter, and filled with 30 cm of 24 ± 1ºC water. Fresh water was used for every mouse. After handling and habituation to the experimenter, drug-naïve mice first underwent a pretest swim to more reliably induce a depressive phenotype in the subsequent FST sessions. Immobility scores for all mice were determined after the pre-test and mice were randomly assigned to treatment groups to generate groups with similar average immobility scores to be used for the following two FST sessions. The next day, the mice received intraperitoneal pretreatment injections of vehicle (saline) or ketanserin (KETSN, 4 mg/kg). 10 minutes later, animals were treated with 5-MeO-DMT (50 mg/kg) or vehicle (saline). The following day, the mice were subjected to the FST and then returned to their home cages. All FSTs were performed between the hours of 8 am and 1 pm. Experiments were video-recorded and manually scored offline. Immobility time—defined as passive floating or remaining motionless with no activity other than that needed to keep the mouse's head above water—was scored for the last 4 min of the 6 min trial.

5.4.6 Sucrose Preference Test (SPT)

Wildtype and 5HT2A KO 129S6/SvEv mice (male and female) ~6 weeks old were individually housed and subjected to a two-bottle choice experiment. Animals were transferred to the vivarium

and given ~1w to adapt. Throughout the experiment, animals were subjected to a two-bottle choice experiment in which animals are individually housed for 24 h and give access to two bottles, water and 1% sucrose with *ad libitum* access to food.

On the first day of the experiment, animals were given the first two-bottle choice session. After the baseline, animals were put back in their group housing and administered 20 mg/kg of corticosterone in DMSO (CORT) daily for 10 days.

After the 10 days of CORT treatment, animals we subjected to the second two-bottle choice experiment (Post-CORT) for 24h and a preference for sucrose was calculated. From this, treatment groups were randomly assigned and balanced to avoid systematic errors. Animals were injected IP with either vehicle (saline) or psilocybin (10 mg/kg). 24h after this injection, animals were again subjected to the third two-bottle choice experiment (Post-Drug).

Sucrose preference was calculated was calculated as the amount of sucrose solution consumed minus the amount of water consumed, divided by the total amount of liquid consumed.

5.4.7 Electrophysiology

Male wildtype 129S6/SvEv mice (~6 weeks old) were given an intraperitoneal injection of either 5-MeO-DMT (50 mg/kg), TBG (50 mg/kg) or vehicle. After 24 h, mice were anesthetized with isofluorane and transcardially perfused with ice-cold artificial cerebrospinal fluid (ACSF), containing 119 mM NaCl, 26.2 mM NaHCO3, 11 mM glucose, 2.5 mM KCl, 1 mM NaH2PO4, 2.5 mM CaCl2 and 1.3 mM MgSO4. Brains were rapidly removed and 300 μm coronal slices from the mPFC were cut on a Leica VT1200 vibratome (Buffalo Grove, IL) with ice-cold ACSF solution. Slices were incubated in 32 °C NMDG solution for 10 minutes, transferred to room temperature ACSF, and held for at least 50 minutes before recording. All solutions were vigorously

perfused with 95% O2 and 5% CO2. Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded at -70 mV in 32 °C ACSF. Cells were patched with 3–5 MΩ borosilicate pipettes filled with intracellular solution containing 135 mM cesium methanesulfonate, 8 mM NaCl, 10 mM HEPES, 0.3 mM Na-GTP, 4 mM Mg-ATP, 0.3 mM EGTA, and 5 mM QX-314 (Sigma, St Louis, MO). Series resistance was monitored throughout experiments; cells were discarded if series resistance varied more than 25%. All recordings were obtained with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Analysis was performed with the Mini Analysis program (Synaptosoft, Decatur, GA) with a 4 pA detection threshold. Data represents individual neurons taken from 3 different animals per treatment. Data acquisition and analysis was performed by experimenters blinded to treatment conditions.

5.5 Contributions & Collaborations

I couldn't have done this alone. Thank you to all my collaborators who helped me get this far. Contributions are outlined below.

Maxemiliano V. Vargas ran the FST assay to assess 5HT2A involvement for psychoplastogenic compounds. Seona D. Patel and LPC ran the sucrose preference assay. LPC and DEO designed all experiments.

Olson Lab

University of California, Davis

LPC, Shekib Jami and John A. Gray helped to run the patch clamp studies in mouse cortical neurons with input from LPC and DEO.

Gray Lab

University of California, Davis

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Chapter 6

CONCLUSION, DISCUSSION & FUTURE DIRECTIONS

6.1 Psychedelics for Treating Mental Health Disorders

An increasing number of reports[1] suggest that psychedelics may have treatment potential for neuropsychiatric disorders like depression, anxiety and PTSD in humans.[2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13] Impressively, several of these studies demonstrated that after treatment with these compounds, depression ratings dropped and remained consistently low for months after treatment.[2] A cornerstone study by Agin-Liebes et al reported low depression levels as far as 3 years after their initial treatment.[14]

Proper placebo-controlled trials with psychedelic compounds can be difficult to perform given the intense perceptual effects caused by these drugs and not in placebos. Many of these studies have now been tested in rodent models of psychiatric disorders.[15, 16, 17]

Despite being promising therapeutics, psychedelic medicine remains hindered by their ability to cause perceptual disturbances. While it is intriguing to argue that hallucinations might be necessary to achieve a "spiritual transformation" and enlightenment, hallucinations may just occur in parallel and may therefore not be necessary for any therapeutic effect. This dissertation has explored whether hallucinations are necessary for the therapeutic action of psychedelics, or whether the two phenomena are dissociable.

6.1.1 Psychedelic Microdosing

One way to investigate whether hallucinations are necessary for the therapeutic effect of psychedelics is to explore psychedelic microdosing. This is the practice of taking chronic, intermittent, sub-hallucinogenic doses of psychedelic compounds with the aim to increase creativity and cognitive flexibility, while decreasing depression and anxiety.[18, 19]

The studies outlined here in Chapter 3 suggest that psychedelic microdosing works in humans in an unblinded and self-medicating manner, but also in rodents who have no preconceived expectation of therapeutic action. Several other studies in both humans[20, 21] and rodents[22] have emerged demonstrating that psychedelic microdosing may be efficacious. My study demonstrated that the main reason people discontinued the practice was due to a difficulty or risk associated with obtaining the required materials.[23] Indeed, Hutten and colleagues demonstrate that BDNF (as detected in blood plasma) increases in healthy volunteers after low doses of LSD.[24]

In general, microdosing seems to be less robust in eliciting therapeutic responses than a fully hallucinogenic dose. Based on pharmacologic principles, it is very possible that taking a lower dose of a drug will receive a smaller therapeutic response (**Figure 6.1.1-1**). Finding an appropriate dose for therapeutic action can be difficult. This could explain why so many rodent

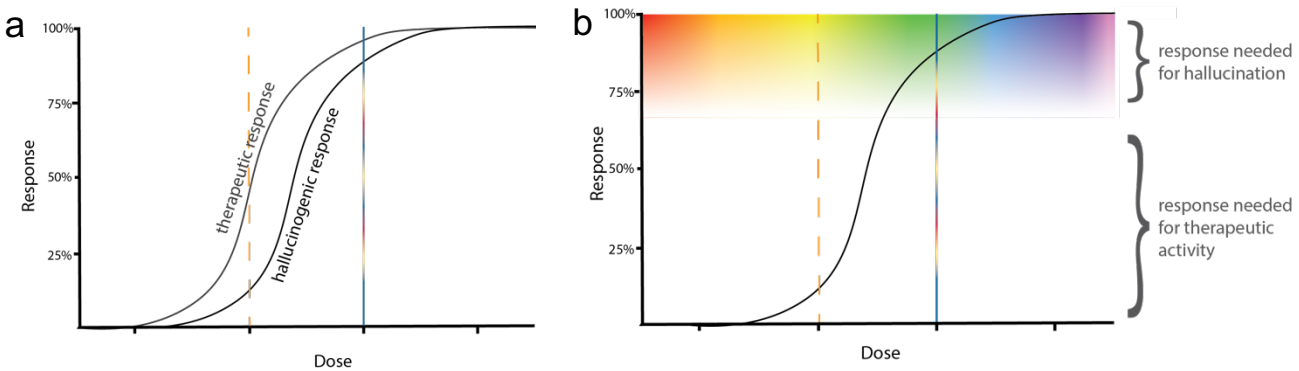

Figure 6.1.1-1*. Schematic of a hallucinogenic versus a therapeutic response for psychedelic microdosing. The full coloured line represents a hallucinogenic dose, and the dotted gold line represents a microdose. Whether the therapeutic and hallucingenic responses can be separated (a) or not (b), decreasing the dose of this drug will ultimately decrease the response in subjects.*

studies—where dose is tightly controlled—show more promising results than human studies. Human studies display more variability, likely because most of these studies are not from survey data, not clinical trials where dose and purity are tightly regulated. Furthermore, differences in body weight composition among individuals, as well as individual differences in metabolism and absorption may play a role in how participants respond to these drugs. Finally, many individuals who microdose have had previously experienced fully-hallucinogenic doses, which may alter both their response to the drug as well as their expectation. In summary, based on basic pharmacological principles, a higher dose would more effectively produce a robust therapeutic response.

A report from Carhart-Harris and colleagues found that positive expectations about psychedelic microdosing could predict improvements in mental health.[25] In the same vein, a foundational study came out this year by Szigeti and colleagues utilizing a clever citizen science approach to study microdosing psychedelics versus placebo.[26] These researchers report that although participants who microdose show improved scores on emotional state, mood and creativity, *these were not different than the placebo-treated group*. This demonstrates that in healthy populations, the anecdotal beneficial effects of psychedelic microdosing may be explained by the placebo effect. Although powerful, this study used healthy individuals and not those in a clinical population. It is possible that patients from a clinical population may respond more robustly to treatment. In fact, studies have demonstrated that the magnitude of therapeutic response to antidepressants depends on how severe depression was to start.[27]

Many studies suggest that psychedelic microdosing appears to have therapeutic effects, though more research needs to be done to be conclusive. It is possible that a higher dose is needed to have more robust therapeutic effects; however, if we are investigating the necessity of hallucinations in achieving the therapeutic effects of psychedelics, it can be difficult to dose higher

without eliciting perceptual effects. Finally, psychedelics also hit 5HT2B which may lead to cardiac valvuopathies, so safety considerations should be explored more deeply.

Interestingly, some studies have used low dose psilocybin (1 mg/70 kg) in lieu of a placebo-control with the intention that doses this low would not be therapeutic.[6] Although the researchers found a more dramatic decrease in depressive scores with higher doses, the low dose elicited physiological changes and was not compared to an inactive group or before-dosing scores. While the data remains murky on the therapeutic efficacy of psychedelic microdosing, researchers should refrain from using low-dose psychedelic compounds in lieu of a proper placebo control.

6.1.2 Non-hallucinogenic analogs as a novel class of psychoplastogens

Though psychedelic microdosing may be effective, it is still using hallucinogenic compounds and is therefore subject to abuse and/or dosing mistakes. The development of novel non-hallucinogenic analogs would increase safety and accessibility of psychoplastogens. Development and testing for a new non-hallucinogenic analog is discussed in Chapter 4. Since the publication of this work, other non-hallucinogenic analogs such as AAZ have been reported.[28] These compounds appear to elicit rapid-acting antidepressant properties, have low abuse potential, minimally impair natural reward seeking, all while reducing hedonic drug seeking.[29] Though these compounds have been validated as non-hallucinogenic by using a HTR and psychLight screening assays,[28] it has yet to be seen if these compounds are truly non-hallucinogenic and therapeutic in humans.

An intriguing line of work would be to delineate a neural signature of hallucinations in rodent brains using fibre photometry, or better yet in vivo calcium imaging in the cortex followed by restimulation using three-dimensional scanless holographic optogenetics with temporal focusing (3D-SHOT; or similar method).[30] Technical challenges include recording cells with an

imaging apparatus attached, as they impair the mouse's ability to engage in HTR behaviour. Using this method may shed light on different neural signatures of hallucinogenic and non-hallucinogenic compounds. Similar studies have found neural signatures of dissociation using anesthetics like ketamine[31] (which do not produce a HTR).

6.1.2.1 Mechanism of action for psychedelics and non-hallucinogenic analogs

If the hallucinogenic and therapeutic properties are indeed dissociable, then you would expect two independent dose-response curves (as in **Figure 6.1.1-1a**). In effect, you would be pushing the hallucinogenic response to the right and/or the therapeutic response to the left. In this way, you would be able to achieve therapeutic responses without hallucinations.

An alternative explanation might be that non-hallucinogenic analogs are "weaker" psychedelics. In other words, it is microdosing a weaker psychedelic. If this were the case, we would assume that the therapeutic and hallucination response curves would both shift right.

An examination of the calcium data demonstrates that TBG is a partial agonist at 5HT2A, whereas a hallucinogenic analog like 5-MeO-DMT is a full agonist (**Figure 4.3.7-2**). This calcium data is measured in HEK cells expressing the 5HT2A receptor.[29] If calcium were necessary for a therapeutic response, it might be that very high concentrations of calcium can cause hallucinations, but that lower amounts are sufficient for cortical growth and behavioural responses (**Figure 6.1.2-1**). This hypothesis would also fit with microdosing, as a subhallucinogenic dose of a hallucinogenic compound may still produce therapeutic effects. While this is a tempting explanation, not all non-hallucinogenic compounds have a decreased potency or efficacy compared to hallucinogenic drugs (for a list of cellular responses to psychedelic and non-hallucinogenic compounds, please refer to **Appendix 2**). For example, DMT elicits a 20.4%

phosphoinositide hydrolysis response (compared to 5HT) and 6-F-DET elicits a 25% response.[32] In this vein, simply the magnitude of calcium release is not enough to explain the dissociation of psychedelic and hallucinogenic effects. Furthermore, something like serotonin itself—which is extremely potent and elicits a maximal calcium response—is not sufficient for growth of cortical neurons or a therapeutic effect. It is possible that there are differences in pharmacokinetics between compounds that create differences when tested in vivo.

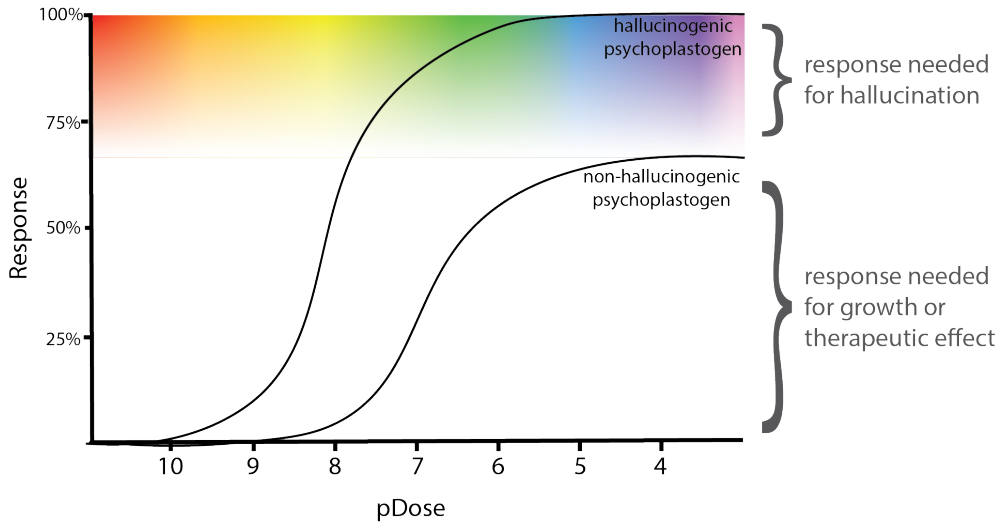

Figure 6.1.2-1. *Non-hallucinogenic psychedelic analogs cannot be explained by simply weaker agonism of their target. Curves here are based off real data of calcium responses in HEK-cells.*

It is possible that the differences between psychedelics and non-hallucinogenic analogs can be explained by differences in functional selectivity. We know that serotonin (a full agonist at G-protein/IP3 hydrolysis/Ca release) and ketanserin (an antagonist in these parameters) are both insufficient to cause neuronal growth and/or therapeutic effects. Most psychedelics and non-hallucinogenic compounds are partial agonists, so it is unclear how exactly how classic G-protein signalling would mediate this. Data from the Roth group using TRUPATH demonstrates that psychedelic compounds (LSD and 25CN-NBOH) as well as serotonin (5HT) are strongly biased to interact with Gq, G11 and G15 with little to no interaction with other G-proteins.[33] Since both

psychedelics and 5HT share these signalling pathways, it is unlikely that the therapeutic responses could come from differential G-protein activation. Furthermore, many psychedelics have a broad range of β-arrestin engagement, with something like LSD being a full agonist while lisuride and DMT barely engage β-arrestin at all (unpublished data, data not shown). Taken together, there seems to be no commonalities between functional selectivity pathways and a compound's ability to increase cortical neuron growth or a therapeutic response.

There is also ample evidence from the Gonzalez-Maeso group that psychedelic compounds engage signaling through a 5HT2A-mGluR2 dimer.[34, 35] It is possible that this dimer complex signals or is stabilized by psychedelic interaction and this does not happen in the presence of a non-hallucinogenic analog. It is also possible that signaling through this dimer actually initiates a therapeutic response—as all the compounds that the Gonzalez-Maeso group have tested are also psychoplastogens—and that hallucinations actually signal through another mechanism, perhaps just increases in neuronal activity, decrease the signal to noise.

Ultimately, I propose that it is the strength and number of receptors that are stimulated that will influence the cells' response. The calcium schematic presented (**Figure 6.1.2-1**) is based off data measured in HEK cells expressing 5HT2A on the cell surface. In neurons, 90% of 5HT2A receptors are intracellular,[36] and so compounds that can easily cross membranes to intracellular targets may have a greater response. In this vein, compounds like serotonin may elicit a maximum response of the receptor but only hit 10% of the receptors. On the other hand, psychedelic compounds may be partial agonists, but they can stimulate both intracellular *and* extracellular receptors, thereby evoking a greater overall response. This hypothesis is being tested now, and further details are elaborated on in **Chapter 5**.

6.1.3 Coupling psychotherapy with psychedelic treatment

Current psychedelic therapy for humans aims to couple drug intake with psychotherapy. This approach aims to develop new thought and behavioural patterns while in a suggestive state. To the best of my knowledge, no one has directly compared no-psychotherapy treatment to psychotherapy treatment in patients administered psychedelic compounds. This may be because patients need to be monitored during their experiences for safety purposes, but also a reflection of clinical trial ethics which aim to "bring no harm" to patients involved.[37]

However, there have been studies in which patients received "standard support" or "high support" during their psychedelic experience.[6] This study demonstrated that patients with high-support showed larger effects across 23 measures than the patient group that received standard support.

A clinical trial in individuals with treatment-resistant depression suggests that social connectedness may be foundational for therapeutic change. Watt and colleagues report that a primary symptom of patients with depression is disconnection from others, and that reconnection was a main theme that was reported after psychedelic use.[38] Individual psychedelic therapy versus group therapy has not been explicitly tested, other than in an inconclusive meta review by Trope et al. which suggests it may be useful but warrants a properly controlled study.[39] A primary goal of group therapy is to facilitate reconnection.[40] In this vein, the psychedelic MDMA has been shown to increase sociability of rodents[41] and cephalopods,[42] though whether this socialization causally leads to decreases in depression (and related pathologies), whether these behaviours are controlled by an overarching neural mechanism, or whether they merely occur in parallel remains to be determined.

Regardless of sociability, the idea of "training" the mind while the brain is in a suggestable state remains open. Studies in our lab have demonstrated that psychedelic treatment before fear extinction training does not augment the training itself, but rather the animals seem to remember the training better when tested the next day.[16] This paradigm nicely parallels with how PTSD is treated in the clinic today with MDMA. Fear conditioning is established in one paradigm, administration of psychedelic and training (or therapy) occur in a different setting, and finally the establishment of that training appears to be better solidified when training occurs with psychedelics on board. Other than fear extinction training, the augmentation of therapeutic effects with psychotherapy is difficult to test in murine models. Future studies in rodents may wish to investigate psychedelic treatment in group versus individually-housed animals, and human studies may wish to directly test the difference between individualized versus group therapy sessions.

Some studies in rodents demonstrate a therapeutic effect without training,[16] suggesting psychotherapy might not be necessary for treatment of depression. For humans, monitoring for safety purposes while the patient is experiencing hallucinations would still be necessary. Interestingly, though psychedelic administration is paired with psychotherapy, ketamine infusions are not and yet still appear to be effective. It is possible that with non-hallucinogenic analogs of psychedelics, monitoring psychedelic sessions to monitor health of the patient are no longer necessary, thus creating a therapy that is cheaper to the patient and facility, and therefore more accessible. That said, it is likely that psychotherapy may enhance the therapeutic effects of these compounds.

6.2 The role of the prefrontal cortex in modulation of subcortical areas

In this dissertation, I discuss the role of psychedelics in mediating therapeutic responses in rodent models. Early reports in the Olson lab demonstrate that psychedelics cause increased dendritogenesis, spinogenesis and synaptogenesis in cortical cultures of neurons, and concomitantly increase their connectivity.[43] Work by Dakic and colleagues also report increases in proteins associated with long-term potentiation and growth after organoids are treated with psychedelics like 5-MeO-DMT.[44]

The PFC is a region of the brain responsible for motivation and goal-oriented behaviour. This region is atrophied and has decreased function in depression, anxiety and addiction.[45, 46, 47, 48, 49, 50] Furthermore, there appears to be a decrease in the number of active synapses in this region,[51] which is concomitant with a decrease in BOLD signal in human imaging studies.[52] Reports of psychoplastogen administration have demonstrated rapid restoration of structure and function of these neurons.[17, 43, 73]

Specifically, the ventral medial PFC (vmPFC), which includes the infralimbic cortex, densely innervates the nucleus accumbens (NAc) medial shell,[53] an area known for responding to aversive stimuli.[54] In addition, it innervates the hypothalamus,[53] ventral tegmental area (VTA)[55, 56]and the amygdala (AMY).[57, 58] Studies have demonstrated a key role for the infralimbic cortex in the acquisition, extinction, and expression of fear memories.[59, 60] Studies have shown that fear extinction increases excitability and activity of neurons in the infralimbic cortex, and consolidation of this extinction is protein-synthesis dependent.[61, 62, 63, 64, 65] Finally, loss-of-function studies using either lesions or pharmacological inactivation demonstrate that infralimbic, but not prelimbic, cortex is necessary for consolidation of extinction learning.[66, 67, 68, 69, 70] Interestingly, infusions of BDNF to the infralimbic cortex facilitates fear extinction in the absence of training,[71] suggesting

that perhaps simply increasing BDNF levels in this region in the absence of training is enough for the expression of extinction behaviour.

6.2.1 The role of the prefrontal cortex in modulating behaviours relevant to depression and anxiety

In depression, neurons in the PFC are atrophied and have decreased excitability. Studies have demonstrated that ketamine—a compound capable of increasing activity in the PFC[72]—is capable of rapidly restoring structure and function to these neurons.[73] Regrowth of structure and maintained activity changes are thought to be due to BDNF release; indeed, an infusion of a BDNF antibody to the PFC blocks the therapeutic actions of ketamine, as measured in rodent behavioural assays.[74] Furthermore, activation of PFC terminals by optogenetics in subcortical regions is sufficient for restoring motivated behaviours.[75]

Like depression, elegant work from the Deisseroth lab has demonstrated that circuits mediating anxiety are also modulated by the vmPFC.[76] Optogenetic activation of the vmPFC, but not dmPFC, facilitate anxiolytic responses in rodent models.

Work in the Olson lab suggests that psychedelic compounds increase the growth and function of cortical neurons after psychoplastogen treatment.[43, 77] In addition, blockade with a TrkB antagonist antagonizes the growth of cortical neurons seen in culture experiments,[43] suggesting psychedelics—like ketamine—work through similar mechanisms.

6.2.2 The role of the infralimbic cortex in modulating behaviours relevant to substance use disorder

There are many parallels between fear extinction learning and drug-extinction learning.[78] Infralimbic cortex is highly active during extinction training,[79, 80] and activity in the infralimbic cortex prevents drug-primed reinstatement and inactivation of the infralimbic cortex results in spontaneous reinstatement of cocaine-seeking responses.[81] In addition, inactivation of this region after extinction training results in a blunted extinction response.[82] Interestingly, these extinction memories can be endogenously modulated via neuromodulators such as adrenaline[82] and dopamine.[83] There are also suggestions to suggest that the NAc medial shell may be strengthened by glutamatergic input from infralimbic cortex after extinction training.[84] Similar to depressive-like behaviours, infusion of BDNF into the mPFC reduces excessive drinking in ethanol-dependent mice.[85, 86]

Both human and rat behavioural studies have described decision making tasks generally utilizing learning strategies encoding the relationship between action and consequence (goal-directed) or stimulus-response associations (habitual). Evidence in humans and rats suggests that goal-directed action is typically governed by frontal cortical regions, whereas control of habitual actions is modulated by the striatum.[87, 88] The prevailing idea is that infralimbic cortex may play a role in the original drug-seeking (goal-directed) behaviours, but over time this behaviour becomes governed by striatal loops. There is also evidence to suggest that the infralimbic cortex initiates some "stop" circuitry via projections to the NAc shell or AMY. [78, 81, 89] Indeed, optogenetic activation of infralimbic terminals in NAc blocks drug seeking behaviour.[90] Importantly, different populations of cells appear to respond during hedonic drug seeking than when seeking more natural rewards like food or sucrose.[91, 92] This suggests that a specific population may be targetable

to decrease drug use without impairing pleasure of natural rewards. We are able to see this in our experiments as TBG treatment is able to blunt self-administration of hedonic drugs like heroin or alcohol, without effecting sucrose or food seeking.[29, 93] We postulate that this occurs due to differences in target populations of PFC neurons by TBG. When using psychoplastogens for these types of studies, detailed thought should be given to when these compounds are administered in relation to the task at hand.

In contrast, the prelimbic cortex is thought to promote drug seeking. Stefanik elegant demonstrated that bilateral optogenetic inhibition of prelimbic fibres in the NAc core inhibited drug seeking.[94] In other words, this projection may drive drug seeking behaviour. Future work by this group also demonstrates that the basolateral amygdala (BLA)—which plays a key role in reinstatement of cocaine seeking—may drive activity in the prelimbic cortex.[95] Inactivation of the prelimbic cortex in rats does not mediate consolidation of instrumental learning, but rather increased responding; this suggests the prelimbic cortex may mediate a form of inhibitory response control.[96] Though most of the evidence seems to agree that activity in infralimbic cortex decreases drug seeking, there are clearly some discrepancies which the field has yet to investigate. This may have to be done systematically, with close attention and consistency with animal models, coordinates of viral injection, which viruses are being used, which cells are being infected, and which projections are being stimulated.

6.3 Sex differences in responsivity to psychedelics

There have been few studies that explicitly explore sex differences in responsivity to psychedelic compounds. I have noticed several different sex differences over the course of my studies, which I shall comment on below.

In humans, females have higher prevalence and severity of depression,[97] and this is correlated with lower serum levels of BDNF than both male healthy and depressed individuals.[98] In studies with ketamine, there are sex differences with respect to the therapeutic response.[99] Evidence suggests the therapeutic potential may depend on the phase of estrus in females.[100]

6.3.1 Sex differences in response to hallucinations

In HTR assays, I have noticed a difference in the sensitivity of mice to psychedelic compounds, specifically that female mice exhibit far more HTRs than males.[101] Both sexes consistently exhibit more head-twitches when administered a psychedelic compound, and trends across sexes are identical, but females exhibit nearly twice as many head-twitches as males. I have found this highly replicable over the years in many studies in the Olson lab. Surprisingly, Alex Kwan's group did not observe differences in HTR when he tested psilocybin in both sexes.[17] Only one study in humans explicitly tested the effects of sex on the subjective experience of MDMA and found that women experience higher intensity of perceptual changes, thought disturbances and fear of loss of body control.[102]

In most human and rodent studies, males and females are combined. If attempting to study the effects of hallucinations in response to psychedelic compounds, attention to sex and hallucination intensity is advised.

6.3.2 Sex differences in therapeutic responsiveness

In our hands, males and females have similar trends in response to psychoplastogens. For both the sucrose preference test and forced swim test, both males and females have similar magnitude of baselines, responses to stress, and rescued behavioural phenotypes after psychoplastogen treatment.

With regards to neuronal structure, I have found that females have a robust response to psychedelic administration in golgi analysis of spines and ephys,[43] and this has been corroborated by Alex Kwan's group who found that females often have a greater increase in the number of spines formed, and also show more robust changes in function.[17] Interestingly, I also found that female rodents are more sensitive to chronic administration of psychedelics, as our psychedelic microdosing study suggested a decrease in the number of spines after treatment, perhaps from overactivation of these neurons.[103]

In trials with psychedelic microdosing, females consistently report improvements after the practice, though these scores are less positive than male counterparts.[104] This may be due to differences in what females experience, however it may also be due to differences in reporting trends.

Female rats with circulating estrogen tend to have a higher response to stress, likely due to greater adrenergic and dopaminergic signaling,[105, 106] and have more dysfunction in the PFC compared to male rats (however this trend is reversed if the females are ovariectomized).[107, 108]

6.4 Health Risks of Psychedelic Medicine

6.4.1 Health risks associated with acute psychedelic intake

Hallucinations are expected after psychedelic intake, and for this reason, patients should be monitored to ensure their safety. Commonly reported acute adverse reactions are strong feelings of dysphoria, anxiety or panic,[109, 110] though these do not typically require pharmacological intervention and do not persist after the drug wears off. This underscores the importance of monitoring patients while they undergo a psychedelic experience.

A common side effect of psilocybin treatment is delayed onset for headaches and migraines.[111] This is particularly interesting, since psilocybin has purported effects for treating cluster headaches.[112]

High doses of psychedelics can lead to cardiovascular issues, as 5HT2A plays a key role in platelet aggregation, smooth muscle contraction, thrombus formation and coronary artery spasms.[113] Depending on the type and dose of psychedelic used, it is possible to elicit vasoconstriction, if 5HT1B receptors are also engaged. Finally many psychedelics engage 5HT2B which can contribute to cardiac valvuopathy. Major clinical studies investigating psilocybin for depression have reported transient moderate increases in systolic and/or diastolic blood pressure after administration of psilocybin (systolic ~146 mm Hg; diastolic ~85 mmHg), as well as a slight increase in heart rate (~90 beats per minute).[2, 3, 4, 5] Across these studies, only one participant reached high enough blood pressure to warrant sublingual nitroglycerin administration.[6] Despite this event, the therapy session went otherwise smoothly, the participant had a positive experience and requested to participate in future sessions (which was denied).

The LD50 of DMT is 47 mg/kg when administered IP in rats and is estimated to be approximately 9 mg/kg for humans (extrapolating from rodent data).[114, 115] When DMT is prepared

as ayahuasca, the LD50 is estimated to be 8 mg/kg. When a group of experienced DMT users were asked about the main risks of DMT, the most common problems reported were a "bad trip" (51%), psychospiritual problems (39%) or physiological problems (26%) which includes respiratory irritation or burns when smoking.[116]

Similar findings have been reported for psilocybin use, though these reports suggest that effects may persist. In a study by Carbonaro et al, researchers found that psilocybin administration may result in persisting fear, anxiety, depression, and paranoia.[117] In fact, 24% of participants reported these symptoms within a week after taking the drugs, and 10% of patients reported symptoms lasting over 12 months after the challenging session. The participants who sought treatment prior to their psilocybin experience was significantly more likely to experience symptoms and seek treatment after the session. In rats, the LD50 of IV psilocybin is above 250 mg/kg.[118] The lethal dose is estimated to be approximately 1000x the effective therapeutic dose.[119]

For LSD, the LD50 is 16.5 mg/kg when administered IV,[120, 121] and no known human deaths have occurred due to LSD overdose. Last year, a woman took 55 mg of LSD intranasally—mistaking it for cocaine—which ended up being 550x the regular dose of LSD. She blacked out and vomited several times but was otherwise healthy with no persisting effects other than a decrease in regular opioid use.[122] In 1974, eight individuals took large amounts of LSD intranasally—again mistaking it for cocaine—and achieved concentrations of 1000-7000 ug/100ml in blood plasma. These individuals suffered collapse, vomiting, hyperthermia, coma, respiratory arrest, and some mild bleeding (due to platelet dysfunction). All patients recovered and had no persistent effects, but this appears to be the upper limit of LSD's safety window.[123]

Ibogaine is less well tolerated in humans. The LD50 for ibogaine in mice is 263 mg/kg.[124]At high doses, convulsions, nervous behaviour and limb paralysis occurs.[124] Rodent

studies suggest it may contribute to cerebellar toxicity,[125, 126] however several studies in primates were not able to replicate the results,[127, 128] suggesting it may be specific to rodents. As mentioned previously, the most clinically significant adverse effects are a decrease in heart rate.[129, 130] Ibogaine is known to block hERG channels responsible for cardiac repolarization, therefore causing long QT syndrome.[131] For this reason, it is highly advised that patients with pre-existing cardiovascular conditions do not use ibogaine. Litjens and Brunt reported in 2016 that 27 deaths occurred several days or even weeks after administration—likely due to the highly lipophilic nature of ibogaine, it can be sequestered in tissue and release slowly over time—all within patients that had pre-existing conditions.[132] Alper and colleagues surveyed individuals between 1990 and 2008 who took ibogaine and similarly found that deaths nearly all resulted from patients with preexisting cardiovascular conditions.[133] Importantly, post-mortem studies of these patients revealed no neurotoxicity.

A meta analysis by Trope and colleagues revealed no reported cases in psychosis, suicide or other serious adverse events associated with psychedelic administration in clinical trials.[39, 134, 135] That said, many large psychedelic trials screen out individuals who have "co-existing psychiatric conditions", which would include conditions such as schizophrenia.[136]

Although it is thought that psychedelic-mediated hallucinations resemble psychosis seen in schizophrenia, there is little evidence to suggest that psychedelics can trigger the onset of schizophrenia. A large study of 130,000 adults in the United States revealed that there was insufficient evidence for a link between psychedelic use and lingering psychosis.[137] Furthermore, there is little evidence to support the existence of hallucinogenic persisting perceptual disorder (HPPD) or 'flashbacks'. Finally, controlled trials with psilocybin have not reported any cases of flashbacks or other visual disturbances.[3, 138, 139]

6.4.2 Health risks associated with chronic psychedelic intake

Studies in Brazil have determined that long-term ayahuasca use is not associated with health risks[140] and lifetime ayahuasca users actually scored significantly better on some neuropsychological tests than controls.[140, 141] Similarly, Johansen and Krebs reported that lifetime use of psychedelic compounds was associated with increased mental health outcomes and decreased psychiatric treatment.[142] Interestingly, Hendricks and colleagues conducted data from 2002-2007 across 25,622 individuals charged with a felony and found that psychedelic use tended to promote abstinence from alcohol and other drug use, as well as increasing prosocial behaviour.[143]

One study found that regular psychedelic use increased the lifetime risk of panic attacks; it should be noted that phenylcyclohexyl piperidine (PCP; an addictive drug) was included in their definition of psychedelic and is known to cause dependence.

A study in rats assessing chronic psychedelic treatment (0.16 mg/kg LSD every other day for over 3 months) resulted in hyperactivity, hyperirritability, as well as increased anhedonia and social impairment. These behavioural traits lasted at least for 3 months after drug discontinuation. Gene expression analyses revealed brain wide changes to neurotransmitter systems as a result of this drug regimen. These behavioural changes resemble psychosis and has been proposed as a model of schizophrenia.[144]

Furthermore, psychoplastogens work through mTOR signaling, and overactivation of mTOR has believed to contribute to the development of autism[145] or Alzheimer's disease.[146] Further research is warranted to determine the exact risks associated with chronic use of psychedelic compounds.

In sum, overuse of psychedelic compounds has potential to develop into persistent schizophrenia-like behaviour or other disorders. Likely, the frequency with which these drugs are used plays a major role in their long-lasting effects. Further studies are needed to investigate this.

6.4.3 Abuse potential

Classic psychedelics are not addictive,[147, 148] and are not considered reinforcing.[149] Animals do not self-administer psychedelic compounds, suggesting that they have low abuse potential.[110, 150, 151] Rhesus monkeys have mixed responses to psychedelics, some of which self-administer and some do not, suggesting that these compounds either have weak reinforcing effects or a mixture of reinforcing and aversive effects.[152]

While acute use of psychedelic compounds do not typically result in long-lasting detrimental effects, chronic use of psychedelics may. For this reason, researchers should emphasize that these drugs be given under medical supervision and only used as needed, not on a regular basis. Most treatment regimens—ours included—typically involves a single dose (or a few doses) to achieve a long-lasting effect, thus minimizing issues related to chronic dosing.

6.5 Conclusion and Discussion

In this dissertation, I sought to understand if hallucinations were necessary in achieving the therapeutic effect of psychedelics, or if these two phenomena are dissociable. Using rodent models, I have demonstrated that the hallucinogenic effects are not necessary to achieve therapeutic responses, though this has yet to be tested in humans. It is possible that although non-hallucinogenic, the actual act of experiencing hallucinations may strengthen or augment the therapeutic effect.

We posit that this occurs by increasing the growth and function of neurons in the prefrontal cortex, strengthening top-down control over subcortical regions involved in emotion.

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Appendix 1

ABBREVIATIONS

25CN-NBOH	4-(2-((2-hydroxybenzyl)amino)ethyl)-2,5-dimethoxybenzonitrile
3D-SHOT	Three-dimensional scanless holographic optogenetics with temporal focusing
5HT	5-hydroxytryptophan; serotonin
5HT2A	Serotonin (5HT) 2A receptor
5-MeO-DMT	5-methoxy-*N,N*-dimethyltryptamine
6-MeO-DMT	6-methoxy-*N,N*-dimethyltryptamine
6-F-DET	6-fluoro-diethyltryptamine
AD	Alzheimer's disease
AMY	Amygdala
ASD	Autism spectrum disorder
AUD	Alcohol use disorder
BDNF	Brain-derived neurotrophic factor
BLA	Basolateral amygdala
BOLD	Blood oxygen level dependent
BROWN	Brown fat
CLAMS	Comprehensive lab animal monitoring system

CPP	Conditioned place preference
CUE	Cued extinction phase (heroin self-administration)
DMT	*N,N*-dimethyltryptamine
DOI	2,5-dimethoxy-4-iodoamphetamine
DOM	2,5-dimethoxy-4-methylamphetamine
dpf	Days post-fertilization
EPI	Epididymal fat
EPM	Elevated plus maze
EXT	Extinction phase (heroin self-administration)
FR1	Fixed ratio 1 phase (heroin self-administration)
FST	Forced swim test
GAD	Generalized anxiety disorder
hERG	Human ether-a-go-go
HPPD	Hallucinogenic persisting perceptual disorder
IBG	Ibogainalog
IBO	Ibogaine
KET	Ketamine
KETSN	Ketanserin
KO	Knockout
LCMS	Liquid chromatography, mass spectrometry
MDD	Major depressive disorder
MDMA	3,4-methylenedioxymethamphetamine
mEPSCs	Miniature excitatory postsynaptic potentials

MES	Mesenteric fat
MTBE	Methyl *tert*-butyl ether
mTOR	Mammalian target of rapamycin
NAc	Nucleus accumbens
NIL	Novelty-induced locomotion
NOR	Novel object recognition
ns	Not significant
PCP	Phenylcyclohexyl piperidine
PFC	Prefrontal cortex
PSI	Psilocybin
PTSD	Post-traumatic stress disorder
RER	Respiratory exchange rate
RP	Retroperitoneal fat
S1BF	Somatosensory cortex, barrel field
SA	Self administration
SAL	Saline
SALT	Spontaneous alternation
SD	Standard deviation
SEM	Standard error of the mean
sEPSC	Spontaneous excitatory postsynaptic currents
SERT	Serotonin transporter
SI	Sertindole
SPT	Sucrose preference test

SNRI	Serotonin and norephinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
SUB	Subcutaneous fat
SUD	Substance use disorder
TBG	Tabernanthalog
TMAK	Trimethylammonium ketamine
TMT	Trimethyltryptamine
TrkB	Tyrosine receptor kinase B
UMS	Unpredictable mild stress
VEH	Vehicle
vmPFC	Ventral medial prefrontal cortex
VR5	Variable ratio 5 phase (heroin self-administration)
VR15	Variable ratio 15 phase (heroin self-administration)
VTA	Ventral tegmental area
WAT	Sum of white adipose tissue
WT	Wildtype
WTD	Whisker-dependent texture discrimination

Appendix 2

Literature Review of Downstream Signaling Paths by Psychedelics

Type of Ligand	Type of Agonist	Cellular Response	% efficacy compared to 5-HT	Assay Type	Cell Type	Reference
5-HT	Agonist	Increased Ca	-	FURA-2/AM	"mammalian cells" (not specified)	Pritchett et al, 1988
5-HT	Agonist	Increased IP3 hydrolysis	-	accumulation of IP and IP2 (metabolite formation)	"mammalian cells" (not specified)	Pritchett et al, 1988
5-HT	Agonist	inward current (depolarization)	-	patch clamp (voltage clamp at -70mV)	xenopus oocyte	Pritchett et al, 1988
5-HT	Agonist	Increased Ca	-	Indo-1 (Ca sensitive dye)	NIH3T3	Julius et al, 1990
5-HT	Agonist	beta-arrestin dependent Internalization	-	beta-arrestin1 and beta-arrestin2 KO cultures compared to WT; media	Mouse embryonic	Schmid et al, 2007

				with serotonin caused internalization in WT (vs surface expression with no serotonin in media), no change in beta-arrestin lines/all 5-HT2A is on cell surface	fibroblasts (MEFs)	
5-HT	Agonist	ERK1/2 phosphorylation (activation)	-	Western blot	Mouse embryonic fibroblasts (MEFs)	Schmid et al, 2007
5-HT	Agonist	ERK1/2 phosphorylation (activation)	-	Western blot	Mouse frontal cortical tissue 15mins after injection	Schmid et al, 2007
5-HT	Agonist	IP Stimulation	-	IP Receptor stimulation	NIH 3T3 cells	Egan et al, 1998
5-HT	Agonist	More [35S]GTPγS Bound	-	[35S]GTPγS Bound	CHO cells	Cussac et al, 2008
5-HT	Agonist	Ca mobilization	-	Fluo-3	CHO cells	Cussac et al, 2008
6-F-DET	Antagonist	No detectable change	25% at 100uM	Phosphoinositide hydrolysis	PC12	Rabin et al, 2002
Apomorphine	Antagonist	no change	0%	Depletion of [3H]PI	CHO cells	Newman-Tancredi et al, 2002
BOL-148	Antagonist	No detectable change	0%	Phosphoinositide hydrolysis	PC12	Rabin et al, 2002

Bromocriptine	Partial agonist		?	Depletion of [3H]PI	SH-SY5Y cells	Mitchell et al, 1998
Bromocriptine	Partial agonist	Decreased [3H] PI	69%	Depletion of [3H]PI	CHO cells	Newman-Tancredi et al, 2002
Bromocriptine	Partial agonist	More [35S]GTPγS Bound	85.1%	[35S]GTPγS Bound	CHO cells	Cussac et al, 2008
Bromocriptine	Partial agonist	Ca mobilization	61.4%	Fluo-3	CHO cells	Cussac et al, 2008
Bromocriptine	Partial agonist	Decreased [3H] PI	79%	Depletion of [3H]PI	CHO cells	Newman-Tancredi et al, 2002
Cabergoline	Agonist	Decreased [3H] PI	94%	Depletion of [3H]PI	CHO cells	Newman-Tancredi et al, 2002
Clozapine	Agonist	5-HT2A internalization		Immunofluorescence	NIH3T3 cells	Willins et al, 1998
Clozapine	Agonist	5-HT2A internalization		Biotin-trap internalization of receptors	NIH3T3 cells	Willins et al, 1998
Clozapine		5-HT2A internalization		Immunofluorescence	Rat cortex	Willins et al, 1998
Clozapine		5-HT2A internalization		Immunofluorescence	Rat (Sprague Dawley) cortex	Willins et al, 1999
Clozapine	Antagonist	no ERK activation	0%	?	Mouse embryonic fibroblasts (MEFs)	unpublished data mentioned in Schmid et al, 2007

Clozapine	Antagonist	no ERK activation	0%	?	Mouse frontal cortical tissue 15mins after injection	unpublished data mentioned in Schmid et al, 2007
DMT	Agonist	[3H] inositol monophosphate formation	90%	3H] inositol phosphate formation	NIH3T3 cells	Smith et al, 1998
DMT	Antagonist?	does not cause 5-HT2A internalization	N/A	5-HT2A internalization	NIH3T3 cells	Smith et al, 1998
DMT	Partial Agonist	PI Hydrolysis	20.4%	Phosphoinositide hydrolysis	PC12	Rabin et al, 2002
DOB	Partial agonist	Calcium release	74%	FLIPR assay	CHO cells	Porter et al, 1999
DOI	Agonist	Head Twitch Response	100%	Head Twitch Response	in vivo mice	Schmid et al, 2007
DOI	Antagonist?	No internalization of 5-HT2A	0%	beta-arrestin1 and beta-arrestin2 KO cultures compared to WT; media with serotonin caused internalization in WT (vs surface expression with no serotonin in media), no change in beta-arrestin lines/all 5-HT2A is on cell surface DOI is internalized despite beta-arrestin or not	Mouse embryonic fibroblasts (MEFs)	Schmid et al, 2007

DOI	Partial Agonist	ERK1/2 phosphorylation (activation)	24%	Western blot	Mouse embryonic fibroblasts (MEFs)	Schmid et al, 2007
DOI	Agonist	ERK1/2 phosphorylation (activation)	100%	Western blot	Mouse frontal cortical tissue 15mins after injection	Schmid et al, 2007
DOI	Agonist	More [35S]GTPγS Bound	93.6%	[35S]GTPγS Bound	CHO cells	Cussac et al, 2008
DOI	Partial agonist	Ca mobilization	81.3%	Fluo-3	CHO cells	Cussac et al, 2008
DOI	Agonist	Internalization	74%	[3H]ketanserin binding after drug treatment	Rat brain	Buckholtz et al, 1988
DOI	Partial agonist	Calcium release	61%	FLIPR assay	CHO cells	Porter et al, 1999
DOI	Partial agonist	activity	1%	patch spontaneous EPSCs (produces 10% EPSCs compared to 5HT)	cortical neurons	Aghajanian and Marek, 1999
DOM	Partial Agonist	PI Hydrolysis	77.4%		PC12 cells	Rabin et al, 2002
DOM	Agonist	PI Hydrolysis	almost 100%	[3H]Arachidonate release	PC12 cells	Rabin et al, 2002
DOM	Partial Agonist	IP Stimulation	88%	IP Receptor stimulation	NIH 3T3 cells	Egan et al, 1998

Ketanserin	Antagonist	Internalization	0%	Immunofluorescence	HEK cells	Raote et al, 2013
Ketanserin	Antagonist	Internalization	0%	Immunofluorescence	GF-62 cells	Berry et al, 1996
Ketanserin	Antagonist	PI Hydrolysis	0% "not change IP accumulation from basal"	[3H]-IP accumulation	C6 glioma cells	Sullivan Hanley and Hensler, 2002
Lisuride	Partial Agonist	Decreased [3H] PI	75%	Depletion of [3H]PI	CHO cells	Newman-Tancredi et al, 2002
Lisuride	Partial Agonist	IP Stimulation	25%	IP Receptor stimulation	NIH 3T3 cells	Egan et al, 1998
Lisuride	Partial agonist	More [35S]GTPγS Bound	40.7%	[35S]GTPγS Bound	CHO cells	Cussac et al, 2008
Lisuride	Partial agonist	Ca mobilization	48.6%	Fluo-3	CHO cells	Cussac et al, 2008
Lisuride	Partial agonist	PI Hydrolysis	15.6%	Phosphoinositide hydrolysis	PC12 cells	Rabin et al, 2002
(R)-Lisuride	Partial agonist	Calcium release	14%	FLIPR assay	CHO cells	Porter et al, 1999
(S)-Lisuride	Partial agonist	Calcium release	20%	FLIPR assay	CHO cells	Porter et al, 1999
LSD	Partial agonist	PI Hydrolysis	32.3%	Phosphoinositide hydrolysis	PC12 cells	Rabin et al, 2002

LSD	Partial agonist	IP Stimulation	32%	IP Receptor stimulation	NIH 3T3 cells	Egan et al, 1998
LSD	Partial agonist	More [35S]GTPγS Bound	71.2%	[35S]GTPγS Bound	CHO cells	Cussac et al, 2008
LSD	Partial agonist	Ca mobilization	84.6%	Fluo-3	CHO cells	Cussac et al, 2008
LSD	Agonist	Internalization	83%	[^{3}H]ketanserin binding after drug treatment	Rat brain	Buckholtz et al, 1988
LSD	Partial agonist	Increased PI Hydrolysis	15%	IP Accumulation	CHO cells	Berg et al, 1998
LSD	Partial agonist	AA Accumulation	30%	[14C]arachidonic acid production	CHO cells	Berg et al, 1998
LSD	Partial agonist	Calcium release	44%	FLIPR assay	CHO cells	Porter et al, 1999
MDL 100,907	Antagonist	5-HT2A internalization				Willins et al, 1999
MDL 100,907	Antagonist	No IP accumulation	0%	IP accumulation	NIH 3T3 cells	Kehne et al, 1996
Mianserin	Antagonist	5-HT2A internalization		Immunofluorescence	NIH3T3	Willins et al, 1999
Olanzapine	Antagonist	5-HT2A internalization		Immunofluorescence	NIH3T3	Willins et al, 1999
Olanzapine	Antagonist	5-HT2A internalization		Immunofluorescence	Rat (Sprague Dawley) cortex	Willins et al, 1999

Pergolide	Agonist	Decreased [3H] PI	87%	Depletion of [3H]PI	CHO cells	Newman-Tancredi et al, 2002
Pergolide	Agonist	More [35S]GTPγS Bound	91.9%	[35S]GTPγS Bound	CHO cells	Cussac et al, 2008
Pergolide	Partial agonist	Ca mobilization	75.6%	Fluo-3	CHO cells	Cussac et al, 2008
Psilocybin	Partial Agonist	PI Hydrolysis	33.8%	Phosphoinositide hydrolysis	PC12	Rabin et al, 2002
Ritanserin	Antagonist	5-HT2A internalization		Immunofluorescence		Willins et al, 1999
Roxindole	Antagonist	no change	0%	Depletion of [3H]PI	CHO cells	Newman-Tancredi et al, 2002

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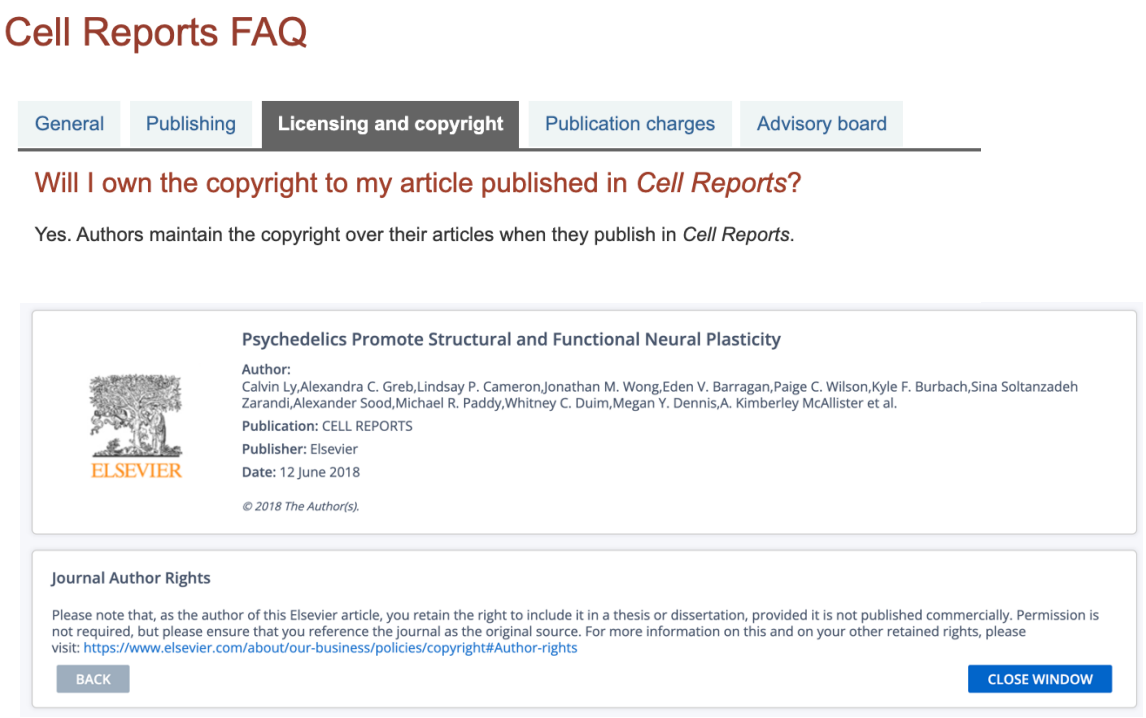

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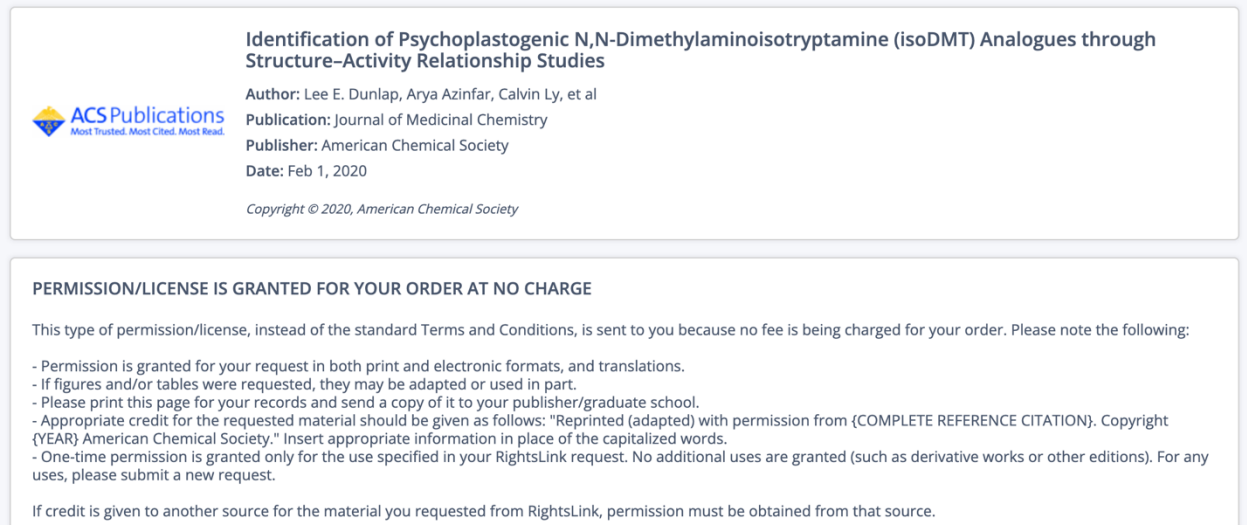

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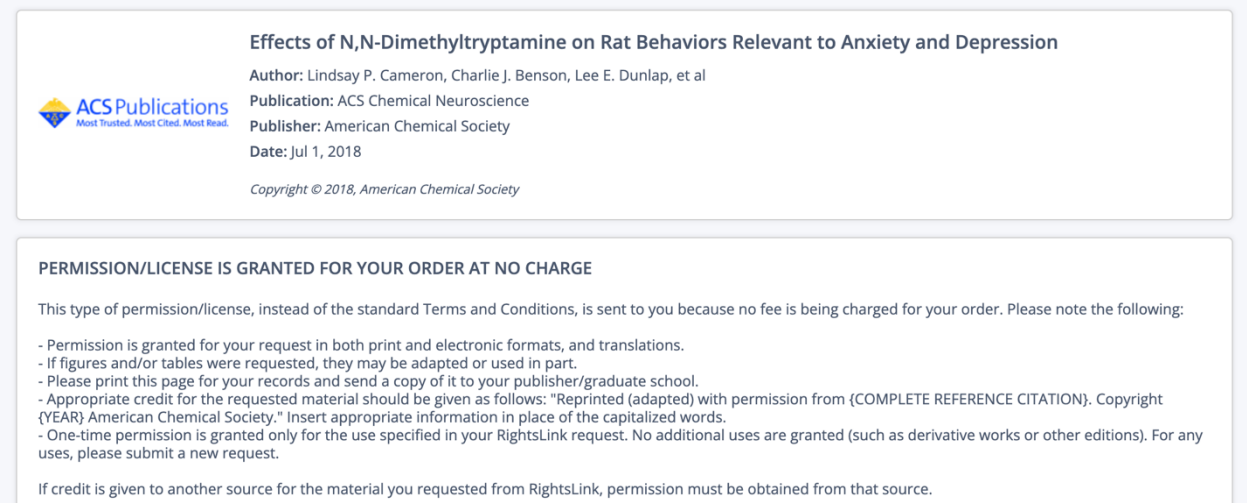

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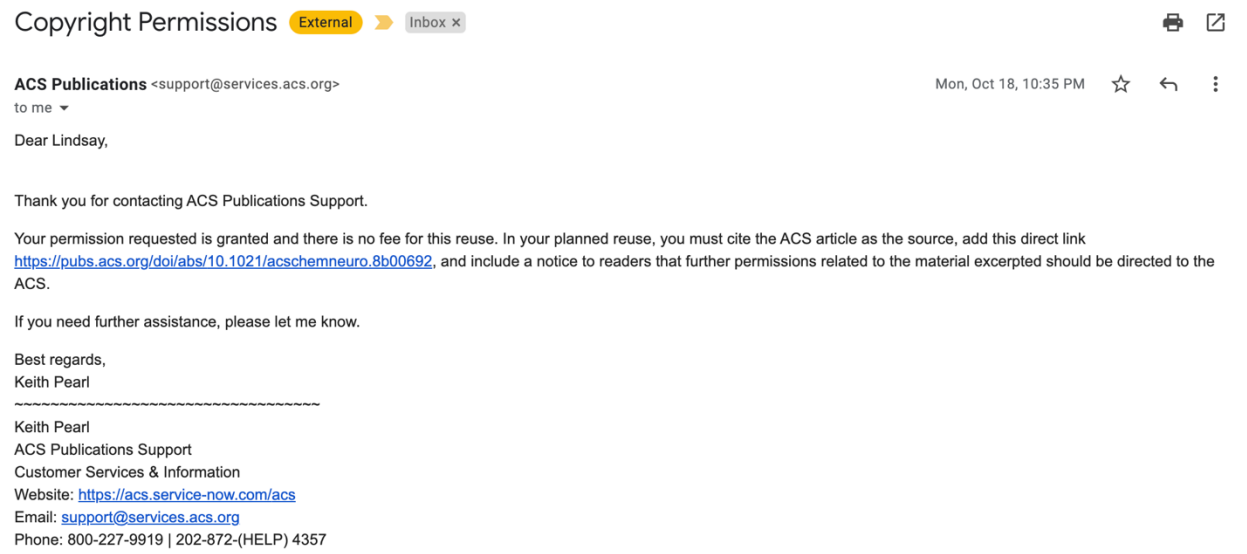

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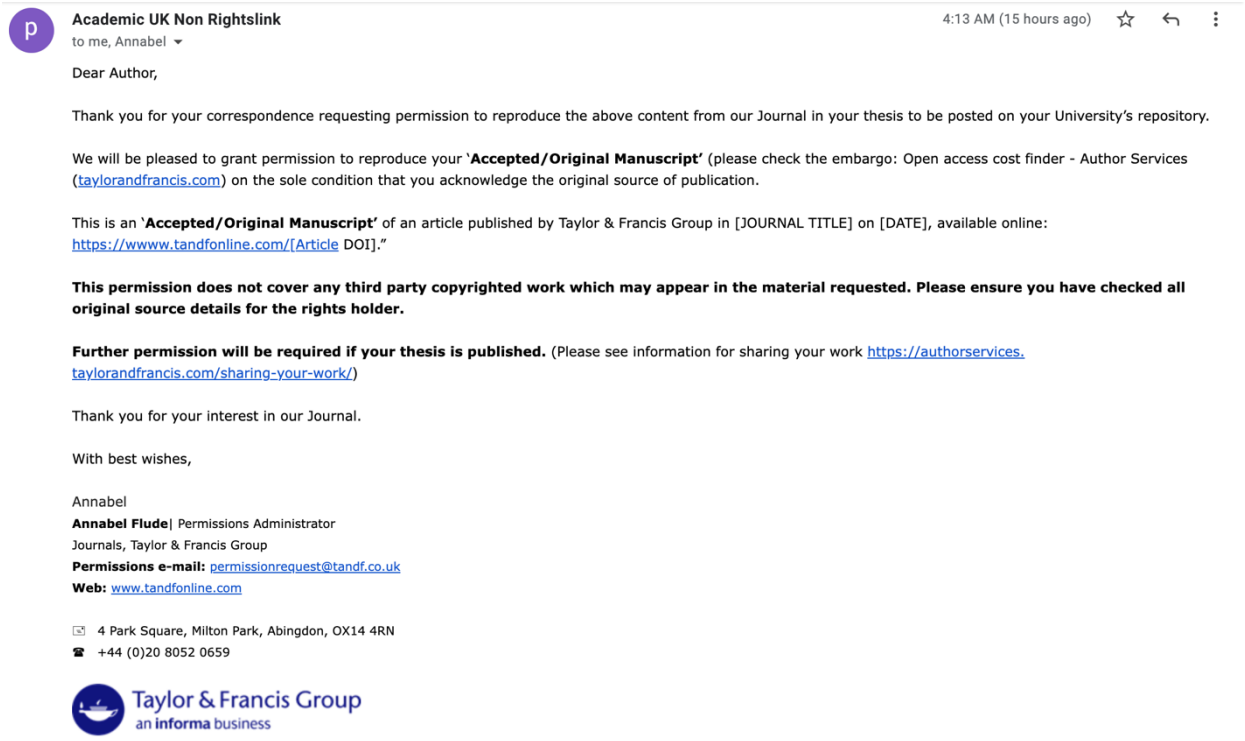

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