Imaging the effects of serotonin 2A receptor modulation on synaptic density in treatment-resistant depression (SYNVEST)

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Principal Investigator: Dr. Ishrat Husain

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Sponsor:	Centre for Addiction and Mental Health Centre for Complex Interventions 1025 Queen Street West, Toronto, Ontario M6J 1H4 416-535-8501 ext. 39412
Principal Investigator:	Dr. Ishrat Husain MBBS, MD (Res.), MRCPsych Centre for Addiction and Mental Health (CAMH) 100 Stokes Street, Room 4285 Toronto, Ontario M6J1H4 416-535-8501 ext. 37838
Funder:	University of Toronto, Department of Psychiatry 250 College Street, 8th Floor Toronto, Ontario M5T1R8 416-970-6948

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STATEMENT OF COMPLIANCE

This clinical trial will be carried out in accordance with the following:

- International Conference on Harmonisation Good Clinical Practice (ICH GCP)
- Tri-Council Policy Statement 2018 (TCPS 2)
- ISO 14155:2020 for Medical Device Clinical Trials
- Personal Health Information Protection Act (PHIPA), 2004; Chapter 3 Schedule A (PHIPA) and applicable regulations
- Food and Drugs Act
  - Part C, Division 5 of the Food and Drug Regulations
- Institutional and REB policies and procedures

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Signature of PI Date

LIST OF ABBREVIATIONS

AE	*Adverse Event*
ALT	*Alanine aminotransferase*
AST	*Aspartate aminotransferase*
ATHF	*Antidepressant Treatment History Form*
CAMH	*Center for Addiction and Mental Health*
CGI	*Clinical Global Impression Scale*
CRF	*Case report form(s)*
C-SSRS	*Columbia-Suicide Severity Rating Scale*
DMT	*5-Hydroxytryptamine*
ECG	*Electrocardiography*
ECT	*Electroconvulsive therapy*
EDI	*Equity, diversity, and inclusion*
GAD-7	*Generalized Anxiety Disorder 7-Item Scale*
HamD-17	*Hamilton Depression Rating Scale*
HDPE	*High-density polyethylene*
HIPPD	*Hallucinogen-induced persistent perceptual disorder*
HPMC	*Hydroxypropyl methyl cellulose*
GCP	*Good Clinical Practice*
ICF	*Informed consent form*
IP	*Investigational product*
IPAC	*Infection prevention and control*
LSD	*Lysergic acid diethylamide*

MADRS	*Mongomery-Åsberg Depression Rating Scale*
MDD	*Major depressive disorder*
MDE	*Major depressive episode*
PAP	*Psilocybin-Assisted Psychotherapy*
PHI	*Personal Health Information*
PHIPA	*Personal Health Information Protection Act*
PI	*Principal Investigator*
QI	*Qualified Investigator*
RCT	*Randomized controlled trial*
REB	*Research ethics board*
SAE	*Serious Adverse Event*
SCID-5	*Structured Clinical Interview for DSM-5*
SETS	*Stanford Expectations of Treatment Scale*
SHAPS	*Snaith-Hamilton Pleasure Scale*
SSRI	*Selective Serotonin Reuptake Inhibitor*
SUSAR	*Suspected unexpected serious adverse reaction*
TASS	*Transcranial Magnetic Stimulation Adult Safety Screen*
TCPS 2	*Tri-Council Policy Statement*
TRD	*Treatment Resistant Depression*
WEMWS	*Warwick-Edinburgh Mental Wellbeing Scale*
WHO-QoL-BREF	*World Health Organization Quality of Life Questionnaire – Brief Version*
5D-ASC	*Five Dimensions of Altered States of Consciousness*

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CLINICAL TRIAL SUMMARY

Title	Imaging the effects of serotonin 2A receptor modulation on synaptic density in treatment-resistant depression (SYNVEST)
Short Title	SYNVEST
Phase	Phase I
Methodology	Open-label clinical trial
Clinical trial Duration	18-months to complete all recruitment, study procedures, and data analysis.
Participating site(s)	Single-Centre

Objectives	To evaluate the feasibility and safety of obtaining PET imaging scans before and after administration of psilocybin (25 mg) with and without risperidone (1 mg) in adults with TRD. To obtain preliminary data on synaptic density (as measured by [^{18}F] SynVesT-1 volume distribution, V_T) in brain regions relevant to MDD (i.e., prefrontal cortex) before and 4 weeks after psilocybin 25 mg vs psilocybin 25 mg plus risperidone 1 mg in adults with TRD. To obtain preliminary data on cortical plasticity, as measured by TMS-EEG and resting EEG in brain regions relevant to MDD (i.e., prefrontal cortex) before, during and after psilocybin 25 mg vs psilocybin 25 mg plus risperidone 1 mg in adults with TRD. To obtain preliminary data on antidepressant effects as measured by the change in the MADRS from Baseline (V2) to 1-week post-treatment (V5). Antidepressant effects will also be measured at Weeks 2 (V6), 3 (V7), and 4 (V8) post-intervention. Response will be defined as a reduction of 50% or more of the MADRS score and remission defined as a score of <7 on the MADRS from Baseline (V1) to Week 1 (V5).
Number of Participants	Twelve participants diagnosed with treatment-resistant depression
Study Intervention Reference Therapy/Comparator	1 mg of risperidone in combination with 25mg of psilocybin taken in conjunction with psilocybin-assisted psychotherapy (PAP) or 25 mg of psilocybin alone taken in conjunction with PAP
Duration of Intervention	One day: 5-6 hours

Statistical Methodology	Characteristics of the trial cohort will be summarized by mean (SD), median (minimum, maximum). Summary raw scores will be presented at each assessment time both numerically and graphically. The small sample size means that conservative nonparametric testing is required in order to address the primary and secondary objectives. Exact paired permutation t-tests will be used to determine whether psilocybin-assisted psychotherapy with or without risperidone achieves a 50% reduction in MADRS.

1.0 INTRODUCTION

1.1 Background

Treatment-Resistant Depression (TRD) affects up to a third of all depressed individuals (Wiles et al., 2013). Major depressive disorder (MDD) is a leading cause of disability worldwide. Although depressive symptoms are amenable to pharmacotherapy, a high proportion of patients experience TRD, typically defined as not responding to two or more adequate antidepressant trials, or relapse during treatment (Gaynes et al., 2020; Rush et al., 2006). TRD is associated with a significant decline in social and occupational functioning and higher rates of death by suicide and all-cause mortality (Reutfors et al., 2018). Persistent symptoms in TRD often translate into substantial increases in work loss, healthcare resource utilization, and costs, compared to more responsive forms of illness (Li et al., 2020). Current pharmacotherapy for TRD, including augmentation of antidepressants with atypical antipsychotics, lithium, or ketamine have high rates of non-response (Carter et al., 2020) and can be associated with problematic adverse effects (e.g., sedation, weight gain, diabetes, tardive dyskinesia) leading to non-adherence (Ho et al., 2017). Electroconvulsive therapy (ECT) is the most efficacious intervention for TRD. However, many patients with TRD refuse ECT because of stigma, lack of access, and fears of cognitive adverse effects (Wilkinson et al., 2021). Transcranial magnetic stimulation (rTMS) is an alternative to ECT but its response and remission rates are similar to pharmacotherapy (Carter et al., 2020), leaving a large proportion of patients with TRD in need of novel interventions.

Effects of Psilocybin on Synaptic Density

Some evidence suggests that psychedelic drugs such as psilocybin promote growth and plasticity of synapses in the brain. These changes in neuronal connections are a potential mechanism for the clinical efficacy of psilocybin in treating major depressive disorder (MDD). Recent advances have allowed quantitative imaging of synaptic density in the living human brain to be performed for the first time using positron emission tomography (PET) with radiotracers selective for the synaptic vesicle glycoprotein 2A (SV2A). Using SV2A PET, synaptic density changes have been identified across a range of neurodegenerative and psychiatric disorders including MDD. This method may offer a way to measure synaptic density changes associated with treatment directly in patients. The goal of this study is to assess synaptic density before and after treatment with psilocybin in patients with treatment-resistant depression (TRD) in order to determine whether psilocybin induces measurable synapse (re)growth.

1.2 Study Intervention

Recently, psilocybin-assisted psychotherapy (PAP) has been gaining traction as a promising potential treatment for many mental illnesses, including end-of-life anxiety and treatment-resistant depression (Perkins et al., 2021). PAP procedures typically involve psychological preparation prior to therapist-supported psilocybin dosing sessions. These sessions are used to establish a therapeutic relationship, inform participants about what to expect, and set expectations for the dosing session. During the psilocybin dosing session, trained therapists support the individual through their experience and psychological integration therapy occurs after the dosing experience. Evidence from recent clinical trials suggest that PAP can help in the reduction of anxiety, depression, and substance use (Carhart-Harris et al., 2021; Griffiths et al., 2016; Grob et al., 2011; Ross et al., 2016). With proper screening and preparation, psilocybin has a safe physiological and psychological profile.

Psilocybin is a chemical compound that naturally occurs in certain species of mushrooms, (for example, in the psilocybe genus, among others). It belongs to a class of drugs referred to as 'psychedelics'. Psilocybin is a tryptamine which is chemically similar to the neurotransmitter, serotonin, and the essential amino acid, tryptophan. It is considered a 5-hydroxytrptamineric (serotonergic) psychedelic along with other similar drugs such as dimethyltryptamine (DMT) and lysergic acid dieythamide (LSD). Psilocybin is a prodrug for the pharmacologically active ingredient psilocin, which readily crosses the blood-brain barrier and acts as a potential partial agonist at serotonin $5HT_{2A}$ and $5HT_{2c}$ receptors in the brain (Halberstadt et al., 2011; Madsen et al., 2019). Typical effects of psilocybin include significantly altered states of consciousness, experienced through visual and sometimes auditory effects, changes in perception, distortions of time, and a range of effects including a sense of awe, novel perspectives, existential and personal insight, dramatically heightened empathy and feelings of compassion, strong emotions, and unitive experience. These mystical experiences are correlated with improvements in mood in healthy volunteers and palliative patients with end-of-life distress (Johnson et al., 2019). However, there is no convincing evidence that these psychedelic effects are required for an antidepressant effect in patients with MDD (Johnson et al., 2019). In fact, in a recent study, 24 participants who received PAP for MDD, showed no associations between psychedelic effects and sustained improvement in depressive symptoms, suggesting that psychedelic effects may not be necessary to harness psilocybin's antidepressant effects (Gukasyan et al., 2022).

In its current form, psilocybin is not suitable for wide scale clinical adoption. Despite published safety data, psilocybin-induced alterations in perception and consciousness remain a major barrier to widespread clinical implementation, necessitating an 8-hour session of intensive psychological support in a clinical setting during administration, in addition to several lengthy preparation and integration sessions pre- and post-dosing. This complex process could be greatly simplified if the psychedelic effects could be diminished without impairing the antidepressant effects. In humans, the intensity of psilocybin-induced perceptual changes correlates with 5-HT2A activation (Madsen et al., 1995). However,

psilocybin's antidepressant effects may be mediated through rapid activation of other critical 5-HT receptors. Two 5-HT2A-receptor antagonists – risperidone (an atypical antipsychotic) and ketanserin (an antihypertensive) – eliminate self-reported psilocybin psychedelic effects in humans (Vollenweider et al., 1998). Thus, this intervention will involve the administration of risperidone followed by PAP to determine if the psychedelic experience of psilocybin is necessary to produce antidepressant effects.

Psilocybin effects on synaptic plasticity

Like many other antidepressants, psilocybin is theorized to exert its therapeutic effects by stimulating synaptic plasticity in brain areas relevant to MDD including prefrontal cortex and hippocampus (Duman et al., 2016). Preclinical and *in vitro* evidence demonstrates that psilocybin and other psychedelic drugs induce increased synaptogenesis and growth of dendritic spines in neurons within the prefrontal cortex (de la Fuente Revenga et al., 2021; Ly et al., 2018; Shao et al., 2021) as well as neurogenesis within the hippocampus (Catlow et al., 2013; Lima da Cruz et al., 2018; Morales-Garcia et al., 2020). However, to date direct evidence of this mechanism in humans is limited and challenging to come by.

Our understanding of the neuroplastic effects of psilocybin in humans and their potential role in therapeutic efficacy remains incomplete. **To date, no study has evaluated psilocybin effects on neuronal growth in humans and no study has evaluated psilocybin effects on synaptic density *in vivo* in any species.**

1.3 Preclinical Data to Date

Animal studies looking into the effects of psychedelics in rodents have found evidence for improvements in behavioral outcomes, assessed by measures of coping strategy and cognitive function. The long-term effects using LSD (Buchborn et al., 2014; Hibicke et al., 2020), psilocin (Horsley et al., 2018), psilocybin (Hibicke et al., 2020), and DMT (Cameron et al., 2019; Cameron et al., 2018) were comparable to traditional treatment antidepressants. Moreover, there is evidence suggesting improvements in dimensions of cognitive function, such as enhanced associative learning (Buchborn et al., 2014; Harvey, 2003) which are commonly impaired in major depressive disorder (Castaneda et al., 2008). In vitro studies with rat brain preparations have demonstrated that risperidone has a 10-20 fold greater affinity for the 5-HT_{2A} receptor than for the D_2 receptor (Meltzer et al., 1989). Similarly, psilocybin also has a high affinity for the 5-HT_{2A} receptor. The intensity of psilocybin-induced perceptual changes is correlated with serotonin 2A receptor (5-HT2AR) activation (Madsen et al., 2019). In a study involving 8-week old mice exposed to a chronic multimodal stress paradigm, hedonic state was assayed with an appetize choice task: a two-bottle sucrose preference test comparing

consumption of a 1% sucrose solution and water (Hesselgrave et al., 2021). Psilocybin injections restored preference for sucrose solution whereas mice given a saline injection retained low sucrose (Hesselgrave et al., 2021). In the second part of the study, researchers pre-treated the mice with a 5-HT2A/2C antagonist prior to the psilocybin or saline injection. The results indicated that the behavioural responses to the sucrose test were not prevented when the antagonist was administered (Hesselgrave et al., 2021). These results indicate that psilocybin's antidepressant mechanism of action may not be dependent on the psychedelic effects (Hesselgrave et al., 2021).

Psilocybin Effects on Synaptic Plasticity

In preclinical models, psilocybin treatment has been shown to increase a range of markers of neuronal plasticity. In mice, a single dose of psilocybin induced rapid changes in gene transcription in the prefrontal cortex, activating pathways involved in cognition, neural growth, and plasticity (Fadahunsi et al., 2022). Similarly, in rats, a single dose of psilocybin increased expression of neuroplasticity-associated genes and proteins in prefrontal cortex and hippocampus (Jefsen et al., 2021). Another recent study examined the effect of psilocybin on synaptic protein density post mortem in pigs using autoradiography with [^{3}H]UCB-J, a radioligand selective for the ubiquitous synaptic vesicle protein SV2A (Raval et al., 2021). This work found increased SV2A binding (synaptic density) 7 days after psilocybin administration in prefrontal cortex (+6.1%) and hippocampus (+9.2%).

Role of 5-HT2A activation

The intensity of psilocybin-induced perceptual changes (psychedelic effects) correlates with 5-HT2A activation (Madsen et al., 1995), but there is limited evidence that psychedelic effects are required for an antidepressant effect in patients with MDD (Johnson et al., 2019). Similarly, it is not yet clear whether 5-HT2A activation is necessary for the synaptogenic effects of psychedelic drugs. Psilocybin's antidepressant effects may be mediated through other pathways, such as rapid activation of other critical 5-HT receptors. Specific blockade of 5-HT2A receptors with drugs such as risperidone and ketanserin can reduce expression of neurotrophic proteins and neurogenesis on their own in rodents (Catlow et al., 2013; Jiang et al., 2016; Vaidya et al., 1997). However, studies examining effects of 5-HT2A blockade on psilocybin-induced plasticity have produced mixed results (Ly et al., 2018; Shao et al., 2021). Presynaptic effects in particular have not been thoroughly investigated, though a recent study in mice found no effect of 5-HT2A pre-block on the synaptic strengthening effects of psilocybin (Hesselgrave et al., 2021). Together this suggests that psilocybin may activate brain plasticity in part through 5-HT2A-independent mechanisms.

PET Imagining of Synaptic Density

Studies in preclinical models have demonstrated sensitivity of SV2A PET to treatment-related changes in synaptic density, notably in mouse models of Alzheimer's disease (Spurrier et al., 2022; Toyonaga et al., 2019). In humans, one pilot study found that in patients with MDD who showed pre-treatment synaptic density deficits, SV2A PET binding was significantly increased following a subanesthetic dose of ketamine, another drug through to exert antidepressant effects in part by stimulating synaptic plasticity. Further, this increase was associated with symptom improvement (Holmes et al., 2022). This provides highly preliminary but intriguing evidence that SV2A PET may provide a clinically-relevant measure of the neuroplastic effects of novel antidepressants. Notably, that pilot study used a single dose of ketamine rather than a typical multi-dose regimen used in treatment of MDD, and so may have underestimated the relevant effect.

1.4 Clinical Data to Date

To date, there are numerous studies supporting the clinical efficacy of PAP for the treatment of depression and end-of-life anxiety (Carhart-Harris et al., 2021; Griffiths et al., 2016; Ross et al., 2016). Currently, there are over 60 studies registered on ClinicalTrials.gov exploring the use of psilocybin to treat various mental health disorders. Several trials have observed rapid and sustained improvements in patients with treatment-resistant depression with one RCT demonstrating similar efficacy between psilocybin compared with an SSRI. Interestingly, secondary outcomes favoured psilocybin (Carhart-Harris et al., 2021). In particular, psilocybin, the chemical component of "magic mushrooms" at doses of 20-30 mg, in conjunction with supportive psychotherapy, has shown large and sustained antidepressant effects in patients with MDD and TRD in contemporary open-label and randomized clinical trials (RCTs) (Carhart-Harris et al., 2021; Carhart-Harris et al., 2016; Davis et al., 2021). For instance, in an open-label trial of psilocybin-assisted psychotherapy (PAP), 63% of 19 participants with TRD responded 1 week after treatment, and 32% were not on any antidepressant or therapy for a further year (Carhart-Harris et al., 2016). Similarly, an RCT of 24 participants with MDD showed large effect sizes for PAP at week 1 (Cohen d = 2.5) and week 4 (Cohen d = 2.6) post-treatment compared with waitlist control (Davis et al., 2021). A long-term follow-up study of the same participants showed response and remission rates of 75% and 58%, respectively, at 12 months (Gukasyan et al., 2022). More recently, a trial comparing PAP with escitalopram in 59 participants with non-refractory MDD showed that PAP was as effective as escitalopram in reducing depressive symptoms with no differences in adverse effects between groups (Carhart-Harris et al., 2016). Recently, an international phase II RCT of PAP for 233 patients with TRD was completed. Preliminary findings indicate that psilocybin 25 mg led to a significant reduction in scores on the Montgomery-Abserg Depression Rating Scale (MADRS) for at least 6 weeks post-treatment, compared to psilocybin 1 mg (an active placebo), without significant differences in serious adverse events between groups (COMPASS, 2021).

Psilocybin Effects on Synaptic Plasticity

Neuroimaging studies in humans using functional magnetic resonance imaging (fMRI) offer indirect support for an effect of psilocybin to promote brain plasticity. Whole-brain functional connectivity between brain regions is persistently increased following a single psilocybin dose in healthy humans (Barrett et al., 2020). Another study found that functional connectivity changes are related to symptom improvement post-psilocybin in TRD (Carhart-Harris et al., 2017). Because synaptic deficits are directly associated with functional connectivity deficits in depression (Holmes et al., 2019), synaptic density increases may be a correlate of this widespread increase in brain connectivity

PET imaging of synaptic density

PET imaging using SV2A-selective radiotracers [^{11}C]UCB-J and [^{18}F]SynVesT-1 (also known as [^{18}F]SynVesT-1) has recently been developed as a method to measure synaptic vesicle density *in vivo* in the human brain (Finnema et al., 2016). SV2A is a vesicle membrane protein expressed at constant levels in virtually all synapses. SV2A PET binding has been validated as a stable measure of synaptic density (Smart et al., 2021). In non-human primates, *in vivo* SV2A binding measures derived from PET correlate closely with *ex vivo* protein density of SV2A and synaptophysin (Finnema et al., 2016; Smart et al., 2021). A growing body of clinical literature has demonstrated that SV2A PET measures are sensitive to synapse loss in neuropsychiatric disorders, including Alzheimer's disease, epilepsy, schizophrenia, cannabis use disorder, and post-traumatic stress disorder (Angarita et al., 2021; Chen et al., 2018; D'Souza et al., 2021; Finnema et al., 2020; Holmes et al., 2019; Matuskey et al., 2020; Onwordi et al., 2021). Most notably for the present work, one SV2A PET imaging study found that lower synaptic density in several brain areas implicated in mood, including dorsolateral prefrontal cortex, anterior cingulate cortex, and hippocampus, is associated worse depressive symptoms in unmedicated patients (Holmes et al., 2019). Each of these brain regions has been previously identified as a site of synaptic, structural, or functional changes following psilocybin treatment (Fadahunsi et al., 2022; Jefsen et al., 2021; Shao et al., 2021).

We propose a proof-of-concept open-label clinical trial to evaluate the feasibility and safety of obtaining PET imaging scans before and after administration of psilocybin (25mg) with and without risperidone (1 mg) in adults with TRD.
In this study, 12 participants with TRD will receive 12 hours of manualized psychotherapy that has previously been used with psilocybin (Guss et al., 2020). They will also receive either psilocybin 25 mg alone or psilocybin 25 mg plus risperidone 1 mg. We are only recruiting patients with TRD, as psilocybin has not demonstrated superiority over first-line antidepressants for non-refractory MDD (Carhart-Harris et al., 2021). We are using the atypical antipsychotic risperidone,

a potent blocker of the 5-HT2A receptor, because a previous study in healthy volunteers has demonstrated that risperidone 1 mg effectively blocks psilocybin's psychedelic effects (Vollenweider et al., 1998). Although risperidone also has actions on dopaminergic receptors, psilocybin has no direct dopaminergic activity (Creese et al., 1975), and psilocybin's psychedelic effects are not blocked by traditional D2-receptor antagonists like haloperidol (Vollenweider et al., 1998). In summary, if the proposed proof-of-concept study indicates that psilocybin's psychedelic effects may not be required for its antidepressant effects, and if this is confirmed in a future RCT, the use of psilocybin could be standardized and safely expanded without the need for intensive psychotherapy, providing a scalable, novel rapid-acting intervention for TRD.

Additionally, this study will include neurophysiological assessments to help to elucidate the biological mechanisms of psilocybin for TRD. Evidence suggests that prefrontal γ-aminobutyric acid (GABA)ergic inhibitory and glutamatergic excitatory neurotransmission may represent important neurophysiological targets in therapeutic interventions for TRD (Levinson 2010, D Voineskos 2018, 2021) These forms of neurotransmission are effectively indexed via TMS-EEG, with the process being refined over the past decade (Tremblay et al Clin Neurophysiol 2019, Chung, Rogasch et al, Brain Stimulation 2015). Evidence of alterations in glutamatergic and GABAergic neurotransmission, measured via TMS-EEG, has been repeatedly demonstrated after rTMS in TRD populations (Voineskos et al Clinical Neurophysiol 2021, Strafella et al, Biological Psychiatry (accepted) 2023). While psilocin, the active metabolite of psilocybin, appears to have its main actions on 5HT receptors, it is known that both GABAergic inhibitory interneurons and glutamatergic pyramidal neurons, contain multiple 5HT receptors (Smausz et al, Journal of Psychopharmacology 2022). However, the direct effects of psilocybin have not yet been elucidated via TMS-EEG. To our knowledge, this would be the first such investigation into the potential neurophysiological impact of psilocybin in a TRD population.

An understanding of the mechanisms of psilocybin would facilitate our understanding of the biological models of TRD, and the method of action of this novel therapeutic approach.

1.5 Risks/Benefits

Possible Benefits

As with any research study, no direct benefit can be promised to research participants. Clinical trials investigating psilocybin-assisted psychotherapy in depression cohorts have indicated rapid and dramatic reductions in participants' symptoms. Therefore, participants may receive some benefit from the study if PAP plus risperidone is effective in improving depressive symptoms. Participants may also benefit from close monitoring of their clinical conditions.

Psilocybin & Risperidone Risks

Expert consensus indicates that psilocybin is safe in human pre-clinical and clinical trial research (Johnson et al., 2008). In addition, in larger RCTs in patients with TRD, risperidone 0.5 – 3.0 mg daily has been safe for use (Cantù et al., 2021). Thus, we expect that a single low dose (1 mg) of risperidone to have minimal adverse effects. Risperidone administered alone has common side effects (>10%) of drowsiness, headache, tremors, twitching or uncontrollable muscle movements, dizziness, nausea, and/or dry mouth. It is possible that participants may experience these side effects but this is unlikely with a single 1 mg dose. Previous studies in healthy volunteers indicate that risperidone, a potent blocker of the 5-HT2A receptor, can effectively blocks psilocybin's psychedelic effects (Vollenweider et al., 1998). This study also demonstrated that the combination of risperidone 1 mg and psilocybin 25 mg is safe to use in healthy volunteers (Vollenweider et al., 1998). Psilocybin given at a dose of 25mg is expected to alter mood, cognition, and perception. Common psychological and adverse effects of psilocybin include transient anxiety, changes in thought form or thought speed (slowing down or speeding up of thought processes), depersonalization, derealization, inattention, impaired concentration, labile mood, altered perception of time, altered visual perception, mild paranoid ideas and unusual thoughts. Previous studies indicate that these events are transient, tolerable, and largely resolved within the timeframe of the 5-6-hour PAP session (Griffiths et al., 2016; Grob et al., 2011; Ross et al., 2016). In addition, these effects are both expected component of the therapeutic response and it is anticipated that these effects will be diminished when given with risperidone. Psilocybin can produce sympathetic system activation resulting in physiological effects such as pupillary dilation and detectable, but moderate increases in blood pressure or heart rate, transient nausea, diarrhea, paresthesia, dizziness, fatigue and headache. In rare cases, hallucinogen-induced persistent perceptual disorder (HlPPD) where individuals experience the effects of psilocybin for longer than expected. These have not been any reported cases of this in modern clinical settings, but it has been rarely reported following recreational use. With proper screening and preparation, psilocybin has a safe physiological and psychological profile. As with any investigational product trial, there is a possibility that some participants may experience a worsening of their mental state after the drug experience. Reports of this are very rare and have not been seen in other similar studies. Published findings on harm profiles associated with drugs most commonly used in the UK and Australia consistently rate 'magic mushrooms' as being one of the least harmful substances to one's self and to society (Nutt et al., 2010). In order to mitigate risks, a preparatory therapy session will be scheduled with each participant to prepare them for what to expect during the experience.

Medication Tapering Risks

There will be a washout period of a minimum of 2 weeks (4 weeks for fluoxetine) for participants taking any concomitant medications. Withdrawing from medications may result in difficulty sleeping, nausea, diarrhea, flu-like symptoms, and jitters. These symptoms are not dangerous and usually pass in a few days. In addition, tapering off antidepressant

medications can result in the worsening of a participant's symptoms. During the tapering period, the participant will be seen clinically by the study physician.

Blood Draw

There may be mild temporary discomfort, minor bruising or irritation, and in rare cases there may be local infection at the vein site. The blood draws are required to establish safety and eligibility for the trial.

ECG

Skin irritation from the ECG electrode pads or pain when removing the sticky pads are possible side effects.

TMS-EEG

Regarding the TMS/EEG visits, the most frequent risk associated with TMS is scalp discomfort, which has been reported in approximately 5% of participants. This discomfort is transitory and typically can be resolved with acetaminophen. EEG recordings are not associated with any known risks to health and there is no evidence that there are either short-term or long-term side effects though some scalp discomfort during the procedure which can occur in a small proportion of patients. Single pulse TMS is in routine clinical diagnostic use in hundreds of neurophysiological laboratories worldwide. The induced electrical current is well below that which is expected to cause harm to nervous tissue. The US FDA has concluded that stimulation at <1 Hz carries virtually no risk of seizure and is therefore classified as a non-significant risk device. There is a minimal but slightly increased risk of discomfort, psychological and physical harm during these neurophysiological tests.

Assessment Measures

Assessment measures are designed to address various aspects of psychopathology and as such, may be distressing. Participants may experience emotional reactions to the questions and when providing responses about the material on the questionnaires and in the interviews. Any distress or discomfort encountered by participants will be addressed by a member of the study team. In addition, the assessments may cause fatigue. These risks will be mitigated by offering breaks throughout the study visits.

Imaging and Related Risks:

[^{18}F]SynVesT-1:

Risks associated with the radiotracer itself are most likely minimal. It is possible, although very unlikely, to have an allergic reaction to the radioactive tracer. The amount of radiotracer injected is very small (microgram) and does not

usually interfere with the normal functioning of the brain and body based on experience with a large number of research studies on many radiotracers.
Participants will be monitored after injection and throughout the scan. A physician will be available on-site for the duration of each scan in case of any issues.

Radiotracer injection:
As with any injection or blood work, there is a small risk of bruising or swelling at the site of injection, and lightheadedness and feeling faint. Radiotracer injection will be performed by qualified personnel who perform these injections routinely. Participants will be monitored after injection and throughout the scan.

Arterial line and blood draw:

There will be a slight discomfort from the insertion of intravenous and arterial lines as well as the possibility of bruising. More serious complications are rare but include aneurysm formation (local ballooning of the artery), dissection (rupture of the inner artery wall) or local thrombosis (blockage) of the artery, which may require surgical intervention to correct. Local anesthesia is used to minimize discomfort during arterial line placement. A qualified professional who routinely puts in arterial lines will put in the line. A physician will be available on-site for the duration of each scan in case of any issues or discomfort. Participants will be instructed to avoid heavy lifting or sudden wrist movements for 24 hours after the arterial line is removed. Participants will be asked if they have donated blood recently and, if so, first PET scan will be scheduled at least 30 days after any donation to minimize the impact of multiple blood collection procedures. Participants will also be advised not to donate blood for at least 30 days after the second PET scan.

PET scan:

Subjects will be exposed to a small amount of radiation (<0.1 mSv) from a brief transmission or CT scan collected for attenuation correction, and will receive ~5 mCi per ^{18}F scan (10 mCi total for the study). The radiation dose is comparable to other nuclear medicine scans. The total radiation dose from [^{18}F]SynVesT-1 during the two PET scans (including the CT or transmission scans) is about 8.4 mSv. These doses have never been associated with any definite adverse effects, but potential long-term risk is unknown.
Mitigation strategies: A secure central registry at the CAMH PET centre tracks research participants who complete PET scans to ensure radiation exposure does not exceed the annual or lifetime limits set by the centre and by the Canadian Nuclear Safety Commission. Participants will also be asked about prior PET scans at their screening visit to capture nuclear medicine procedures done at other centres or in clinical care. Participants will be asked to void after each PET scan to clear radioactive material from the body faster and limit exposure.

Reproductive health and pregnancy:

It is advised that pregnant people avoid radiation to minimize any risk to the fetus. Participants will be informed of these risks and asked to notify study personnel if they are or become pregnant or are breast-feeding during the study. A urine pregnancy test will be administered to all female participants in this study prior to each PET scan, and pregnant women will not be scanned.

MRI scan:

Certain metal objects may lead to injuries during the MRI. Some people may feel uncomfortable lying still in the confined space of the MRI scanner, tingling sensations are felt by some people during certain scans, or dizziness may be felt for a few minutes at the end of the MRI study. Participants will be asked about any metal implants or objects in the body and any tattoos. Participants with any metal implants or objects that are not safe for the 3T MRI will not be allowed to enrol or continue in the study. Research study staff and the MR technologist will monitor the patient during the scan and pause or end the scan in case of anxiety or severe discomfort, or at the participant's request.

2.0 CLINICAL TRIAL OBJECTIVES

2.1 Primary Objective

To evaluate the feasibility and safety of obtaining PET imaging scans before and after administration of psilocybin (25mg) with and without risperidone (1 mg) in adults with TRD.

Hypothesis 1a (feasibility): We will be able to recruit 12 participants within 12 months with a retention of >90%.
Hypothesis 1b (safety): Obtaining PET imagining scans before and after administering psilocybin 25mg with and without 1mg of risperidone will not be associated with serious adverse events.

2.2 Exploratory Objectives

- To obtain preliminary data on synaptic density (as measured by [^{18}F] SynVesT-1 volume distribution, V_T) in brain regions relevant to MDD (i.e., hippocampus, prefrontal cortex) before and 4 weeks after psilocybin 25 mg vs psilocybin 25 mg plus risperidone 1 mg in adults with TRD.

- To obtain preliminary data on cortical plasticity, as measured by TMS-EEG and resting EEG in brain regions relevant to MDD (i.e., hippocampus, prefrontal cortex) before, during and after psilocybin 25 mg vs psilocybin 25 mg plus risperidone 1 mg in adults with TRD.

- To obtain preliminary data on antidepressant effects as measured by the change in the MADRS from Baseline (V2) to 1-week post-treatment (V5). Antidepressant effects will also be measured at Weeks 2 (V6), 3 (V7), and 4 (V8) post-intervention. Response will be defined as a reduction of 50% or more of the MADRS score and remission defined as a score of <7 on the MADRS from Baseline (V1) to Week 1 (V5)

3.0 CLINICAL TRIAL DESIGN

3.1 Overall Design

This study is an open-label, proof of concept study study evaluating the feasibility and safety of obtaining PET imaging scans before and after administration of psilocybin (25 mg) with and without risperidone (1 mg) in adults with TRD. The preliminary clinical data will help understand the mechanism of antidepressants effects of psilocybin (with and without serotonin 2A receptor agonism) in individuals with treatment resistant depression, and inform the development of larger mechanistic studies.

Overview of Study Design:
Participants will be assigned sequentially to one of two open-label groups: (Group 1) 25 mg of psilocybin combined with psychotherapy, (Group 2) 25 mg of psilocybin + 1 mg of risperidone combined with psychotherapy. The first six recruited participants will be assigned to Group 1 and the second six recruited participants will be assigned to Group 2. Each participant will undergo a screening assessment where they will complete lab tests, and clinical and psychiatric assessments to determine eligibility. Following the screening visit, participants will undergo a washout period where they will be tapered off concomitant medication over a period of 2 to 6 weeks. The length of the tapering period will depend on the type of medication the participant is being tapered off (based on the half-life of the medication) and the participant's

preference for the length of the tapering period. Most medications will require a minimum of a 2-week tapering period with the exception of fluoxetine which will require a minimum of 4-weeks. Additional time may be added at the discretion of the study investigator. During this period, there will be weekly check-ins with the study physician. Participants who are not on any prohibited medications will undergo a two-week monitoring period prior to baseline session (Visit 2). At study Visit 2 (Baseline, V2), participants will complete a series of questionnaires and assessments,undergo pre-intervention clinical blood work and preparatory therapy with trained study therapists. To reduce participant burden, baseline can be broken up into multiple days, however all assessments must be completed within 7-days of the intervention. Ideally, baseline occurs the day before the intervention is administered. At study Visit 3 (V3), 12 participants will receive either 25 mg of psilocybin in conjunction with supportive therapy (Group 1: first six recruited participants) or 1mg of risperidone and 60-minutes later will receive an active dose (25mg) of psilocybin in conjunction with supportive therapy. On the day after the dosing session (Visit 4, V4) and one-week after the dosing session (Visit 5, V5), participants will be asked to complete the same questionnaires that were done at Baseline (V2) and will undergo an integrative therapy session with the trained study therapists. At Visit 4, participants will undergo post-intervention clinical bloodwork. At Visit 6 (V6), Visit 7 (V7), and Visit 8 (V8), participants will complete follow-up assessments similar to those administered at Baseline (V2).

Additionally, participants will undergo neurophysiolpogical testing (TMS-EEG) at baseline (visit 2), intervention (visit 3) 1-2 hours after receiving the intervention and at the end of the intervention day), and at follow-up (visit 5).

Participants will also undergo three scans: (1) A pre-treatment *[^{18}F]SynVesT-1 PET scan (up to 4 hours), (2) An MRI scan* used for co-registration of PET images (up to 1 hour) and (3) a post-treatment *[^{18}F]SynVesT-1 PET scan (up to 4 hours).*

Pre-treatment imaging scan will be collected before the study treatment visit. Post-treatment scan will take place between 1 and 6 weeks after treatment. All study procedures are expected to be completed within 3 months of enrolment, depending on scheduling of the trial treatment visit.

<u>Positron emission tomography</u>

PET scans will be acquired with a GE Discovery MI 5-ring PET/CT scanner or with a high-resolution research tomograph (HRRT). Before each emission scan, following the acquisition of a scout view for accurate positioning of the participant, a low dose (about 0.1 mSv) CT scan (for the PET/CT scanner) or transmission scan (for the HRRT) will be acquired and used for attenuation correction. Otherwise, procedures will be identical for both scanners. Custom-made thermoplastic facemasks (Orfit, Antwerp, Belgium) together with a head-fixation system will minimize head movement in the PET scanner. Up to 5 mCi (+/- 10%) of the radioactive tracer will be injected as a bolus into an intravenous line placed in the antecubital vein. Participants will undergo up to 125-minute acquisition scan.

PET scans will use an arterial line to obtain samples of arterial blood (up to 160 mL) throughout the scan, which are used in conjunction with the PET scan measure in the brain to calculate and validate the binding measure. Prior to insertion, lidocaine, a local anesthetic is applied in a small dose to numb the area and to make the insertion of the catheter easier. The arterial line is inserted by a registered respiratory therapist with a long-standing expertise in arterial line insertion, as is customary at our Centre. Arterial blood will be drawn automatically from a radial artery using an automated blood sampling system (ABSS) pump throughout the scan (total automatic volume, approx. 81 mL) and manually at discrete time points (-5, 2.5, 7, 12, 15, 20, 30, 45, 60, 90, and 110 minutes, where t=0 is scan start time; total manual volume, approx. 61 mL). The removal of the arterial line will be done by a qualified health care professional. The procedure will include the application of pressure at the site of arterial line insertion for about 25 minutes and/or until the puncture site has stopped bleeding (whatever happens first). After that, an elastic bandage will be applied to the wrist where the line was inserted. Participants will be instructed to avoid activities that involve sudden wrist movements or lifting heavy items with this hand for 24 hrs.

In the event that an arterial line cannot be placed or fails before scan start, the scan may proceed without an arterial line at the discretion of the investigators and alternate analysis methods will be used (i.e., reference region quantification using the simplified reference tissue 2 (SRTM2) model, which provides a comparable measure of synaptic density using only brain data – see *Data Analysis*). In the event of radiochemistry failure or equipment malfunction, the PET scan will be rescheduled if the participant agrees and if schedules allow.

Magnetic resonance imaging:
MRI imaging will take place at the CAMH Brain Health Imaging Centre. The MRI will take approximately 1 hour including scanner set-up.

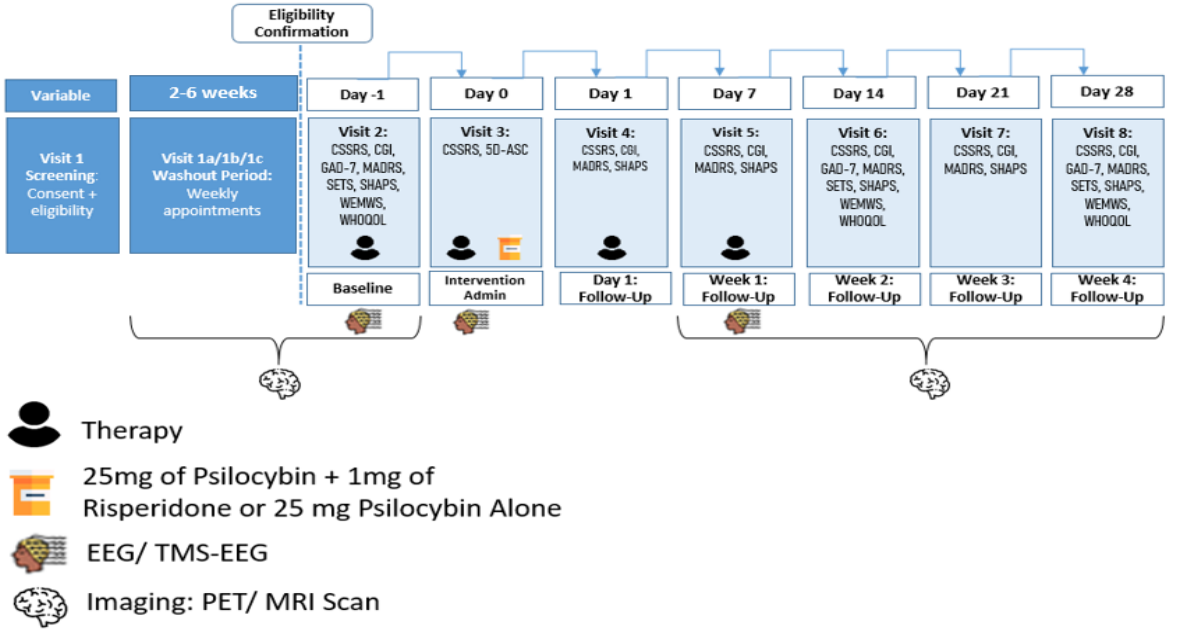
A 3 T GE Discovery MR750 MRI system (General Electric, Milwaukee, WI) will be used to acquire whole-brain scans for spatial normalization of parametric PET images (anatomical T1-weighted and proton density-weighted MRI scan). If time allows within the one-hour imaging session (including setup), scans measuring brain activity at rest will also be acquired (resting state functional MRI scan, approximately 15 minutes). Throughout anatomical and functional imaging scans, participants will rest comfortably on the scanner bed in a dimly lit room monitored by MR technicians.

Neurophysiology:
TMS will be administered over the frontal cortex, with simultaneous EEG recording. Cortical inhibition will be assessed via the Short Intracortical Inhibition (SICI) paradigm. SICI is a paired-pulse paradigm that can be used to index inhibitory

of the cortex, whereby a conditioning stimulus (CS) at 80% of RMT is presented at 2ms before a test stimulus (TS) at 120%RMT. SICI is mediated by fast-acting ionotropic GABAA receptors [Ilic et al 2002]. This suggests that SICI arises when the conditioning stimulus (CS) activates a low-threshold inhibitory system, leading to hyperpolarizing inhibitory postsynaptic potentials (IPSPs) that suppress the cortical output evoked by the subsequent test stimulus (TS). Each testing session will involve the administration of up to 500 TMS pulses using a randomized sequence of single (TS) and paired (SICI) paradigms.
Biphasic TMS pulses will be administered using a Magventure B65 coil. Each TMS session will include the establishment of the individual threshold for the resting motor threshold (RMT). The RMT will be determined according to the protocol outlined by Rossini et al. The coil is held tangentially to the head, with the handle of the coil pointing backward and 45 degrees laterally from the midline. The RMT is defined as the minimum stimulus intensity that elicits an MEP of more than 50 mV in five of ten trials.

In addition, before the TMS-EEG paradigms, we will collect 5 minutes each of resting-state, eyes-closed and eyes-open EEG.

Figure 1. Study Schematic:



Timeline

In total, there are a minimum of 12 study visits (8 study visits, 2 imaging visits and a minimum of 2 check-in visits during the washout period). There may be more study visits scheduled at the discretion of the study team or the participant. These study visits will take place over the span of approximately 2.5 months. The total expected duration of the clinical trial from the time the study team starts recruiting until data analysis has been complete is 18-months. Following a 3-month startup period, the study team will recruit approximately 1 participant per month over the period of 12 months. Study interventions and follow-up assessments will be completed by month 14. This leaves approximately 4 months for data analysis which will be completed at month 18.

3.2 Primary Endpoints

Safety endpoints will be evaluated using standardized adverse events monitoring at all time points. Adverse event monitoring will be prioritized to closely and thoroughly evaluate the acute and sub-acute psychological safety profile. Constant observation by therapists will monitor for adverse events during the dosing sessions. An on-call psychiatrist will be available at all times to further assess as needed for acute concerns. The safety endpoint is the number and severity of adverse events reported throughout the duration of the trial.

Feasibility endpoints include the recruitment and retention rates. Dropout rates during three periods will be evaluated, namely: 1) screening period in which the participant undergoes medication tapering and washout; 2) during the acute course of the study intervention; and 3) during the follow-up after the intervention. Throughout all three periods, we will also evaluate adverse events including psychological distress and serious adverse events (e.g., hospitalization, suicide attempt, death).

4.0 PARTICIPANT SELECTION AND WITHDRAWAL

4.1 Target Population

The target population for this study are adults aged 18-65 who are diagnosed with major depressive disorder and experiencing a clinically significant depressive episode that has failed to respond to at least two adequate trials of antidepressants. Participants must meet all inclusionary/exclusionary study criteria as confirmed by the study investigator. In order to be eligible, these criteria must be met at the Baseline visit (V2). For participants on concomitant medications, confirmation of eligibility occurs after a successful washout period in which the participant has been tapered off concomitant medications for a period of at least 2-weeks prior to baseline (4-weeks for fluoxetine), as confirmed by the study investigator.

4.2 Participant Recruitment and Screening

The target sample size is 12 participants (N=12) diagnosed with treatment-resistant depression. The study will take place at a single site: the Centre for Addiction and Mental Health (CAMH) in Toronto, Ontario.

The source of participants in this study will come from CAMH outpatient units and external healthcare providers. Clinicians at CAMH may identify potential research participants and obtain verbal permission from these potential participants for a member of the research team to approach them. Potential participants that are interested in participating in the study will be prescreened by a member of the study team, as outlined below.

The CAMH Research Registry will also be used to recruit participants for this clinical trial. Upon REB approval to use the Research Registry as a recruitment strategy, authorized research personnel will search and contact potential research participants included within the member database of the Research Registry for study participation. This clinical trial will also be posted on the Research Registry website, as well as the public CAMH website. The recruitment material posted on these websites will be reviewed and approved by Research Communications as well as the REB prior to posting. Once posted, interested participants can use the "Find a CAMH study" feature to explore clinical trials that they are interested in.

Prescreening Procedures

Once a potential participant contacts the research team or is referred to the research team as an interested potential participant, a research team member will schedule a phone call. This phone call will be referred to hereafter as the Pre-Screening conversation. During the pre-screening conversation, a brief description of the study is provided to the potential participant and then, if the person agrees, the following eligibility criteria is obtained:

- Contact information (phone number and/or email)
- Partial date of birth
- Ability to read and speak English
- Whether they have a clinical diagnosis of major depressive disorder
- Whether they are currently experiencing a major depressive episode
- Treatments taken for major depressive episode (frequency and type of treatment)
- Whether the potential participant has been diagnosed with psychotic disorder, bipolar disorder, paranoid personality disorder, schizoaffective disorder, or borderline personality disorder;
- Diagnosis of a substance use disorder (excluding tobacco) within the previous 6-months;
- If they are currently undergoing therapy and if they are, the date that they started
- Whether the potential participant would be willing to discontinue current antidepressant medications
- Whether they are seeing a doctor on a regular basis for a medical problem
- If they are currently taking medications for the treatment of a physical health problem
- Difficulty with giving blood or needles
- Currently nursing or pregnant
- Willingness to take contraceptives for the duration of the study starting atleast one month prior to screening visit (Visit 1)
- History of allergy or adverse reaction to risperidone
- Currently enrolled in another study involving an investigational product or device
- If they are able to take medication orally
- History of traumatic brain injury, neurological or neurodegenerative disorder
- Willingness to participate in PET and MRI scans
- History of claustrophobia

- History of pacemaker implantation
- Blood disorders, disorders of coagulation, or ongoing use of anticoagulant medication
- Any disability that may prevent the participant from completing study requirements (e.g., non-correctable clinically significant sensory impairment such as not hearing well enough to communicate with study personnel during scans, or physical disability that does not allow them to lie still on the scanner bed for 1-2 hours
- A history of metal implants in the body

The information collected during this conversation will be recorded on the pre-screen form which will be reviewed by the study PI. If the potential participant does not meet any exclusionary criteria as listed on the Pre-Screen form, then the potential participant is called back to invite them to schedule a consent and screening visit.

If the person meets any exclusionary criteria during the pre-screening conversation or as determined by the study investigator, then the person is asked whether they would be interested in participating in any other studies (current or future) within our program. If they are interested in other studies within our department, their name and contact information will be transferred to a password protected log that is only accessible by Centre for Complex Interventions staff. If they fail the pre-screen and do not wish to be contacted, their pre-screen form will be discarded in the confidential shredding bin which will then be securely disposed of. However, their name will be kept in a password protected log along with the date and result (pass/fail) of their pre-screen so if they contact us again (e.g. to inquire about their eligibility) we can refer back to it.

Compensation

Participants will not be charged for research-only services for their participation in this study. All research-only services, such as clinical assessments, blood work, and the IP will be provided at no cost to the participant.

Participants will be reimbursed for the cost of parking incurred at each study visit. To receive reimbursement for parking expenses incurred at each study visit, participants must provide the research team with a parking receipt. In addition, reimbursement will be provided if the participant used public transit for transportation to and/or from study appointments.

Participants will also be reimbursed for the time spent at study visits occurring after the screening and washout period where treatment (therapy or the intervention) is not administered. Participants will be reimbursed $10 per hour for each study visit that they attend (V6, V7, and V8). Participants will also be compensated for their scans: 150.00 for

each PET scan and 60.00 for the MRI scan. In total, if participants complete all study visits, they may be reimbursed up to $385 for their time.
Compensation will be provided at the end of the sessions via e-gift card, if the session is conducted virtually. No payment will be provided in advance.

Study Visit:	Duration:	Compensation:
PET scan 1: [^{18}F]SynVesT-1 scan with arterial line	~4 hours	$ 150
PET scan 2: [^{18}F]SynVesT-1 scan with arterial line	~4 hours	$ 150
MRI scan	~1 hour	$ 60
Follow-Up: Visit 6	~1 hour	$10
Follow-Up: Visit 7	~ 30 min	$5
Follow-Up: Visit 8	~1 hour	$10

4.3 Equity, Diversity and Inclusion Considerations

Equity, diversity, and inclusion (EDI) are important to ensuring the study design is ethically sound. No exclusions will be made based on race, ethnicity, religion, sex, or gender.

4.4 Eligibility Criteria

4.4.1 Inclusion Criteria

The participant must meet all of the inclusion criteria to eligible for this clinical trial:

1. Adults 18 to 65 years old;
2. Must be deemed to have capacity to provide informed consent;
3. Must sign and date the informed consent form;
4. Stated willingness to comply with all study procedures;
5. Ability to read and communicate in English, such that their literacy and comprehension is sufficient for understanding the consent form and study questionnaires, as evaluated by study staff obtaining consent;
6. Primary DSM-5 diagnosis of non-psychotic MDD, single or recurrent, based on the Structured Clinical Interview for DSM-5 (SCID-5) administered at the first screening visit;
7. Participants diagnosed with treatment-resistant depression defined as individuals with a baseline HamD-17 score > 14 and that have not responded to two or more separate trials of antidepressants at an adequate dosage and duration (an antidepressant resistance rating score of three or more is considered an adequate trial) based on the Antidepressant Treatment History Form (ATHF); there is no upper limit on the number of treatment failures;
8. Ability to take oral medication;

9. Individuals who are capable of becoming pregnant: use of highly effective contraception for at least 1 month prior to screening and agreement to use such a method during study participation;
10. Individuals who are willing to and have tapered off current antidepressant and antipsychotic medications for a minimum of 2-weeks (or more depending on the medication) prior to Baseline (V2) and whose physician confirms that it is safe for them to do so;
11. Agreement to adhere to Lifestyle Considerations (section 4.5) throughout study duration.

4.4.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this clinical trial:

1. Pregnant as assessed by a urine pregnancy test at Screening (V1) and Baseline (V2) or individual's that intend to become pregnant during the study or are breastfeeding;
2. Treatment with another investigational drug or other intervention within 30 days of Screening (V1);
3. Have initiated psychotherapy in the preceding 12 weeks prior to Screening (V1);
4. Have a DSM-5 diagnosis of substance use disorder (use of tobacco is permitted) within the preceding 6 months;
5. Have active suicidal ideation with intent and plan as determined by item 3 of the HamD-17;
6. Any DSM-5 lifetime diagnosis of a schizophrenia-spectrum disorder; obsessive-compulsive disorder, psychotic disorder (unless substance induced or due to a medical condition), bipolar I or II disorder, paranoid personality disorder, borderline personality disorder, or neurocognitive disorder as determined by medical history and the SCID-5 clinical interview;
7. Any first-degree relative with a diagnosis of schizophrenia-spectrum disorder; psychotic disorder (unless substance-induced or due to a medical condition); or bipolar I or II disorder as determined by the family medical history form and discussions with the participant;
8. Presence of a relative or absolute contraindication to psilocybin, including a drug allergy, recent stroke history, uncontrolled hypertension, low or labile blood pressure, recent myocardial infarction, cardiac arrhythmic, severe coronary artery disease, or moderate to severe renal or hepatic impairment.
9. Presence of baseline prolonged QTc or Torsade de Pointes as measured by the ECG or a history of long QTc syndrome or related risk factors;
10. History of allergy or contraindication to risperidone
11. Current or past traumatic brain injury or other neurological/neurodegenerative disorder
12. Unable or unwilling to undergo PET or MRI scanning (e.g. claustrophobia, pacemaker);
13. Blood disorders, disorders of coagulation, or ongoing use of anticoagulant medication

14. Any disability that may prevent the participant from completing study requirements (e.g., non-correctable clinically significant sensory impairment such as not hearing well enough to communicate with study personnel during scans, or physical disability that does not allow them to lie still on the scanner bed for 1-2 hours);
15. Participant exceeds the annual or lifetime amount of radiation
16. Any other clinically significant physical illness including chronic infectious diseases or any other major concurrent illness that, in the opinion of the investigator, may interfere with the interpretation of the study results or constitute a health risk for the participant if they take part in the study.

4.5 Lifestyle Considerations

During this clinical trial, participants are asked to:

- Abstain from alcohol for 24 hours before baseline and for up to 6hrs after intervention administration (V3).
- Abstain from the use of any prescribed opioids, benzodiazepines, or sleep aids (Z-drugs) within 12hrs prior to the intervention (V3) and for up to 6hrs after administration.
- Abstain from any illicit drugs (e.g. cocaine, ecstasy/MDMA, hallucinogens) and/or cannabis for the duration of the study. Presence of these substances will be assessed at a urine drug screening at Visit 1 and Baseline (V2)
- Abstain from driving or operating heavy machinery for up to 24hrs after the intervention.

4.6 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but do not meet one or more eligibility criteria required for participation. The screening period for participants in this study occurs before Baseline (V2) and eligibility for the study cannot be confirmed until the participant has tapered off any concomitant medication. In order to be eligible, the participant must meet all eligibility criteria as outlined in Section 4.4. The information collected about the participant during the screening process including demography, screen failure details, eligibility criteria not met, and any AEs/SAEs will be used for the purposes of transparent reporting. Participants who are deemed ineligible will continue with their usual standard of care or may be referred to other research protocols for TRD.

4.7 Participant Withdrawal Criteria

4.7.1 When and How to Withdraw Participants

Participants are free to withdraw from participation in the clinical trial at any time.
An investigator will discontinue or withdraw a participant from the clinical trial for the following reasons:

- Pregnancy or if participants cease effective contraception;
- Significant study intervention non-compliance;

- If any adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the clinical trial would not be in the best interest of the participant;
- Disease progression which requires discontinuation of the study intervention;
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation; or
- If the participant develops or is found to have any condition that may confound interpretation of the primary PET outcome measure, the participant will be withdrawn from the study.
- Participants developing severe claustrophobia during imaging (MRI or PET) will be withdrawn to prevent any risk of severe anxiety to the participant

The reason for participant discontinuation or withdrawal from the study will be recorded within the participant's research record, and/or health record at CAMH.

Participants that are withdrawn from the study will be replaced using the same recruitment methods as outlined in Section 4.2: Participant Recruitment and Screening.

4.7.2 Follow-up for Withdrawn Participants

If a participant withdraws consent, the information that was provided by the participant and recorded by the study team before they withdrew consent will not be destroyed. However, once withdrawn from the clinical trial, no further research procedures or evaluations will be performed, or additional research-specific data collected on the participant. Reasonable effort will be made to obtain permission to document the reason for withdrawal.

Withdrawn participants will be seen clinically by the study investigator to ensure a plan for continued care outside of the study is established. If the participant is interested in hearing about other treatment options, they may be offered a referral to the CBT group at CAMH and/or a consultation with a psychiatrist to discuss pharmacotherapy options.

4.7.3 Early Termination Visit

If a participant withdraws from the clinical trial, every effort should be made to perform an Early Termination Visit.

Participants that withdraw after the first dosing session:
If the participant is willing to attend an early termination visit, the following information will be documented:

- Assessment of new and ongoing AEs;
- Assessment of any complications following the study intervention;
- Documentation of all concomitant medications;

The PI will also ensure the participant is appropriately transitioned/followed for any additional care as required.

4.7.4 Participants who are Lost to Follow-up

A participant will be considered lost to follow-up if they fail to return for 2 or more scheduled visits and is unable to be contacted by the research team.

The following actions will be taken if a participant fails to attend a required study visit:

- The research team will attempt to contact the participant and reschedule the missed visit 7 days, counsel the participant on the importance of maintaining the assigned visit schedule, and reconfirm whether the participant wishes to and/or should continue in the clinical trial.
- Before a participant is deemed lost to follow-up, the research team will make every effort to regain contact with the participant via 2 different methods of contact (e.g. telephone and email). These contact attempts should be documented in the participant's research record and/or legal health record.
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the clinical trial with a primary reason of lost to follow-up.

5.0 STUDY INTERVENTION

5.1 Description

Pharmacokinetics and Psilocybin Effects

Psilocybin is detectable in plasma 20 to 40 minutes after oral administration of 0.224 mg/kg (10-20mg total dose) (Hasler et al., 1997). Orally ingested psilocybin is metabolized in the liver, and primarily transformed into the active hydroxyl metabolite, psilocin. Psilocybin is essentially a prodrug and psilocin represents the pharmacologically active agent in systemic circulation. The elimination half-life of psilocybin is 50 minutes (Lindenblatt et al., 1998). Psilocin's half-life ranges between 2 and 3 hours and it detectable 6 hours after oral administration (Hasler et al., 1997; Hasler et al., 2004; Lindenblatt et al., 1998). Both psilocin and psilocybin are detectable in human urine, unmodified and particularly conjugated with glucuronic acid(Hasler et al., 2002). The majority of psilocybin recovered in urine is excreted within 3 hours after oral administration and is completely eliminated from the body within 24 hours (Hasler et al., 2002).

As a $5HT_{2A/2C}$ agonist, psilocin is regarded as a "classical" psychedelic; in humans, it commonly elicits significantly altered states of consciousness, experienced through visual and sometimes auditory effects, changes in perception, distortions of time, and a range of effects including a sense of awe, novel perspectives, existential and personal insight, dramatically heightened empathy and feelings of compassion, strong emotions, and non-dual or unitive experience. A number of these 'peak' experiences have been

associated with improved quality of life and improvement in mood (Griffiths et al., 2016; McClain et al., 2003; Visser et al., 2010). For a more detailed explanation on the effects of psilocybin and its mechanism of action, please refer to the investigators brochure.

Pharmacokinetics and Risperidone Effects

Risperidone is an atypical antipsychotic with antagonist binding affinity at serotoninergic, dopaminergic, adrenergic, and histaminergic receptors (Schatzberg & Nemeroff, 2009). It has a very high affinity for the 5-HT_{2A} receptor (Meltzer et al., 1989) and moderately high affinity for the D_2 dopamine receptor (Schatzberg & Nemeroff, 2009). Risperidone is taken orally and almost entirely absorbed in the GI tract and metabolized in the liver (Schatzberg & Nemeroff, 2009). The plasma half-life is approximately 3 hours in extensive metabolizers and 20 hours in poor metabolizers (Broder et al., 2012). Clearance values for risperidone and the active metabolite are estimated to be 4.6 and 6L/hr, respectively (Vandenberghe et al., 2015). In general, risperidone is well-tolerated. It does have a broad range of common side effects (>10%) which include somnolence, nausea, dizziness, dry mouth, and tremor (Chopko & Lindsley, 2018). Risperidone has been shown to improve depression in SSRI-resistant patients (Correia & Vicente, 2007). In healthy subjects, risperidone (1 mg) blocked (98-99%) the psychedelic effects of psilocybin including the perceptual disturbances and hallucinatory phenomena (Vollenweider et al., 1998).

Psilocybin-Assisted Psychotherapy

The participant will attend 1 preparatory session that occurs within 7-days of the first psilocybin dosing session (V3) to develop a therapeutic alliance, set intentions for the experience, and learn what to expect during the dosing session. An additional preparatory therapy session can be added at the discretion of the study therapists and study investigator. In addition, the participant will undergo 2 integrative therapy sessions after the intervention (V4 & V5). During dosing, there will be two therapists present. A physician will be available at all times during the dosage session to assess and manage any medical or psychiatric adverse events (either on-call or as a therapist in the room with the patient). Efforts will be made to ensure that the therapists remain the same at every therapy session, however the only day that requires two therapists is dosing. Therapy sessions will not be video recorded.

Each therapist will undergo training using the Yale Manual for Psilocybin-Assisted Therapy of Depression, an evidence informed protocol for PAP. For a full description of each therapy session and PAP, please refer to the Yale Manual for Psilocybin-Assisted Therapy of Depression. For a full description of each therapy session and PAP, please refer to the Yale Manual for Psilocybin-Assisted Therapy of Depression. Therapy sessions will not be video recorded. At least one of the therapists will be a clinician and will be available at all times during the dosage session to assess and manage any medical or psychiatric adverse events.

In addition, therapists will undergo protocol-specific training for the study. Therapists involved in the trial must be licensed to provide therapy by a regulatory body. Unlicensed therapists will be directly supervised by a licensed therapist.

Requirements for lead study therapists:

Licensed to provide psychotherapy in the province of Ontario:

Social worker

Psychologist

Psychiatrist or physician (M.D or equivalent) with psychotherapy training

Nurse with psychotherapy training (e.g. nurse psychotherapist certificate)

Registered with the appropriate regulatory body in Ontario:

College of Registered Psychotherapists of Ontario

College of Psychologists of Ontario

Ontario College of Social Workers and Social Service Workers

The College of Physicians and Surgeons of Ontario

College of Nurses of Ontario

Previous experience administering PAP

Unlicensed therapists who are in the process of becoming licensed or therapists who have not had any PAP experience must be supervised under the direct supervision of a lead therapist.

How the study intervention will appear:
The psilocybin will be provided by Filament Health Corp. (Burnaby, British Columbia, Canada). The dose of psilocybin used in this study will be 25mg. The psilocybin will be administered in size 2 hydroxypropyl methyl cellulose (HPMC), white capsules. The risperidone (where applicable) will be provided by CAMH pharmacy and will appear as a 1mg oral tablet.

5.2 Treatment Regimen

There will be one intervention day (V3 – Day 0) following Baseline (V2) and after the participant has been deemed eligible to participate. The procedures are outlined below:

Each participant will be assigned 1 treatment bottle containing 1 capsule of 25mg psilocybin and, where applicable, 1 treatment bottle containing 1 tablet of 1mg of risperidone. For participants allocated to Group 2, under the supervision of a clinician, the 1mg tablet of risperidone will be taken orally and the 25 mg capsule of psilocybin will be taken 60 minutes after with a glass of water. For participants in Group 1, under the supervision of a clinician, the 25 mg capsule will be taken orally with a glass of water. There will be no modifications to the dosage, each participant will receive the same

dosage of psilocybin. All participants in Group 2 will receive the same dose of risperidone. However, if adverse effects are experienced, the study clinician may make changes to the intervention, which include therapy after each session. The study clinician may decide that participants may require more therapy sessions. In addition to the psilocybin, two study therapists trained in psilocybin-assisted psychotherapy will be supporting the participant during the dosing session. There will be 1 therapist present at all times throughout the dosing session. The total treatment time will be 5-6 hours when the acute effects of the psilocybin have passed.

5.3 Method for Assigning Participants to Treatment Groups

The first six recruited participants will be assigned to the Group 1: 25 mg of psilocybin combined with adjunct supportive therapy. The second six recruited participants will be assigned to Group 2: 25 mg of psilocybin plus 1 mg of Risperidone combined with supportive therapy.

5.4 Administration of Study Intervention

The IPs will be prepared by the CAMH pharmacy and picked up by a trained research staff member. The IPs will be given to the participant by the study clinician who will supervise the participant orally take the medication. Participants in Group 1 will receive a capsule containing 25mg of psilocybin which will be taken orally, with water. Participants in Group 2 will receive a tablet of 1mg of risperidone taken orally followed 60-minutes later by a capsule containing 25mg of psilocybin which will also be taken orally, with water. The remaining 10 participants will receive one 25mg tablet of psilocybin, which will be taken orally with water. The capsules should not be opened or chewed.

After taking the IPs, the participant will lie down on a bed in a non-clinical environment. Therapists will encourage participants to focus their attention inward and stay with any experience that arises. To enhance inward reflect, a pre-selected music playlist will be played quietly. Two study therapists trained in PAP will be supporting each participant during the dosing session with at least one therapist being present at all times to respond to the emotional and physical needs of the participant. Constant observation by therapists will monitor for adverse events during the dosing sessions. At least one member of the dyad will be a clinician. An on-call psychiatrist will be available at all times to further assess as needed for acute concerns.

Where applicable, the effects of risperidone will take approximately 60 minutes after administration. The effects of psilocybin usually start about 20 to 30 minutes after administration, becoming the most intense in the first 90 to 120 minutes and gradually subsiding in 5 to 6 hours. The participants will be asked to remain in the room for the duration of the session regardless of the intensity of the effects, preferably lying down and mostly silent unless they have a concern or need to communicate a discomfort or seek reassurance from the therapist, or use the restroom. The therapists will ‘check-in’ with the participant (i.e., ask how the participant is doing) in 30 to 60 minute intervals post-

dosing. Light snacks will be available, though participants are encouraged to bring their own lunch. At approximately 1-2 hours after receiving the intervention, participants will undergo neurophysiological testing

About 5 to 6 hours after dosing, the trained therapists will discuss the IP administration experience with the participant. Additional neurophysiological testing will take place at the end of the intervention day prior to participant dishcharge. The participant will be discharged 5-6 hours post-dosing when, in the opinion of the investigator, the acute effects of psilocybin are resolved. The participant must be accompanied home by a caregiver who will remain with the participant for up to 24hrs after the intervention was given. The study team is to be notified that the participant has arrived home safely via phone call. In the absence of receiving a phone call, the study staff will directly contact the participant.

5.5 Participant Compliance Monitoring

The IP will be administered to the participant in front of study personnel. Thus, administration of the IP will be supervised by study personnel to ensure compliance.

5.6 Concomitant Therapy

All prescription and non-prescription medications (e.g. over-the-counter drugs and herbal supplements) that participants report taking during the 30 days prior to Screening (V1) will be assessed and recorded at V1. For each medication, documentation should list the trade or generic name, the total daily dose including units (or the dose, units, and scheduled and actual frequency of administration if the medication is not taken daily), the route of administration, and the reason for use. Where applicable, medication reports should be corroborated with participant medical records. All as-needed (*pro re nata,* PRN) prescriptions should be converted to reflect the actual number of pills or dose taken per day.

Concomitant medication refers to all drugs and therapies used from the time the ICF is signed through until the end of study participation. Changes, additions, or discontinuations to medications and/or therapy will be assessed, recorded, and verified with participants in the data collection forms during each study visit.

Permissible Medications

Medications for the management of concurrent anxiety and insomnia, or non-psychiatric medications that have a potential psychotropic effect are permitted within the following limitations.

For the initial Screening Visit (V1) through to the final study visit (V8), participants are permitted to use benzodiazepines (up to 2mg of lorazepam equivalent per day for insomnia and anxiety if it is not taken within 12 hours before the psilocybin dose. Prescription and nonprescription medications with psychoactive properties that are used

as needed for non-psychiatric conditions (e.g. pseudoephedrine for allergies or cold, zoplicone for sleep disorders) should be used no more than 2 times a week and not within 12 hours before any study assessment. Documentation of the use of adjunctive anxiolytics, hypnotics, or medication with potential psychotropic properties (including over-the-counter preparations) will be obtained at each visit.

Permissible Contraceptive Methods

A woman/female or person who is not of childbearing potential is considered to be postmenopausal after at least 12 months without menstruation. The participant must be on a permissible contraceptive for a minimum of 1 month prior to screening and for the duration of the study. The following methods of contraception, if used properly and used for the duration of the study, are permissible:

- Combine estrogen-and progestogen-containing hormonal contraception associated with inhibition of ovulation:
  - Oral
  - Intravaginal
  - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  - Oral
  - Injectable
  - Implantable
- Intrauterine device
- Intrauterine hormone-releasing system
- Bilateral tubual occlusion
- Vasectomized partner
- Sexual abstinence
- Tubal ligation/ occlusion

Periodic abstinence (e.g. calendar, symptothermal, or postovulation methods) is not an acceptable form of contraception for this study.

These methods of contraception also apply to partners of male participants.

The investigator (or delegate) and each participant will determine the appropriate method of contraception for the participant during the participation in the study. This will be documented at Screening (V1).

Prohibited Medications

Participants are to be discontinued from antidepressants and/or antipsychotic medications at least 2 weeks prior to Baseline (V2). Participants on fluoxetine will be taped off the medication at least 4-weeks prior to Baseline (V2). Additional time may be required as determined by the study investigator. Medications that must be discontinued include the following 2 classes of the Anatomical Therapeutic Chemical (ATC)

Classification System: NO5A Antipsychotics & NO6A Antidepressants. Methylphenidate is also included in this list. In addition, participants must also taper off inhibitors of 5'-diphospho-glucuronosyltransferase (UGT)1A9 and 1A10, aldehyde dehydrogenase inhibitors (ALDHs), and alcohol dehydrogenase inhibitors (ADHs) for a minimum of 2-weeks (or more depending on the medication) prior to Baseline (V2).

These medications should not be re-introduced until after Week 4 (V8) when the study is complete. If the medications are re-introduced, the study investigator must be notified and the medications will be documented in the data collection form. Participants who require concomitant medication(s) specifically for the treatment of depression at any time through the duration of the study will be assessed for reasons of resuming their medications.

Rescue Medication

The decision to medicate a participant will depend on if the therapists and study investigator determine the safety of the patient and others can be maintained without medical intervention. The final decision will be made by the study investigator.

- Benzodiazepine anxiolytics
    - The preferred pharmacological intervention of choice in case of acute psychological distress (e.g. medications such as lorazepam or alprazolam that have a rapid onset, a short time until peak plasma concentration, and a short duration of therapeutic action).
    - The oral route is preferable because IV injection procedures may further exacerbate the participant's anxiety.
- Antipsychotic medications (e.g. additional risperidone) should be available in the event that an adverse reaction escalates to unmanageable psychosis.
- Management of blood pressure:
    - Asymptomatic with blood pressure (BP) < 180/100
        - Reassure, ensure lights are dim or off, tilt head of bed 15 degrees up and continue to monitor
        - Increase blood pressure measurement frequency to q15min until BP has partly normalized (sBP = 100-159; dBP = 60-99)
    - Asymptomatic with BP >180/100 for >30 minutes
        - Administer captopril* 12.5mg PO/SL x 1 with MD order
        - Increase blood pressure measurement frequency to q15min until BP has partly normalized (sBP = 100-159; dBP = 60-99)
    - Asymptomatic with BP persisting at >180/100 for >60 minutes post-dose, despite administering first captopril dose:
        - Consider potential transfer to ER – decision to be made by study investigator
        - Administer 2nd dose of captopril 12.5mg x 1 with MD order
    - Management of severe treatment emergent hypertension:

- Consider potential transfer to ER – decision to be made by study investigator
- Administer captopril 25mg PO/SL x 1
- Call 911 immediately for patients experiencing symptoms of a hypertensive crisis (e.g., chest paint, shortness of breath) or hypertensive encephalopathy (e.g., sudden severe headache, visual disturbances, seizures, diminished consciousness, or focal neurological deficit)

- Note: if there are contraindications to captopril, substitute for hydralazine 10mg PO

In case of development of acute anxiety or psychotic symptoms requiring pharmacological intervention, the participant will be managed under the care of the onsite psychiatrist. The participant may be discharged from the clinic when, in the opinion of the investigator, the condition has stabilized. The participant will be accompanied home. The participant is to notify the site when they have returned home safely. In the absence of receiving a phone call, site staff will directly contact the participant.

Information for how to manage subjects during difficult psychological states are detailed in the Yale Manual for Psilocybin-Assisted Therapy of Depression. All therapists will undergoing training with the study investigator using this manual.

5.7 Packaging

Where applicable, the risperidone 1 mg will be provided be provided and packaged by CAMH pharmacy. The psilocybin will be provided by Filament Health Corp (Burnaby, British Columbia, Canada). The dose of psilocybin used in this study will be 25mg. The entire shipment for this trial will be sent in bulk (i.e. 20 capsules x 25mg each). Psilocybin capsules will be packaged individually in high-density polyethylene bottles (30cc). The dose for each participant will be stored in individual boxes labelled with the protocol number, trial name, lot number, unique box number, and a statement that the drug is for clinical use only. The IP will only be removed from the safe for one participant at a time on the day of their session. For a description of safety reporting, please see Section 8.3.1 of the protocol.

5.8 Blinding of Study Intervention

Not applicable.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Study Intervention Supplies

Upon receipt of the study intervention supplies, an inventory will be performed and a receipt log filled out and signed by the person accepting the shipment. Designated research staff/pharmacy must count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study intervention in a given shipment (active drug or comparator) will be documented in the clinical trial files.

5.9.2 Storage

The risperidone will be stored with CAMH pharmacy. All psilocybin will be kept in a locked area with limited access. The high-density polyethylene (HDPE) bottles of psilocybin are to be stored as indicated in the investigators brochure. Bottles must be maintained at room temperature (15 to 30 degrees Celsius) in a locked, secure location within research pharmacy. Deviations of storage temperature outside this required range should be documented and the study investigator should be notified immediately. Bottles of psilocybin should not be frozen. If any component of the psilocybin is damaged, the PI must be notified immediately. Any storage deviations that meet criteria for reporting will be reported to the REB as a protocol deviation.

5.9.3 Dispensing of Study Intervention

Each participant in Group 1 will be assigned either 1 treatment bottle containing 1 capsule of 25mg of psilocybin. Each participant in Group 2 will be assigned one treatment bottle containing 1 tablet of 1 mg of risperidone and one treatment bottle containing 1 capsle of 25 mg of psi;locybin. For Participants in Group 2, the risperidone will be administered first, followed 60-minutes later by psilocybin. The IP will be dispensed and administered to the participants only by an appropriately qualified study therapist or delegated clinician. The study intervention will be administered orally, with water. First, risperidone will be administered followed 60-minutes later by psilocybin. The psilocybin capsules should be administered in an unaltered state, by mouth, with water. The capsules should not be opened, chewed, or held in the mouth for an extended period without swallowing.

The investigator must keep an accurate accounting of the number of psilocybin capsules delivered to the site, and the IPs administered to participants, and destroyed during and at the completion of the study. The IPs (psilocybin and risperidone) is to be used in accordance with the protocol by participants. The study team, overseen by the PI, should maintain records that adequately document that the participants were administered the IPs dose specified by the protocol.

Regular study intervention reconciliation will be performed to document study intervention assigned, consumed, and remaining. This reconciliation will be logged on an accountability log (i.e. drug accountability log), and signed and dated by delegated research and/or pharmacy staff.

5.9.4 Return or Destruction of Study Intervention

At the completion of the clinical trial, there will be a final reconciliation of the study intervention shipped, consumed and remaining. This reconciliation will be logged on an accountability form, and signed and dated by delegated research and/or pharmacy staff. Any discrepancies noted will be investigated, resolved, and documented prior to destruction of unused study intervention. Intervention destroyed on site will be documented in the clinical trial's files.

6.0 RESEARCH PROCEDURES

6.1 Research Visits

Description of Measures

Screening Measures

Structured Clinical Interview for DSM-5 (SCID-5). The SCID-5 is a semi-structured diagnostic interview for ascertaining DSM-5 diagnoses. It will be administered by a trained study staff member.

Hamilton Depression Rating Scale (HamD-17). HamD-17 is a clinician-rated measure of depressive symptoms that consists of 17 items rated using a semi-structured interview. Eight of the 17 items are rated on a 5-point scale (0=absent; 1=doubtful or mild; 2=mild to moderate; 3=moderate to severe; 4=very severe), while the remaining 9 items are rated on a 3-point scale (0=absent; 1=doubtful or mild; 2=clearly present), yielding a minimum total score of 0 (least severe) and a maximum score of 52 (most severe) (Hamilton, 1960).

Safety Measures

Columbia-Suicide Severity Rating Scale (C-SSRS): The C-SSRS will be used to assess suicide potential or tendency as a study entry criteria and monitored throughout the study. The C-SSRS is a semi-structured interview designed to assess the severity and intensity of suicidal ideation, suicidal behavior, and non-suicidal self-injurious behavior over a specified time period. The measurement of suicidal ideation is based on 5 "yes" or "no" questions with accompanying descriptions arranged in order of increasing severity. If the patient answers "yes" to either questions 1 or 2, the intensity of ideation is assessed in 5 additional questions related to frequency, duration, controllability, deterrents, and reasons for the most severe suicidal ideation. Suicidal behavior is assessed by asking questions categorizing behaviors into actual, aborted, and interrupted attempts; preparatory behavior; and non-suicidal self-injurious behavior. If any item(s) on the C-SSRS are answered "yes", the primary investigator or physician investigator must review the patient's responses in order to:

(a) During the screening period and at Baseline to determine the patient's study eligibility and potential need for referral to a mental health professional, and

(b) During the study evaluate the patient's need for appropriate medical management such as a referral to a mental health professional.

Outcome Measures:

Clinical Global Impression scale (CGI). The CGI is a brief observer-rated instrument that measures the clinician's view of the patient's global functioning prior to and after initiating a study medication. It consists of two one-items measures that evaluate 1) severity of psychopathology from 1 ('normal – not at all ill, symptoms of disorder not present in the past seven days') to 7 ('among the most extremely ill patients – pathology drastically interferes in many life functions;) and 2) change from the initiation of treatment on a similar seven-point scale (1 = 'very much improved' and 7 = 'very much worse'). It has been used in both research and clinical practice (Busner & Targum, 2007).

Generalised Anxiety Disorder 7-item (GAD-7) scale. The GAD-7 is a brief self-report measure of generalised anxiety, it consists of 7 items rated from 0 ('not at all sure') to 3 ('nearly every day'). It has good psychometric properties and is a widely used research instrument in assessing adult anxiety (Spitzer et al., 2006).

Montgomery-Åsberg Depression Rating Scale (MADRS;(Montgomery & Asberg, 1979)). The MADRS is a clinician rated measure of severity of depressive symptoms. It consists of 10-items rated from 0 to 6. It is one of the most widely used clinician-rated assessments of depressive severity with well-established psychometric properties.

Snaith-Hamilton Pleasure Scales (SHAPS). The SHAPS is a 14-item self-report scale that measures hedonic capacity. Participants are asked to rate themselves on a Likert scale from 0 ('strongly disagree') to 3 ('strongly agree'). It is both a reliable and valid measure that is frequently used in research and clinical settings (Snaith et al., 1995).

Stanford Expectancy of Treatment (SETS). The SETS is an instrument to measure positive and negative treatment expectancies in clinical trials. It contains two subscales for both negative and positive expectancies. There are 6-items which are participant rated from 'strongly disagree' to 'strongly agree'. Items 7 to 10 are direct questions asked to the participant (Younger et al., 2012)

World Health Organization Quality of Life Questionnaire – Brief Version (WHO-QoL – Bref). This 26-item, self-report measure was developed by the WHO in order to assess quality of life in the following areas: physical, psychological, level of independence, social relationships, environment, and spirituality/religion/personal beliefs. Responses are rated on a 5-point Likert scale rating from 1 = (not at all, over poor, very dissatisfied, never) through to 5 = (very good, very satisfied, an extreme amount, completely, always).

Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS). The WEMWBS consists of a 12-item scale used to assess the mental wellbeing of people. The self-report scale consists of positively worded statements covering feelings and functioning aspects of mental wellbeing. Responses are rated on a 5-point Likert scale ranging from 1 ('none of the time') to 5 ('all of the time').

Five Dimensions of Altered States of Consciousness (5D-ASC). The 5D-ASC is a 94-item scale that consists of five subscales: 1) oceanic boundlessness; 2) anxious ego dissolution; 3) visionary re-structuralization; 4) auditory alterations; 5) reduction of vigilance (Dittrich, 1998; Studerus et al., 2010). It is well validated and widely used to characterize the subjective effects of psychedelic drugs. This self-rated scale appears as at 10-item Likert scale ranging from 1 to 10.

Outline of Study Procedures

Visit 1 (V1) – Screening Visit

- Administered by trained study staff:
  - Informed consent
  - Review of medical history, family medical history, and demographics
  - ATHF
  - SCID-5
  - HamD-17
  - Vital Signs (blood pressure, pulse)
  - Height and weight
- Clinician administered:
  - Review of prior and current medications; the participant will be tapered from prohibited medications (see Section 5.6), if any, under the supervision of the study clinician
    - The study clinician will discuss options of tapering off medications with the participant and their healthcare provider.
    - Participants will be given a choice of how quickly they would like to come off the medications, but participants must be off concomitant medications (see Section 5.6) at least 2 weeks prior to the Baseline Visit (V2). Some medications may require a longer tapering period.
  - Review of eligibility criteria, medical history, and family medical history
  - Review of assessments
  - Documentation of contraceptive method to be used by the participant
  - C-SSRS

- Biological specimen collection and laboratory evaluations collected at the Queen Street CAMH laboratory:
  - <u>Clinical laboratory tests:</u>
    - Approximately 20 mL blood will be drawn to conduct the following evaluations:

- *Haematology:* hemoglobin, hematocrit, red blood cell count, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, white blood cell count (with differential), and platelet count.
- *Chemistry:* albumin, alkaline phosphatase, alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), bicarbonate, bilirubin (direct, indirect, and total), calcium, chloride, creatine kinase, creatinine, gammaGT, glucose, lactate, dehydrogenase, lipase, magnesium, phosphate, potassium, protein-total, sodium, urea (blood urea nitrogen), and uric acid.

▪ Urine Samples:
- *Urinalysis:* a dipstick urinalysis will be performed for blood, glucose, ketone, protein, pH, specific gravity, nitrite, leukocytes, bilirubin, and urobilinogen
- *Urine drug screen:* for illicit drugs or drugs of abuse. Results of a positive drug screen will be reviewed by the study clinician for pattern of use.
- *Urine pregnancy test* for all women/people of childbearing potential

▪ ECG: Standard 12-lead ECG to check heart function

Washout Period: Minimum of 2-Weeks

Participants who are on concomitant medications (Section 5.6) must be tapered off at least 2 weeks prior to Baseline (V2). The plan for tapering off medications will be determined at the first screening visit (V1) with the participant and the study physician. During the washout period, the study physician will have weekly appointments with the participant to check how they are doing and ensure they are safe. The weekly appointments can be scheduled in-person or remote (via telephone/WebEx) based on the participant's preference and at the discretion of the study physician. Participants will be assessed for suicidality with the C-SSRS at each contact/visit.

Any safety assessment visits during the washout period will be called V1a, V1b, etc. During these visits, the C-SSRS and any changes in medications since the previous visit will be obtained in addition to other assessments at the study clinician's discretion.

Visit 2A (V2A)- Imaging Visit- between Visit 1 and Visit 2B

Visit 2A will occur at any time after Visit 1- Screening and Visit 2B-Baseline, depending on participant preference and appointment availability.

- A [^{18}F]SynVesT-1 scan with arterial line will be performed by CAMH imaging staff at the CAMH CAMH Brain Health Imaging Centre (250 College Street), as well as an MRI scan (1 hour).
  - Note: The PET scan and MRI scan can be performed on the same day or separated into different days, depending on participant preference and appointment availability. The MRI scan must occur within 2 weeks of the PET scan.

Visit 2B (V2B) - Baseline Visit - Day -7 to Day -1

The Baseline visit (V2) will occur approximately 2-6 weeks after the initial Screening (V1) when the participant has successfully been tapered off any concomitant medication. At the Baseline Visit (V2), the participant's eligibility will be confirmed by the study investigator by reviewing the Inclusion/Exclusion Criteria (Section 4.4) and updating the medical history. The Baseline visit (V2) can occur within 7 days before the anticipated psilocybin session and may be split over multiple days to reduce the burden on the participant (additional study visits will be labelled V2a, V2b etc). The following procedures will be performed and recorded at the Baseline visit (V2):

- Administered by trained study staff:
  - Vital Signs (blood pressure, heart rate)
  - C-SSRS
  - CGI
  - GAD-7
  - MADRS
  - SETS
  - SHAPS
  - WHOQOL-BREF
  - WEMWBS
- Clinician administered:
  - Confirmation of eligibility criteria
- Optional laboratory evaluations collected at the Queen Street CAMH laboratory:
    - Blood: 20 mL of blood will be withdrawn under fasting conditions (minimum 6-hour fast) from participants. The plasma will be separated by centrifuging the blood at 1500 rpm for 10 min at 4°C, and subsequently stored at -80°C for future analysis. This is optional for all participants.
  - *Biomarkers:*
    - Mitochondrial biomarkers: (1) circulating cell-free mitochondrial DNA (ccf-mtDNA): We will use the QIAmp 96 DNA Blood kit (Qiagen, Valence, USA) to extract DNA from 200µL of the plasma, according to manufacturer protocols for blood and body fluids. The total DNA will be quantified using spectrophotometric analysis at 260/280 nm in NanoPhotometer ® P-Class (Implen, Westlake Village, CA, US). Quantitative analysis of the ccf-mtDNA will be performed using a real-time polymerase chain reaction (RT-PCR). The PCR reaction will be performed using SYBR Green Technology (Thermo Fisher Scientific, Waltham, MA, USA). Each 20 µL reaction contained 5 µL of template, 1 µL of each primer (10 µM), 10 µL SYBR MIX (2× Sensifast, Bioline, London, UK), and 3 µL of nuclease-free water. Each reaction will be run in triplicate on CFX96 Touch Real-Time PCR Detection System (Bio-rad, Hercules, California, USA). PCR program: initial denaturation at 95 ◦C for 10 min, followed by 45 cycles consisting of 95 ◦C in 10 s (melting), 65 ◦C for 10 s (annealing), and 72C for 10sec (extension). The program ended with a melting curve analysis measuring

fluorescence continuously from 60 to 97 ◦C. We will use primers to amplify the mtDNA genes MT-ND1 and MT-ND4, as well as nuclear β-2 microglobulin.

- (2) mtDNA next-generation sequencing: Genomic DNA will be purified from blood samples (10 ml) (ChemagicTM MSM I DNA extractor; Perkin-Elmer, Waltham, MA) following manufacturer protocol. Briefly, whole mtDNA will be amplified in one large fragment of 16.6kb in length using long-range polymerase chain reaction approach. The mtDNA next generation sequencing (NGS) will be performed on Illumina MiSeq v3 device using 2x300 read length. Libraries will be prepared using standard Illumina DNA Preparation for NGS. The CAMH Biobank team will prepare the samples (pre-sequencing preparation), and send them to the Ontario Institute for Cancer Research facility for DNA sequencing.We will use in-house pipeline for raw data cleaning and variant call. (3) mtDNA-copy number: The mtDNA copy number will be assayed using the relative quantification method using a mtDNA amplicon and a nuclear single copy gene (β-2 microglobulin). The ratio of mtDNA to nuclear DNA will be quantified by 2−ΔCt method using primers for mtDNA and nuclear β-2 microglobulin as well as thermal cycler condition as standard. All qPCR will be performed on the BioRad CFX96 RT-PCR detection system (Bio-Rad Laboratories, Inc.). Each sample will be run in triplicate using 0.6ng of DNA in a 20µl reaction. The PCR reactions will be performed on separate runs with the same samples in the same well positions. Primers will be the same as describe for ccf-mtDNA.
- Neuroinflammation markers: Brain Derived Neurotrophic Factor (BDNF) and Inflammatory Cytokines,
- Plasma levels of Tryptophan, 5-HT and Kynurenine

- *Urine drug screen:* for illicit drugs or drugs of abuse. Results of a positive drug screen will be reviewed by the study clinician for pattern of use.
- *Urine pregnancy test* for all women of childbearing potential

- The Pre-Psilocybin Neurophysiology visit will occur at Baseline (V2B), within 7-days of Psilocybin Session Dose (V3). Neurophysiology procedures will be reviewed with participant prior to the following procedures being performed and recorded:
  Administered by trained study staff with a responsible Clinician available on site:
  - Assessment of TMS contraindications
  - Assessment of participant's resting motor threshold (RMT)
  - TMS-EEG stimulating left FC (SICI Paradigm)

- Preparatory session (up to 4 hours) with the study therapists which will involve building a therapeutic alliance, psychoeducation about the psychedelic experience, and setting intentions for the intervention.
  - Documentation of participant preference and consent for therapeutic touch
  - Note: Therapists will have the option to schedule an additional preparatory session at their discretion.

- For a more detailed explanation of the preparatory therapy session, please refer to the Yale Manual for Psilocybin-Assisted Therapy of Depression.
- Prep therapy can occur in-person or via secure videoconferencing software (i.e. WebEx).

- Clinical Laboratory Tests:
  - ECG
  - Urine Drug Screen

Visit 3 (V3) – Intervention – Day 0

The intervention will occur the day after Baseline (V2). The participant may have this session ≤7 days following the Baseline visit (V2). If the participant is out of the ≤7 day window, all baseline assessments are to be repeated. On the day of the intervention the following procedures will take place:

- Review and confirmation of participant preference and consent for therapeutic touch
- Review of neurophysiology tests with participant prior to dosing
- Study intervention administration (Section 5.0): 1 oral dose of 1mg of risperidone, followed 60-minutes later by 1 oral dose of 25mg of psilocybin, or 1 oral dose of 25mg of psilocybin alone, administered in conjunction with supportive therapy (PAP).
- Vital signs (body temperature, blood pressure and pulse) will be taken three times during this session (once prior to psilocybin administration, once at one hour post-administration and once at the end of the intervention session).
- At least one therapist will be present in the room at all times during PAP and be available to respond to participants' physical and emotional needs
- Participants will be instructed to lie on a bed in a non-clinical environment, and therapists will encourage participants to focus their attention inward and stay with any experience that arises. To enhance inward reflection, a pre-selected music playlist will be played. Administration of questionnaires or other instruments to be completed at the end of the dosing session when the acute effects of psilocybin have resolved:
  - Study team or therapist administered:
    - 5D-ASC to assess the acute drug effects using 5 primary dimensions and respective sub dimensions.
    - C-SSRS
  - Neurophysiology Testing: (Administered by trained study staff with a responsible clinician available on site):
    - 1 measurement approximately 1-2 hours after psilocybin administration
    - 1 measurement at the end of the visit

- The participant will be discharged 5-6 hours post-dosing when, in the opinion of the study PI (or delegate), the acute effects of psilocybin are resolved. The participant must be accompanied home by a caregiver who will remain with them for up to 24hrs after the dose was administered.
- Rescue medications are permitted during this visit as outlined in Section 5.6.

Visit 4 & Visit 5 (V4 & V5) – Post-Intervention – Day 1 & Day 7

- Administered by trained study staff or therapist/clinician:
  - CSSRS
  - CGI
  - MADRS
  - SHAPS
- Integrative psychotherapy session will occur with the study therapists. The participant will discuss their experience during the dose session including their thoughts, feelings, and experiences. Integration therapy can occur in-person or via secure videoconferencing software (i.e. WebEx). For more detailed information on the integrative therapy sessions, please refer to the therapists manual (Yale Manual for Psilocybin-Assisted Therapy of Depression).

On *Visit 4 (V4)* only

- Optional biological specimen collection and laboratory evaluations collected at the Queen Street CAMH laboratory:
  - Blood: 20mL of blood will be withdrawn under fasting conditions (minimum 6-hour fast). The plasma will be separated by centrifuging the blood at 1500 rpm for 10 min at 4°C, and subsequently stored at -80°C for future analysis. This is optional for all participants.
  - *Biomarkers:*
  - Mitochondrial biomarkers: (1) circulating cell-free mitochondrial DNA (ccf-mtDNA): We will use the QIAmp 96 DNA Blood kit (Qiagen, Valence, USA) to extract DNA from 200µL of the plasma, according to manufacturer protocols for blood and body fluids. The total DNA will be quantified using spectrophotometric analysis at 260/280 nm in NanoPhotometer ® P-Class (Implen, Westlake Village, CA, US). Quantitative analysis of the ccf-mtDNA will be performed using a real-time polymerase chain reaction (RT-PCR). The PCR reaction will be performed using SYBR Green Technology (Thermo Fisher Scientific, Waltham, MA, USA). Each 20 µL reaction contained 5 µL of template, 1 µL of each primer (10 µM), 10 µL SYBR MIX (2× Sensifast, Bioline, London, UK), and 3 µL of nuclease-free water. Each reaction will be run in triplicate on CFX96 Touch Real-Time PCR Detection System (Bio-rad, Hercules, California, USA). PCR program: initial denaturation at 95 ◦C for 10 min, followed by 45 cycles consisting of 95 ◦C in 10 s (melting), 65 ◦C for 10 s (annealing), and 72C for 10sec (extension). The program ended with a melting curve analysis measuring fluorescence continuously from 60 to 97 ◦C. We will use primers to amplify the mtDNA genes MT-ND1 and MT-ND4, as well as nuclear β-2 microglobulin.
  - (2) mtDNA next-generation sequencing: Genomic DNA will be purified from blood samples (10 ml) (ChemagicTM MSM I DNA extractor; Perkin-Elmer, Waltham, MA) following manufacturer protocol. Briefly, whole mtDNA will be amplified in one large fragment of 16.6kb in length using long-range polymerase chain reaction approach. The mtDNA next generation sequencing (NGS) will be performed on Illumina MiSeq v3 device using 2x300 read length. Libraries will be prepared using standard Illumina DNA

Preparation for NGS. The CAMH Biobank team will prepare the samples (pre-sequencing preparation), and send them to the Ontario Institute for Cancer Research facility for DNA sequencing.We will use in-house pipeline for raw data cleaning and variant call. (3) mtDNA-copy number: The mtDNA copy number will be assayed using the relative quantification method using a mtDNA amplicon and a nuclear single copy gene (β-2 microglobulin). The ratio of mtDNA to nuclear DNA will be quantified by 2−ΔCt method using primers for mtDNA and nuclear β-2 microglobulin as well as thermal cycler condition as standard. All qPCR will be performed on the BioRad CFX96 RT-PCR detection system (Bio-Rad Laboratories, Inc.). Each sample will be run in triplicate using 0.6ng of DNA in a 20µl reaction. The PCR reactions will be performed on separate runs with the same samples in the same well positions. Primers will be the same as describe for ccf-mtDNA.

- Neuroinflammation markers: Brain Derived Neurotrophic Factor (BDNF) and Inflammatory Cytokines
- Plasma levels of Tryptophan, 5-HT and Kynurenine

- **On *Visit 5 (V5)* only,** post-psilocybin neurophysiology assessments will be completed. The following procedures will be performed and recorded:
  Administered by trained study staff with a responsible Clinician available on site:
  - TMS-EEG (Visit 5 only) (Allowable window of +/- 3 days in case of scheduling issues)

Visit 5B (V5B)- Follow-up Imaging Visit

- A post-treatment *[^{18}F]SynVesT-1* scan with arterial line will be performed by CAMH imaging staff at the CAMH Brain Health Imaging Centre (250 College Street).
- This post-treatment scan can take place between 1 and 6 weeks after treatment administration.

Visit 6 (V6), Visit 7 (V7) & Visit 8 (V8) – Follow-Up: Week 2, Week 3 & Week 4

Follow-up visits occur at Weeks 2 (V6), 3 (V7), and 4 (V8) after the intervention. The following assessments will occur at each visit:

- Administered by trained study staff or clinician:
  - CSSRS
  - CGI
  - GAD-7 (except Visit 7)
  - MADRS
  - SHAPS
  - WHOQOL-BREF (except Visit 7)
  - WEMWBS (except Visit 7)
- Clinician administered:
  - Review of safety assessments

6.2 Schedule of Events

Procedures	Screening (Visit 1)	Washout period[1] (2-6 weeks)	Visit 2A (V2A)- Imaging Visit- between Visit 1 and Visit 2B	Baseline[2] (Visit 2B, Day -7 to Day -1)	**Intervention (Visit 3, Day 0)**	1-Day Post-Intervention (Visit 4, Day 1)	1-Week Post-Intervention (Visit 5A, Day 7)	Visit 5B (V5B)- Follow-up Imaging Visit	2-Weeks Post-Intervention (Visit 6, Day 14)	3-Weeks Post-
Location of Visit	Clinic	Clinic or Remote	CAMH Imaging Centre	Clinic	Clinic	Clinic	Clinic	CAMH Imaging Centre	Clinic or Remote	C R
Allowable Window		Weekly			≤7 days from Baseline	None	±3 day		±3 day	
Informed Consent	✓									
Demographics	✓									
Medical history	✓			✓						
Prior/concomitant medication review	✓			✓	✓	✓	✓		✓	
Inclusion/Exclusion Criteria Review	✓	✓		✓						
ATHF	✓									
CGI				✓		✓	✓		✓	
CSSRS[3]	✓	✓		✓	✓	✓	✓		✓	
HamD-17	✓									
MADRS				✓		✓	✓		✓	
SCID-5	✓									
Vital signs (blood pressure, pulse)	✓			✓	✓					
Vital signs (body temperature)					✓					
Weight	✓									
Height	✓									
ECG	✓									

Clinical laboratory tests[4]	✓			✓		✓				
Urinalysis	✓									
Urine drug screening	✓			✓						
Urine pregnancy test[5]	✓			✓						
Documentation of birth control	✓									
Preparatory/Integrative therapy & psychoeducation[6]				✓	✓	✓	✓			
Intervention (1mg of risperidone + 25mg of psilocybin / 25mg of psilocybin)					✓					
Neurophysiology (TMS-EEG)				✓	✓		✓			
Adverse event and serious adverse event review and evaluation	✓	✓		✓	✓	✓	✓		✓	
Source documentation & CRF completion	✓	✓		✓	✓	✓	✓		✓	
5D-ASC[7]					✓					
GAD-7				✓					✓	
SETS				✓						
SHAPS				✓		✓	✓		✓	
WEMWBS				✓					✓	
WHO-QOL-BREF				✓					✓	
Pre-Treatment PET scan[9]			✓							
MRI[9]			✓							
Post-Treatment PET scan[8]								✓		

1. Additional visits may be needed during the washout period to ensure adequate time for discontinuation of medication. Visits will occur on a weekly basis during this period (V1a, V1b, etc.). Review of medications and assessments for suicidality will occur in addition to other assessments at the discretion of the study investigator.
2. Baseline assessments can occur on separate days (≤7 days from the day of the intervention) to reduce the burden on participants. These visits will be V2a, V2b etc.
3. The "Last 12 Months" version will be administered at Screening and the "Since Last Visit" version will be administered at all other visits.
4. See Section 6.0: Research Procedures for complete list of required laboratory tests to be performed.
5. For women/females and people of child-bearing age only
6. Additional therapy visits may be scheduled at the discretion of the study therapists and/or the study investigator and prep as well as integration therapy can occur in-person or via WebEx.
7. To be administered immediately after the acute effects of psilocybin have subsided.
8. Can be administered anytime between 1-6 weeks post intervention administration (Visit 3)
9. Can be administered anytime between Screening (Visit 1) and Baseline (Visit 2)

Instruments:
ATHF: Antidepressant Treatment History Form; CGI: Clinical Global Impression; CSSRS: Columbia Suicide Severity Rating Scale; ECG: Electrocardiogram; GAD-7: Generalized Anxiety Disorder assessment form; HamD-17: Hamilton Depression Rating Scale; MADRS: Montgomery-Asberg Depression Rating Scale; SCID-5: Structured Clinical Interview for DSM-5; SETS: Stanford Expectations of Treatment Scale; SHAPS: Snaith Hamilton Anhedonia Scale; WEMWBS: Warwick-Edinburgh Mental Wellbeing Scale; WHO-QoL-BREF: World Health Organization Quality of Life abbreviated scale; 5D-ASC: 5-Dimensional Altered States of Consciousness Rating Scale.

7.0 STATISTICAL PLAN

7.1 Sample Size Determination

A formal sample size calculation was not conducted for the purposes of this feasibility/pilot study. We believe that the proposed sample of N = 12 participants will be suitable to establish feasibility to inform the development of a larger, adequately powered PET imaging study.

7.2 Statistical Methods

Characteristics of the trial cohort will be summarized by mean (SD), median (minimum, maximum). Summary raw scores will be presented at each assessment time both numerically and graphically. The small sample size means that conservative nonparametric testing is required in order to address the primary and secondary objectives. Exact paired permutation t-tests will be used to determine whether psilocybin-assisted psychotherapy with or without risperidone achieves a 50% reduction in MADRS.

PET analyses will be conducted using the region of interest (ROI) method in the PMOD platform. This software, in concert with SPM, (i) transforms a standard brain template with a set of predefined ROIs to match individual high-resolution MR images, (ii) refines the ROIs from the transformed template based on the gray matter probability of voxels in the individual MR images (segmentation step), and (iii) co-registers the individual MR images to the PET images so that the individual refined ROIs are transformed to the PET image space. The ROI template is based on the anatomical Hammers brain atlas(Hammers et al., 2003) as well as cytoarchitecturally-defined PFC subregions developed by Rajkowska(Chiuccariello et al., 2014; Rajkowska & Goldman-Rakic, 1995).

The primary ROIs are the hippocampus and prefrontal cortex subdivisions, the dorsolateral prefrontal cortex, orbitofrontal cortex, and medial prefrontal cortex. PET outcome measures include volume of distribution, V_T (primary outcome measure), and binding potential, BP_{ND} (secondary outcome measure). These will be determined within each ROI at each time point using arterial plasma input functions and the one-tissue compartment model (Naganawa et al., 2021). In the case that arterial blood sampling data is not available, data will be analyzed using SRTM2 to generate estimates of BP_{ND} (Naganawa et al., 2022).

Statistical analysis will be performed in R software. Change in synaptic density ([^{18}F]SynVesT-1) from pre- to post-treatment will be compared within subjects using a mixed effects linear model with subject as a random effect factors and ROI and session (baseline vs. post-treatment) as fixed effects. Post hoc paired t tests will assess synaptic density changes in each of the primary ROIs. Effects will be considered significant at the threshold of $p < 0.05$. Exploratory analyses will be performed in other brain regions relative to MDD and psilocybin treatment including hypothalamus, amygdala, and insula, as well as in voxel-wise whole brain analyses in order to identify other relevant regions and guide inquiry in a future, fully-powered study.

8.0 SAFETY AND ADVERSE EVENTS

8.1 Definitions

Adverse Event

An **adverse event** (AE) is any untoward medical occurrence in a research participant administered an investigational product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the investigational product.

AE severity can be defined as:

- *Mild:* discomfort noticed but no disruption of normal activity
- *Moderate:* discomfort sufficient to reduce or affect normal daily activity
- *Severe:* interferes significantly with the participant's normal activity or course of illness

Serious Adverse Event

A **serious adverse event** (SAE) is any AE that is:

- Fatal;
- Life-threatening;
- Requires or prolongs hospital stay;
- Results in persistent or significant disability or incapacity;
- A congenital anomaly or birth defect; or
- An important medical event (events that may not be life threatening but are of major clinical significance, such as a drug overdose or seizure that did not result in in-patient hospitalization).

Adverse Drug Reactions

An adverse drug reaction is any noxious, unintended or undesirable response to a medicinal product related to any dose.

Unexpected Adverse Reactions

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure).

Adverse Event Collection Period

AEs occurring as of the first screening visit and until the end of the study will be collected. AEs recorded during this period will be followed through to resolution, or until the event is assessed as chronic or stable.

Preexisting Condition

A preexisting condition is one that is present at the start of the clinical trial. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period. At the Screening Visit (V1), any clinically

significant abnormality will be recorded as a preexisting condition in source documentation. Where applicable and at the consent of the participant, additional information from the participant's healthcare provider including medical records, may be requested. Throughout the clinical trial, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

Post-study Adverse Event

At the last scheduled visit, the PI and/or QI should instruct each participant to report any subsequent event(s) that the participant believes might reasonably be related to participation in this clinical trial. The PI and/or QI should notify Health Canada of any death or adverse event (meeting reporting criteria) occurring at any time after a participant has discontinued or terminated participation that may reasonably be related to this clinical trial. Health Canada and Filament Health Corp. should also be notified if the PI and/or QI should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a participant that was involved in this clinical trial.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions are met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality;
- The abnormality suggests a disease and/or organ toxicity;
- The abnormality is of a degree that requires active management (e.g. change of dose, discontinuation of the study intervention, more frequent follow-up assessments, further diagnostic investigation, etc.); or
- Any laboratory abnormalities assessed as being clinically significant by a study physician or qualified individual.

8.2 Recording of Adverse Events

All adverse events occurring during the study period must be recorded. At each contact with the research participant, the research team must seek information on adverse events by specific questioning. Information on all adverse events should be recorded immediately in the data collection form and/or legal health record, and recorded in the adverse event log. All adverse events will be assessed the PI for relatedness, expectedness, seriousness, and severity in relation to the study intervention. All clearly related signs, symptoms, and abnormal diagnostic procedure results should be recorded in the data collection form and/or legal health record and assessed by the PI in a timely manner allowing sufficient time to meet required reporting timelines for SAEs and SUADRs (severe unexpected adverse drug reactions) if needed. Adverse events related to the study drug will be reported to Filament Health Corp within 24hrs of the study team becoming aware of the event. . These reports should not contain PHI.

8.3 Reporting of Serious Adverse Events

8.3.1 Investigator Reporting: Notifying the Sponsor

The sponsor for this study is CAMH and the PI/QI (Dr. Ishrat Husain), therefore no notification is necessary. However, Filament Health Corp. is the supplier of the psilocybin used in this trial. Filament Health Corp. will be sent safety reports on adverse events and serious adverse events within 24hrs of the study team becoming aware of the event. None of these safety reports will contain PHI and all data will be coded.

8.3.2 Investigator Reporting: Notifying the REB

The process for notification to the REB for applicable serious adverse events (SAEs) must be completed as per REB reporting requirements. SAEs and unanticipated events must be recorded and reported to the REB in accordance with the REB's reporting requirements and timelines. Copies of each report and documentation of REB notification and REB receipt/acknowledgement must be kept in the Investigator Study Binder.

8.3.3 Sponsor Reporting of SUADRs: Notifying Health Canada

The sponsor for this study is CAMH & the study PI/QI. The study PI/QI is responsible for reporting the safety information to Health Canada as required. The SUADR report must be reported to Health Canada in the following cases:

- Where the ADR is neither fatal nor life-threatening, within 15 days after becoming aware of the information
- Where it is fatal or life-threatening, immediately where possible and, in any event, within 7 days after becoming aware of the information
- And within 8 days after having informed Health Canada of the ADR, submit as complete as possible, a report which includes an assessment of the importance and implication of any findings.

8.3.4 Sponsor Reporting of SUADRs: Notifying Sites

Not applicable.

8.4 Reporting of Device Deficiencies

Not applicable.

8.5 Safety Management Plan

Safety of the participants (including data confidentiality) and the scientific integrity of the project will be ensured by the research team led by the PI. Participant safety will be monitored at each study visit by asking the participant about their experience and about any adverse events from the last study visit. All adverse events will be reviewed by the study PI and reported to the REB and/or Health Canada in accordance with the regulatory guidelines as outlined by each entity. Adverse events will be recorded and/or reported as

outlined in Section 8.2 and 8.3. Safety reports on AEs and SAEs will be provided to Filament Health within 24hrs of the study team becoming aware of the event. In addition, all safety data related to the psilocybin will be provided to Filament Health. . None of these safety reports will contain PHI and all data will be coded. The study team will also use a published Suicide Risk Management Protocol to as*sess* and reduce suicide risk (Herbeck et al., 2015). Participants experiencing a serious adverse event will be immediately withdrawn from the study. In the case of increased suicidality, the study physician will conduct an urgent psychiatric assessment with the participant.

The study investigator and study team will meet regularly to review the accrued data, data confidentiality, recruitment, and participants complaints. Participant confidentiality will be maintained through the use of code numbers to identify all participants. All research records will be kept in a locked file and no participants will be identified in any published report.

Participants may be removed from the study at the discretion of the PI. Reasons for possible withdrawal from the clinical trial are outlined in Section 4.7.1.

Therapeutic Risk Management Measures for Psychological Harm

Psychological well-being will be closely monitored by the study team and study therapists throughout the trial. Study therapists will provide the participant with information about what might be experienced during the dosing session, including physiological, sensory and psychological effects, and the possibility of challenging experiences. The role of the therapists at each of the therapy sessions is to provide support for the participant and create a psychologically safe environment. Therapists will work with the participant to develop grounding exercises according to the participant's preference (e.g., deep breathing, breath-focused awareness, progressive muscle relaxation etc.). These grounding techniques will be re-reviewed on the day of the intervention. Following dosing, integration therapy sessions will be conducted where participants can reflect on their dosing experience with the study therapists. For a full description of therapeutic safety monitoring procedures, refer to the Yale Manual for Psilocybin-Assisted Therapy of Depression. Participants will continue to be followed by the study team for up to 4-weeks after the intervention. Psychological well-being (including suicidality) and adverse event monitoring will be assessed at all time-points.

Remote Assessment Safety Procedures

All remote assessments will be conducted in a private room. The research team will not require identification from the participant as the research team will already be familiar with the participant and will be able to identify them visually through WebEx. The sessions occuring over WebEx or over the phone will not be recorded. If the assessment requires screen sharing, the individual administering the assessment will ensure that any documents or windows on the desktop containing PHI or personal information will be closed. The individual administering the assessment will also have access to necessary communication technology in order to communicate with relevant research supports or emergency services in case of an emergent situation. When sending invitations for

remote assessments or communicating via email, the research team will limit personal information in all emails by avoiding full names ot direct identifiers in the subject line of the email or meeting invitation.

8.6 Unblinding Procedures

Not applicable.

8.7 Data and Safety Monitoring Board

9.0 CLINICAL TRIAL DISCONTINUATION AND CLOSURE

9.1 Clinical Trial Discontinuation

This clinical trial may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause (i.e. closure based on PI decision, sponsor/funder decision, REB or other oversight bodies' decision; review of serious, unexpected and related AEs; noncompliance; futility). Notification, which includes the reason for study suspension or termination, will be provided by the suspending or terminating party to research participants, the PI, funding agency, CAMH, and regulatory authorities. If the clinical trial is prematurely terminated or suspended, the PI will promptly inform research participants, the REB, and the sponsor, and will provide the reason(s) for the termination or suspension. All communication with participants for this purpose will go through REB review and approval. Research participants will then be contacted, as applicable, and be informed of changes to the study visit schedule.

10.0 DATA HANDLING AND RECORD KEEPING

10.1 Source Documents & Case Report Forms

Source documentation will be recorded in data collection forms.
We will also collect health card information from all participants that will be securely transferred to ICES, which is a prescribed entity under section 45 of Ontario's Personal Health Information Privacy Act that is permitted to collect personally identifiable information for the purposes health system management and evaluation without individual consent or research ethics approval. The purpose of this transfer will be to link the individual's clinical data with health administrative databases to study long-term safety and efficacy outcomes. The maximum access date will be 15 years to evaluate long-term safety and efficacy outcomes.

REDCap

Data for this clinical trial will be managed using REDCap electronic case report forms. This system is maintained on central CAMH servers, with data backed up daily, and is supported by the Research Informatics department.

10.2 Protocol Deviations

No deviations from or changes to the protocol will be implemented without approval from the REB or Health Canada, unless to eliminate an immediate hazard to a participant. All study staff will monitor the study procedures to detect any potential protocol deviations. All potential protocol deviations will be reviewed by the study PI. The protocol deviation will be reported if any of the following criteria are met:

- Deviations that, in the opinion of the PI, jeopardize the safety of research participants, or that jeopardize the research efficacy or data integrity
- Any change in the approved process for obtaining consent
- Any deviations that lead to a serious adverse event or unanticipated problem
- Any unauthorized collection, use, or disclosure of personal health information (PHI)

10.3 Record Retention

Research records pertaining to this clinical trial will be retained for 15 years.

10.4 Clinical Trial Registration

In accordance with TCPS 2, a description of this trial will be registered on www.clinicaltrials.gov before the start of recruitment activities, and the content will be updated throughout the duration of the clinical trial. All results, including negative results should be entered at the completion of the clinical trial.

11.0 STUDY MONITORING, AUDITING, AND INSPECTING

11.1 Study Monitoring Plan

Site monitoring is conducted to ensure that the rights and well-being of research participants are protected, the reported trial data are accurate, complete, and verifiable, and the conduct of the clinical trial is in compliance with the currently approved protocol/amendment(s), ICH GCP, and applicable regulatory requirement(s). Reference the study monitoring plan for specific monitoring information.

11.2 Auditing and Inspecting

The PI and site will permit study-related audits, and inspections by the REB, CAMH, sponsor, and applicable granting agencies or regulatory bodies, including access to all study-related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The PI will ensure the capability for audits/inspections of

applicable study-related facilities (e.g. research pharmacy, clinical laboratory, imaging facility, etc.).

12.0 ETHICAL CONSIDERATIONS

12.1 Research Ethics Board (REB) Approval

Research Ethics Board (REB) approval will be obtained prior to beginning any research-specific procedures. Following initial ethics approval, ongoing ethical approval will be maintained and the clinical trial will undergo REB review at least annually, in accordance with regulatory and REB requirements. The clinical trial will be conducted in accordance with the REB-approved study documents and the determinations (including any limitations) of the REB, and in compliance with REB requirements. Any amendments to the protocol will require review and approval by the REB before the changes are implemented in the clinical trial, unless to eliminate an immediate hazard to the participant.

Whenever new information becomes available that may be relevant to participant consent, a consent form and/or consent for addendum will be presented to the REB for review and approval prior to its use. Any revised written information will receive REB approval prior to use.

12.2 Informed Consent Process & Documentation

Informed consent is a process that is initiated prior to the individual agreeing to take part in the clinical trial and continues throughout their participation.

Informed consent will be obtained from each participant prior to their participation in the clinical trial. Informed consent will be obtained by appropriately trained and qualified CAMH research personnel who do not have an existing clinical relationship with the participant or caregiver The PI will not obtain participant consent. Informed consent will be obtained in-person.

Each participant will be provided with a current copy of the REB approved ICF prior to the consent discussion. Research personnel will explain the clinical trial to the participant and answer any questions that may arise. This discussion will include an explanation of the clinical trial purpose, procedures, potential risks and benefits, confidentiality considerations and participant rights (e.g. participants will not be penalized or lose any benefits regardless of what they decide and they have the right to withdraw from the clinical trial at any time). Participants may take as much time as they need to make their decision, and may consult with others (e.g. family members, other health care providers, etc.) if they like. Following the consent discussion, and once the participant has decided to take part, the participant, and the person conducting the consent discussion will personally sign and date the ICF. Each participant will be provided with a complete (fully signed) copy of the ICF. The original ICF(s) and the informed consent process will be documented in the source documents.

Each study visit occurring onsite, including the consent visit, will follow the most current institutional IPAC guidelines put forth by CAMH to ensure staff and participants are protected against COVID-19 and other infectious diseases (e.g. participant screening upon entry, frequent hand-washing, masks for participants and staff).

13.0 PRIVACY AND CONFIDENTIALITY

All clinical trial-related documents and data will be held in strict confidence and stored at CAMH or on CAMH servers, and will follow CAMH policies and procedures to ensure participant privacy and confidentiality.

All research activities will be conducted in as private a setting as possible. The study team (including the PI), the study monitor, representatives of the REB, and Health Canada may inspect all documents and records required to be maintained by the PI, including but not limited to, medical records and pharmacy records for the participants in this clinical trial. The participant's contact information will be securely stored at CAMH for internal use during the clinical trial. At the end of the clinical trial, all records will continue to be kept in a secure location in accordance to applicable institutional and regulatory requirements. Safety reports on AEs and SAEs will be reported to Filament Health within 24hrs of the event occurring. In addition, safety data generated from the trial regarding the use of psilocybin and a report on the safety and efficacy of the clinical trial data will be provided to Filament Health Corp. None of these safety reports will contain PHI and all data will be coded.

There is a potential risk of breach of confidentiality that is inherent in all research protocols. Breach of confidentiality will be minimized by the research staff who will maintain research data (identified only by participant code number not related to name, or date of birth). A list of participant names, their ID numbers, and information about how they can be reached will be kept in a separate locked cabinet with access only to study personnel authorized by the PI. To minimize the risk of breach of confidentiality formal training sessions for all research staff emphasizing the importance of confidentiality will be conducted and formal mechanisms limiting access to information that can link data to individual participants will be monitored and established by study personnel. All information obtained from participants will be kept as confidential as possible. Computer-based files/data will be entered into password-secured databases (details below) and paper-based files will be stored in a secure location. These data will only be accessible to personnel involved in the study and they will abide by confidentiality regulations of the REB.

In unusual cases, a participant's research record may be released in response to a court order. If the research team learns that a participant or someone with whom the participant is involved with is in serious danger or harm, an investigator will inform the appropriate agencies.

Data from this study will be entered into a secure REDCap database. At point-of-entry, data values will undergo consistency edits (e.g., ID validation, range verification, duplicate detection) and personnel will be required to correct errors. Reports will be created via the REDCap program. Data management staff will run logic error programs to check for accuracy and irregularities within and across data structures and within and across sites. Quality assurance checks will be conducted regularly by study personnel. Although unlikely, instances may occur where REDCap is not available. In the case that this happens, we will follow the CAMH REDCap Downtime Procedures.

Imaging Data
Digital files of PET and MRI data will be considered source documents and will be stored on a password-locked computer without personal identifying information. The PSF will note the day and time of the PET scan, its number in the PET Centre Database, the injected radioactivity and a note to the effect that the scan was completed with no technical problems.

A secure central registry at the CAMH PET centre tracks research participants who complete PET scans to ensure radiation exposure does not exceed the annual or lifetime limits set by the centre and by the Canadian Nuclear Safety Commission. This registry records participants' name, date of birth, and participation in this and (if applicable) other studies. This information is maintained in a secure system not linked to other medical records and is accessible only by the PET Centre's Nuclear Safety Officer.

In accordance with Health Canada regulations for Basic Research Applications involving Positron-Emitting Radiopharmaceuticals, all data and all appropriate documentation will be stored for 10 years after the completion of the study, which meets the requirement for retention of study documents for at least 5 years after completion of the study.

14.0 CLINICAL TRIAL FINANCES

14.1 Funding Source

This study is funded by the Centre for Complex Interventions and the CAMH Brain Health Imaging Centre at the Centre for Addiction and Mental Health (CAMH).

14.2 Conflict of Interest

The research team does not have any conflicts of interest to disclose.

15.0 PUBLICATION POLICY/DATA SHARING

In the publication of the results of research, the investigators are obliged to preserve the confidentiality of all research participants. Participants will not be identified in any publication of research results. The results of this study will be published as group data without the use of characteristics that would identify individual participants. The study investigator will hold the primary responsibility for the publication of the results of the clinical trial. All publications will follow CAMH policies associated with publications.

15.1 Future Secondary Use of Data

De-identified data from this project may be used for future research by internal and/or external project collaborators in the future. The research team may share de-identified data with other researchers at CAMH or with collaborators around the world. Coded data that has been collected may also be combined with data collected from other people on other studies or it may be saved in a database. This is an optional part of the study for participants. On the ICF, participants can indicate whether they consent to allowing their data to be shared and/or pooled in the future.

16.0 REFERENCES

Broder, M. S., Bates, J. A., Jing, Y., Hebden, T., Forbes, R. A., & Chang, E. (2012). Association between second-generation antipsychotic medication half-life and hospitalization in the community treatment of adult schizophrenia. *J Med Econ, 15*(1), 105-111. https://doi.org/10.3111/13696998.2011.632042

Buchborn, T., Schröder, H., Höllt, V., & Grecksch, G. (2014, Jun). Repeated lysergic acid diethylamide in an animal model of depression: Normalisation of learning behaviour and hippocampal serotonin 5-HT2 signalling. *J Psychopharmacol, 28*(6), 545-552. https://doi.org/10.1177/0269881114531666

Busner, J., & Targum, S. D. (2007, Jul). The clinical global impressions scale: applying a research tool in clinical practice. *Psychiatry (Edgmont), 4*(7), 28-37.

Cameron, L. P., Benson, C. J., DeFelice, B. C., Fiehn, O., & Olson, D. E. (2019, Jul 17). Chronic, Intermittent Microdoses of the Psychedelic N,N-Dimethyltryptamine (DMT) Produce Positive Effects on Mood and Anxiety in Rodents. *ACS Chem Neurosci, 10*(7), 3261-3270. https://doi.org/10.1021/acschemneuro.8b00692

Cameron, L. P., Benson, C. J., Dunlap, L. E., & Olson, D. E. (2018, Jul 18). Effects of N, N-Dimethyltryptamine on Rat Behaviors Relevant to Anxiety and Depression. *ACS Chem Neurosci, 9*(7), 1582-1590. https://doi.org/10.1021/acschemneuro.8b00134

Cantù, F., Ciappolino, V., Enrico, P., Moltrasio, C., Delvecchio, G., & Brambilla, P. (2021, Feb 1). Augmentation with Atypical Antipsychotics for Treatment-Resistant Depression. *J Affect Disord, 280*(Pt A), 45-53. https://doi.org/10.1016/j.jad.2020.11.006

Carhart-Harris, R., Giribaldi, B., Watts, R., Baker-Jones, M., Murphy-Beiner, A., Murphy, R., Martell, J., Blemings, A., Erritzoe, D., & Nutt, D. J. (2021, Apr 15). Trial of Psilocybin versus Escitalopram for Depression. *N Engl J Med, 384*(15), 1402-1411. https://doi.org/10.1056/NEJMoa2032994

Carhart-Harris, R. L., Bolstridge, M., Rucker, J., Day, C. M., Erritzoe, D., Kaelen, M., Bloomfield, M., Rickard, J. A., Forbes, B., Feilding, A., Taylor, D., Pilling, S., Curran, V. H., & Nutt, D. J. (2016, Jul). Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. *Lancet Psychiatry, 3*(7), 619-627. https://doi.org/10.1016/s2215-0366(16)30065-7

Carter, B., Strawbridge, R., Husain, M. I., Jones, B. D. M., Short, R., Cleare, A. J., Tsapekos, D., Patrick, F., Marwood, L., Taylor, R. W., Mantingh, T., de Angel, V., Nikolova, V. L., Carvalho, A. F., & Young, A. H. (2020, Aug-Sep). Relative effectiveness of augmentation treatments for treatment-resistant depression: a systematic review and network meta-analysis. *Int Rev Psychiatry, 32*(5-6), 477-490. https://doi.org/10.1080/09540261.2020.1765748

Castaneda, A. E., Tuulio-Henriksson, A., Marttunen, M., Suvisaari, J., & Lönnqvist, J. (2008, Feb). A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. *J Affect Disord, 106*(1-2), 1-27. https://doi.org/10.1016/j.jad.2007.06.006

Chopko, T. C., & Lindsley, C. W. (2018, Jul 18). Classics in Chemical Neuroscience: Risperidone. *ACS Chem Neurosci, 9*(7), 1520-1529. https://doi.org/10.1021/acschemneuro.8b00159

COMPASS. (2021, November 9, 2021). *COMPASS Pathways announces positive topline results from groundbreaking phase IIb trial of investigational COMP360 psilocybin therapy for treatment-resistant depression*. COMPASS Pathways plc. Retrieved April 20 from https://ir.compasspathways.com/news-releases/news-release-details/compass-pathways-announces-positive-topline-results/

Correia, C., & Vicente, A. M. (2007, Aug). Pharmacogenetics of risperidone response and induced side effects. *Per Med, 4*(3), 271-293. https://doi.org/10.2217/17410541.4.3.271

Creese, I., Burt, D. R., & Synder, S. H. (1975, Dec 1). The dopamine receptor: differential binding of d-LSD and related agents to agonist and antagonist states. *Life Sci, 17*(11), 1715-1719. https://doi.org/10.1016/0024-3205(75)90118-6

Davis, A. K., Barrett, F. S., May, D. G., Cosimano, M. P., Sepeda, N. D., Johnson, M. W., Finan, P. H., & Griffiths, R. R. (2021, May 1). Effects of Psilocybin-Assisted Therapy on Major Depressive Disorder: A Randomized Clinical Trial. *JAMA Psychiatry, 78*(5), 481-489. https://doi.org/10.1001/jamapsychiatry.2020.3285

Dittrich, A. (1998). The standardized psychometric assessment of altered states of consciousness (ASCs) in humans [doi:10.1055/s-2007-979351]. *Pharmacopsychiatry, 31*, 80-84. https://doi.org/10.1055/s-2007-979351

Gaynes, B. N., Lux, L., Gartlehner, G., Asher, G., Forman-Hoffman, V., Green, J., Boland, E., Weber, R. P., Randolph, C., Bann, C., Coker-Schwimmer, E., Viswanathan, M., & Lohr,

K. N. (2020, Feb). Defining treatment-resistant depression. *Depress Anxiety, 37*(2), 134-145. https://doi.org/10.1002/da.22968

Griffiths, R. R., Johnson, M. W., Carducci, M. A., Umbricht, A., Richards, W. A., Richards, B. D., Cosimano, M. P., & Klinedinst, M. A. (2016, Dec). Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial. *J Psychopharmacol, 30*(12), 1181-1197. https://doi.org/10.1177/0269881116675513

Grob, C. S., Danforth, A. L., Chopra, G. S., Hagerty, M., McKay, C. R., Halberstadt, A. L., & Greer, G. R. (2011, Jan). Pilot study of psilocybin treatment for anxiety in patients with advanced-stage cancer. *Arch Gen Psychiatry, 68*(1), 71-78. https://doi.org/10.1001/archgenpsychiatry.2010.116

Gukasyan, N., Davis, A. K., Barrett, F. S., Cosimano, M. P., Sepeda, N. D., Johnson, M. W., & Griffiths, R. R. (2022, Feb). Efficacy and safety of psilocybin-assisted treatment for major depressive disorder: Prospective 12-month follow-up. *J Psychopharmacol, 36*(2), 151-158. https://doi.org/10.1177/02698811211073759

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Halberstadt, A. L., Koedood, L., Powell, S. B., & Geyer, M. A. (2011). Differential contributions of serotonin receptors to the behavioral effects of indoleamine hallucinogens in mice. *Journal of Psychopharmacology, 25*(11), 1548-1561. https://doi.org/10.1177/0269881110388326

Hamilton, M. (1960, Feb). A rating scale for depression. *J Neurol Neurosurg Psychiatry, 23*(1), 56-62. https://doi.org/10.1136/jnnp.23.1.56

Harvey, J. A. (2003). Role of the serotonin 5-HT2A receptor in learning. *Learning & Memory, 10*(5), 355-362.

Hasler, F., Bourquin, D., Brenneisen, R., Bär, T., & Vollenweider, F. X. (1997, Jun). Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. *Pharm Acta Helv, 72*(3), 175-184. https://doi.org/10.1016/s0031-6865(97)00014-9

Hasler, F., Bourquin, D., Brenneisen, R., & Vollenweider, F. X. (2002, 2002/09/05/). Renal excretion profiles of psilocin following oral administration of psilocybin: a controlled study in man. *Journal of Pharmaceutical and Biomedical Analysis, 30*(2), 331-339. https://doi.org/https://doi.org/10.1016/S0731-7085(02)00278-9

Hasler, F., Grimberg, U., Benz, M. A., Huber, T., & Vollenweider, F. X. (2004, Mar). Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose-effect study. *Psychopharmacology (Berl), 172*(2), 145-156. https://doi.org/10.1007/s00213-003-1640-6

Hesselgrave, N., Troppoli, T. A., Wulff, A. B., Cole, A. B., & Thompson, S. M. (2021, Apr 27). Harnessing psilocybin: antidepressant-like behavioral and synaptic actions of psilocybin are independent of 5-HT2R activation in mice. *Proc Natl Acad Sci U S A, 118*(17). https://doi.org/10.1073/pnas.2022489118

Hibicke, M., Landry, A. N., Kramer, H. M., Talman, Z. K., & Nichols, C. D. (2020, Mar 18). Psychedelics, but Not Ketamine, Produce Persistent Antidepressant-like Effects in a Rodent Experimental System for the Study of Depression. *ACS Chem Neurosci, 11*(6), 864-871. https://doi.org/10.1021/acschemneuro.9b00493

Ho, S. C., Jacob, S. A., & Tangiisuran, B. (2017). Barriers and facilitators of adherence to antidepressants among outpatients with major depressive disorder: A qualitative study. *PLoS One, 12*(6), e0179290. https://doi.org/10.1371/journal.pone.0179290

Horsley, R. R., Páleníček, T., Kolin, J., & Valeš, K. (2018). Psilocin and ketamine microdosing: effects of subchronic intermittent microdoses in the elevated plus-maze in male Wistar rats. *Behavioural pharmacology, 29*(6), 530-536.

Johnson, M., Richards, W., & Griffiths, R. (2008, Aug). Human hallucinogen research: guidelines for safety. *J Psychopharmacol, 22*(6), 603-620. https://doi.org/10.1177/0269881108093587

Johnson, M. W., Hendricks, P. S., Barrett, F. S., & Griffiths, R. R. (2019, May). Classic psychedelics: An integrative review of epidemiology, therapeutics, mystical experience, and brain network function. *Pharmacol Ther, 197*, 83-102. https://doi.org/10.1016/j.pharmthera.2018.11.010

Li, G., Zhang, L., DiBernardo, A., Wang, G., Sheehan, J. J., Lee, K., Reutfors, J., & Zhang, Q. (2020). A retrospective analysis to estimate the healthcare resource utilization and cost associated with treatment-resistant depression in commercially insured US patients. *PLoS One, 15*(9), e0238843. https://doi.org/10.1371/journal.pone.0238843

Lindenblatt, H., Krämer, E., Holzmann-Erens, P., Gouzoulis-Mayfrank, E., & Kovar, K.-A. (1998, 1998/05/29/). Quantitation of psilocin in human plasma by high-performance liquid chromatography and electrochemical detection: comparison of liquid–liquid extraction with automated on-line solid-phase extraction. *Journal of Chromatography B: Biomedical Sciences and Applications, 709*(2), 255-263. https://doi.org/https://doi.org/10.1016/S0378-4347(98)00067-X

Madsen, M. K., Fisher, P. M., Burmester, D., Dyssegaard, A., Stenbæk, D. S., Kristiansen, S., Johansen, S. S., Lehel, S., Linnet, K., Svarer, C., Erritzoe, D., Ozenne, B., & Knudsen, G. M. (2019, Jun). Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels. *Neuropsychopharmacology, 44*(7), 1328-1334. https://doi.org/10.1038/s41386-019-0324-9

Madsen, P. L., Hasselbalch, S. G., Hagemann, L. P., Olsen, K. S., Bülow, J., Holm, S., Wildschiødtz, G., Paulson, O. B., & Lassen, N. A. (1995, May). Persistent resetting of the cerebral oxygen/glucose uptake ratio by brain activation: evidence obtained with the Kety-Schmidt technique. *J Cereb Blood Flow Metab, 15*(3), 485-491. https://doi.org/10.1038/jcbfm.1995.60

McClain, C. S., Rosenfeld, B., & Breitbart, W. (2003, May 10). Effect of spiritual well-being on end-of-life despair in terminally-ill cancer patients. *Lancet, 361*(9369), 1603-1607. https://doi.org/10.1016/s0140-6736(03)13310-7

Meltzer, H. Y., Matsubara, S., & Lee, J. C. (1989). The ratios of serotonin2 and dopamine2 affinities differentiate atypical and typical antipsychotic drugs. *Psychopharmacol Bull, 25*(3), 390-392.

Montgomery, S. A., & Asberg, M. (1979, Apr). A new depression scale designed to be sensitive to change. *Br J Psychiatry, 134*, 382-389. https://doi.org/10.1192/bjp.134.4.382

Nutt, D. J., King, L. A., & Phillips, L. D. (2010, Nov 6). Drug harms in the UK: a multicriteria decision analysis. *Lancet, 376*(9752), 1558-1565. https://doi.org/10.1016/s0140-6736(10)61462-6

Perkins, D., Sarris, J., Rossell, S., Bonomo, Y., Forbes, D., Davey, C., Hoyer, D., Loo, C., Murray, G., Hood, S., Schubert, V., Galvão-Coelho, N. L., O'Donnell, M., Carter, O., Liknaitzky, P., Williams, M., Siskind, D., Penington, D., Berk, M., & Castle, D. (2021, Dec). Medicinal psychedelics for mental health and addiction: Advancing research of an emerging paradigm. *Aust N Z J Psychiatry, 55*(12), 1127-1133. https://doi.org/10.1177/0004867421998785

Reutfors, J., Andersson, T. M., Brenner, P., Brandt, L., DiBernardo, A., Li, G., Hägg, D., Wingård, L., & Bodén, R. (2018, Oct 1). Mortality in treatment-resistant unipolar depression: A register-based cohort study in Sweden. *J Affect Disord, 238*, 674-679. https://doi.org/10.1016/j.jad.2018.06.030

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

Rush, A. J., Trivedi, M. H., Wisniewski, S. R., Nierenberg, A. A., Stewart, J. W., Warden, D., Niederehe, G., Thase, M. E., Lavori, P. W., Lebowitz, B. D., McGrath, P. J., Rosenbaum, J. F., Sackeim, H. A., Kupfer, D. J., Luther, J., & Fava, M. (2006, Nov). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry, 163*(11), 1905-1917. https://doi.org/10.1176/ajp.2006.163.11.1905

Schatzberg, A. F., & Nemeroff, C. B. (2009). *The American psychiatric publishing textbook of psychopharmacology*. American Psychiatric Pub.

Snaith, R. P., Hamilton, M., Morley, S., Humayan, A., Hargreaves, D., & Trigwell, P. (1995, Jul). A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale. *Br J Psychiatry, 167*(1), 99-103. https://doi.org/10.1192/bjp.167.1.99

Spitzer, R. L., Kroenke, K., Williams, J. B., & Löwe, B. (2006, May 22). A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med, 166*(10), 1092-1097. https://doi.org/10.1001/archinte.166.10.1092

Studerus, E., Gamma, A., & Vollenweider, F. X. (2010, Aug 31). Psychometric evaluation of the altered states of consciousness rating scale (OAV). *PLoS One, 5*(8), e12412. https://doi.org/10.1371/journal.pone.0012412

Vandenberghe, F., Guidi, M., Choong, E., von Gunten, A., Conus, P., Csajka, C., & Eap, C. B. (2015, Dec). Genetics-Based Population Pharmacokinetics and Pharmacodynamics of Risperidone in a Psychiatric Cohort. *Clin Pharmacokinet, 54*(12), 1259-1272. https://doi.org/10.1007/s40262-015-0289-8

Visser, A., Garssen, B., & Vingerhoets, A. (2010, Jun). Spirituality and well-being in cancer patients: a review. *Psychooncology, 19*(6), 565-572. https://doi.org/10.1002/pon.1626

Vollenweider, F. X., Leenders, K. L., Oye, I., Hell, D., & Angst, J. (1997, Feb). Differential psychopathology and patterns of cerebral glucose utilisation produced by (S)- and (R)-ketamine in healthy volunteers using positron emission tomography (PET). *Eur Neuropsychopharmacol, 7*(1), 25-38. https://doi.org/10.1016/s0924-977x(96)00042-9

Vollenweider, F. X., Vollenweider-Scherpenhuyzen, M. F., Bäbler, A., Vogel, H., & Hell, D. (1998, Dec 1). Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport, 9*(17), 3897-3902. https://doi.org/10.1097/00001756-199812010-00024

Wiles, N., Thomas, L., Abel, A., Ridgway, N., Turner, N., Campbell, J., Garland, A., Hollinghurst, S., Jerrom, B., Kessler, D., Kuyken, W., Morrison, J., Turner, K., Williams, C., Peters, T., & Lewis, G. (2013, Feb 2). Cognitive behavioural therapy as an adjunct to pharmacotherapy for primary care based patients with treatment resistant depression: results of the CoBalT randomised controlled trial. *Lancet, 381*(9864), 375-384. https://doi.org/10.1016/s0140-6736(12)61552-9

Wilkinson, S. T., Kitay, B. M., Harper, A., Rhee, T. G., Sint, K., Ghosh, A., Lopez, M. O., Saenz, S., & Tsai, J. (2021, Jul 1). Barriers to the Implementation of Electroconvulsive Therapy (ECT): Results From a Nationwide Survey of ECT Practitioners. *Psychiatr Serv, 72*(7), 752-757. https://doi.org/10.1176/appi.ps.202000387

Younger, J., Gandhi, V., Hubbard, E., & Mackey, S. (2012, Dec). Development of the Stanford Expectations of Treatment Scale (SETS): a tool for measuring patient outcome expectancy in clinical trials. *Clin Trials, 9*(6), 767-776. https://doi.org/10.1177/1740774512465064