# Study Protocol with Statistical Analysis Plan

**Study Title**: Persisting Effects of Psilocybin

**Clinical Trials ID (NCT number)**: NCT02971605

**Last Protocol Approval Date**: April 24, 2017

This open-label, non-blinded study will involve 12 volunteers who will receive a high dose of psilocybin under supportive conditions. Assessments (questionnaires and emotional tasks) will be conducted during screening sessions, and 1 day before, 1 week after, and 1 month after the high-dose psilocybin session. Functional magnetic resonance imaging (fMRI) will also be conducted 1 day before, 1 week after, and 1 month after the psilocybin session.

Primary objectives:

1. Assess the effects of psilocybin on the neural circuitry of emotion processing as measured by functional magnetic resonance imaging (fMRI) at 1 week and 1 month timepoints after psilocybin administration

Secondary objectives:

1. Assess the effects of psilocybin on emotional processing at 1 week and 1 month timepoints after psilocybin administration, well after acute subjective effects of the drug have subsided
2. Assess the effects of psilocybin on personality at 1 week and 1 month timepoints after psilocybin administration

**Inclusion/Exclusion Criteria**

Overview of Screening Procedures: Participants will be screened via telephone to determine whether they meet major inclusion/exclusion criteria, and thus whether they are eligible for an in-person screening session. Participants who do not fail telephone screening will be invited to BPRU on the Johns Hopkins Bayview Campus for in-person screening.

Consent will be obtained at the BPRU at a scheduled meeting after participants have passed telephone screening. Participants will be allowed to take as much time as necessary to decide whether or not to sign consent. Study staff will discuss the consent form with the participant after the participant has read the consent form. Study staff will ask questions to assess the individual's understanding of the consent form. Participants may take the consent form home to review and return to sign consent if they wish. Participants who might be eligible for a future study will be asked to complete IRB-approved HIPAA-IRB Form 3 "Authorization to contact you about future research studies". Participants' files will be kept in a locked room and treated as confidential research material. All data will be collected using the unique identification code numbers assigned to each participant upon entry to the study. No individual identifiable information will be released without written authorization. These procedures have been used in the past to protect confidentiality of study data and no instance of loss of confidential information has occurred.

Participants will be physically and psychologically healthy adult participants (approximately equal numbers of males and females) 18 to 45 years old. Non-English speakers and those with language or hearing impairments will not participate in the study. Participants will be recruited primarily from the Baltimore/Washington DC metro area. Participants will have used hallucinogens in the past, although preference will be given to those who have had minimal prior use (e.g. 1 to 2 prior uses) that was at least 3 months ago. Potential participants will be carefully screened to eliminate those with significant medical or psychiatric illnesses (see below for specific inclusion/exclusion criteria).

We expect to consent up to 100 volunteers at Johns Hopkins (some of whom will not pass the in-person screening), in order to achieve 12 participants who provide complete data.

Screening evaluation will include a history and physical examination, ECG, a 30 cc blood draw for medical screening (e.g., complete blood count and Quest Test 18T Comprehensive metabolic panel with eGFR), a personal and family medical history questionnaire, psychiatric interview (SCID-5), and a urine drug test.

As per standard BPRU screening procedures, screening physical examination and ECGs will be performed by BPRU medical staff (nurse, mid-level, or physician). ECGs will be interpreted by a cardiologist credentialed and privileged to read ECGs at Bayview Medical Center to determine if a significant abnormality exists. Participants will be requested to refrain from illicit drug use during the course of the study and a urine test will be conducted before the drug session (e.g., various opioids, stimulants and sedatives). Pregnant or nursing women are ineligible; female participants will receive a urine pregnancy test at intake and before the drug session, and must use effective methods of contraception during the study. With regard to psychiatric screening, clinical interviews and a structured psychiatric diagnostic interview (e.g., SCID) will be used to obtain baseline psychological data, including psychiatric history (including family history), psychoactive drug-use history, and information about employment status and current functioning (including mood and psychological and psychosomatic symptoms). Participants will be eligible if they are judged to be psychologically stable and do not meet any of the psychiatric exclusion criteria (as defined by DSM-V). Finally, participants will be required to meet standard inclusion/exclusion guidelines for brain imaging protocols (see below). All inclusion and exclusion criteria are described below:

Inclusion criteria:

- 18 to 45 years old
- Have given written informed consent
- Have at least a high-school level of education or equivalent (e.g. GED), and be fluent in English
- Be healthy and psychologically stable as determined by screening for medical and psychiatric problems via a personal interview, a medical questionnaire, a physical examination, an electrocardiogram (ECG), and routine medical blood and urinalysis laboratory tests
- Agree to consume approximately the same amount of caffeine-containing beverage (e.g., coffee, tea) that he/she consumes on a usual morning, before arriving at the research unit on the morning of the drug session day. If the participant does not routinely consume caffeinated beverages, he/she must agree not to do so on the session day.
- Agree to refrain from using any psychoactive drugs, including alcoholic beverages and nicotine, within 24 hours of each drug administration. The exception is caffeine.
- Agree not to take any PRN medications on the mornings of drug sessions
- Agree not to take sildenafil (Viagra®), tadalafil, or similar medications within 72 hours of each drug administration.
- Agree that for one week before each drug session, he/she will refrain from taking any nonprescription medication, nutritional supplement, or herbal supplement except when approved by the study investigators. Exceptions will be evaluated by the study investigators and will include acetaminophen, non-steroidal anti-inflammatory drugs, and common doses of vitamins and minerals.
- Have limited lifetime use of hallucinogens (at least one prior use of a hallucinogen, but preference will be given to those with the fewest number of prior exposures to a hallucinogen)
- Last hallucinogen use was at least 3 months ago (but preference will be given to those with a greater duration of time since last hallucinogen use)

General medical exclusion criteria:

- Women who are pregnant (as indicated by a positive urine pregnancy test assessed at intake and before each drug session) or nursing; women who are of child-bearing potential and sexually active who are not practicing an effective means of birth control.
- Cardiovascular conditions: coronary artery disease, stroke, angina, uncontrolled hypertension, a clinically significant ECG abnormality (e.g., atrial fibrilation), artificial heart valve, or TIA in the past year
- Epilepsy with history of seizures
- Insulin-dependent diabetes; if taking oral hypoglycemic agent, then no history of hypoglycemia

- Currently taking psychoactive prescription medication on a regular (e.g., daily) basis
- Currently taking on a regular (e.g., daily) basis any medications having a primary centrally-acting serotonergic effect, including MAOIs. For individuals who have intermittent or PRN use of such medications, psilocybin sessions will not be conducted until at least 5 half-lives of the agent have elapsed after the last dose.
- More than 20% outside the upper or lower range of ideal body weight according to Metropolitan Life height and weight table

Psychiatric Exclusion Criteria:

- Current or past history of meeting DSM-5 criteria for schizophrenia spectrum or other psychotic disorders (except substance/medication-induced or due to another medical condition), or Bipolar I or II Disorder
- Current or past history within the last 5 years of meeting DSM-5 criteria for a moderate or severe alcohol or drug use disorder (excluding caffeine and nicotine)
- Have a first or second-degree relative with schizophrenia spectrum or other psychotic disorders (except substance/medication-induced or due to another medical condition), or Bipolar I or II Disorder
- Has a psychiatric condition judged to be incompatible with establishment of rapport or safe exposure to psilocybin

Additional MRI Exclusion Criteria:

- Head trauma
- Claustrophobia incompatible with scanning
- Cardiac pacemaker
- Implanted cardiac defibrillator
- Aneurysm brain clip
- Inner ear implant
- Prior history as a metal worker and/or certain metallic objects in the body -- must complete MRI screening form (see eIRB Study Documents) and be approved by MRI technologist before each scan

Cardiovascular screening: There will be at least four blood pressure assessment occasions over at least two separate days. Within a day, assessment occasions will be separated by at least 15 minutes. Each assessment occasion will involve two or more blood pressure readings. To qualify for the study, the mean blood pressure (mm Hg) of the four or more assessment occasions will not exceed 140 systolic and 90 diastolic.

Blood pressure will be taken while subjects are at rest and have been seated or supine for at least 5 minutes. As recommended by the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, these assessments will involve the average of 2 or more readings separated by two minutes. If the first 2 readings differ by more than 5 mm Hg, additional readings will be obtained and averaged. On one or more of the blood pressure measurement occasions, the volunteer will be acclimated to the automated blood pressure monitoring equipment by repeatedly taking blood pressure (at least 3 readings) with the device. Because it has been our experience that time-to-time blood pressure readings with the automated equipment can be variable due to measurement artifact, any reading that initially exceeds our threshold value will be reassessed twice within 4 minutes to assure accuracy.

Individuals who do not pass screening or choose not to participate will be asked whether they want us to keep their personal contact information. Identifiers for those individuals who do not want us to retain their information will be destroyed.

**Drugs/ Substances/ Devices**

Psilocybin dose: The dose of psilocybin administered in this study will be a moderately high dose (25 mg/70 kg). Psilocybin doses will be calculated based on body weight, which is consistent with our previous published studies (Griffiths et al. 2006, Griffiths et al. 2011, Johnson et al. 2014) , controlled studies of psilocybin conducted in other research laboratories (Studerus et al. 2011) , and our ongoing protocols. Evidence from our psilocybin dose-effects study (Griffiths et al., 2011) and a study of psilocybin compared to an active control drug (Griffiths et al., 2006) suggests that similar doses (20 mg/70 kg to 30 mg/70 kg) are associated with strong psychoactive effects, mystical-type effects, and enduring positive effects on mood and emotions that last for at least 14 months (Griffiths et al., 2008; Griffiths et al., 2011). The proposed 25 mg/70 kg dose is less than the maximum dose that has been administered in our laboratory, and it is in the range of doses examined extensively in previous studies with hallucinogen-naïve individuals (Malitz et al. 1960, Metzner, Litwin & Weil 1965, Pahnke 1969, Pahnke et al. 1969, Leuner 1962) . The dose in the current study is considerably less than that (49 mg/70kg) administered safely under a previously authorized IND (Strassman 1992, 1998).

**Study Statistics**

Emotion processing tasks: Three well–established emotional processing tasks will be assessed in the fMRI scanner: an emotion recognition task, an emotional conflict Stroop task, and an emotional discrimination task. In the emotion recognition task (Gur et al. 2002, Moser et al. 2007, Gur et al. 2010) , participants identify positive and negative emotional facial expressions, with identification of the gender of actors as a control task. The primary outcome measures of this task are accuracy, response time, and average BOLD response to positive and negative facial expressions during emotion identification compared to gender identification. In the emotional conflict Stroop task (Etkin et al. 2006, Egner et al. 2008) , participants identify the valence of emotional facial expressions (targets) with overlaid emotional words (distractors). Targets and distractors may have congruent or incongruent emotional valence. Primary outcome measures are response time, accuracy, and average BOLD response to incongruent compared to congruent trials, separated by the emotional valence of the distractor. The emotional discrimination task consists of selecting between neutral and negative facial expressions (Hariri et al. 2002, Hariri et al. 2003, Kraehenmann et al. 2015) , with selection of geometric figures as a control task. The primary outcome measure is BOLD response in the amygdala to facial affect discrimination compared to the control task (psilocybin was not shown to acutely affect accuracy and response time) (Kraehenmann et al. 2015) . Participants will practice these tasks at each preparation meeting (Visits 3 and 4), before the baseline fMRI scan.

Resting-State Scans: Blood-oxygen dependent level (BOLD) data will be collected for 12 minutes during a period of rest. The participant will be asked to simply rest with eyes opened and focused on a single point in the participant's view. This scan will allow us to assess the long-term effects of psilocybin on resting-state brain function, and it will also allow us to assess static and dynamic functional connectivity between brain regions and compare results to numerous other fMRI studies of resting-state brain activity.

Longitudinal Emotion and Social Questionnaires: A battery of questionnaires will be completed one day before, one week after, and one month after psilocybin to assess emotional and social changes. This includes general [the Positive and Negative Affect Scale - X (Watson, Clark 1994) and the Profile of Mood States (McNair, Lorr & Droppleman 1992) ] and emotion-specific [the Dispositional Positive Emotions Scale (Shiota, Keltner & John 2006) , the Depression Anxiety Stress Scale (Lovibond, Lovibond 1995) , and the State Trait Anxiety Inventory (Spielberger et al. 1983) ] measures, as well as a measure of social cognition [the Social Value Orientation test (Murphy, Ackermann, & Handgraaf, 2011)]. Outcome measures will consist of the validated scale scores for each questionnaire.

Longitudinal and Follow-Up Questionnaires: Participants will complete the Big Five Inventory (BFI) (John, Naumann & Soto 2008) , the Brief Affective Neuroscience Personality Scales (BANPS) (Barrett, Robins & Janata 2013) , the Tellegen Absorption Scale (Tellegen, Atkinson 1974) , and the Emotion Regulation Questionnaire (Gross, John 2003) which are validated measures of personality traits. These questionnaires will be administered during screening and at the 1-month post-psilocybin imaging session, in order to test the hypothesis that openness and positive emotional personality traits will increase, and negative emotional personality traits will decrease, after psilocybin administration. A prior study in our laboratory found changes in the domain of openness following psilocybin (MacLean, Johnson & Griffiths 2011) .

**Statistical plan**

Assessing the effects of psilocybin on emotional processing. We hypothesize that psilocybin administration will lead to impaired recognition of negative emotional facial expressions, decreased emotional conflict to incongruent emotional stimuli during an emotional conflict processing task, decreased negative affect, and increased positive affect one week and one month after psilocybin administration, relative to baseline. Outcome measures of the emotion recognition task and emotional conflict Stroop task will be separately submitted to two-way repeated measures ANOVA, with assessment time-point (baseline, one week and one month post-psilocybin) and emotional valence of stimuli (positive vs negative) as within-subject factors. F-tests will be used to assess whether there is a main effect of assessment time point and an effect of the interaction between assessment time point and emotional valence on each outcome measure. Planned comparisons will be used to test the hypothesis of impaired responding to negative emotional stimuli (confirmed by finding increased reaction time and decreased accuracy), and facilitated responding to positive emotional stimuli (confirmed by finding decreased reaction time and increased accuracy) at each post-psilocybin time point (one week, one month) compared to baseline. Outcome measures of the longitudinal questionnaires will be separately submitted to a one-way repeated measures ANOVA, with assessment time-point as a within-subject factor. An F-test will be used to assess whether there is a main effect of assessment time point on each outcome measure.

Assessing the effects of psilocybin on the neural circuitry of emotion processing. We hypothesize that psilocybin administration will reduce amygdala BOLD response to negative emotional stimuli during emotion recognition and discrimination tasks, and ACC BOLD response during emotional conflict processing, one week and one month post-psilocybin. Whole-brain BOLD EPI data will undergo standard preprocessing (realignment/motion correction, co-registration across runs, normalization to the MNI template, smoothing, motion sensoring or “scrubbing” (Power et al. 2012) , physiological artifact correction (Behzadi et al. 2007) , 36-parameter confound regression and spike regression (Satterthwaite et al. 2013) ). Regions of interest (ROI) analysis will be performed, with ACC and amygdala as seed regions, to test the hypothesis of decreased BOLD response to negative stimuli and increased BOLD response to positive stimuli in these regions one week and one month post-psilocybin. The first eigenvector of the signal from all voxels in each region will be submitted to a mixed effects repeated measures ANOVA, with time point and the emotional valence of stimuli as within-subject main effects, and subject as a random effect. Subject-level contrasts will assess effects of task condition at each time point. Second-level t-tests will be used for planned comparisons to test the hypothesis of lower BOLD response to negative stimuli and higher BOLD response to positive stimuli post-psilocybin. We will repeat these analyses as whole-brain voxel-wise analyses to evaluate change outside of hypothesized regions. Preprocessing and whole-brain statistical analysis will be conducted using Statistical Parametric Mapping (SPM; http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB. ROI analysis will be conducted using MarsBaR (http://marsbar.sourceforge.net/).

Secondary Outcome Questionnaire Measures: Repeated-measures ANOVA will be used when appropriate to assess change between groups and over time within groups in secondary outcome measures. Planned

comparisons will investigate change in questionnaire test scores between pre-session and post-session timepoints.

Power Analysis: Assuming a one-way ANOVA with three levels (day before, one week and one month after) and 12 subjects, we have 75.8% power to observe significant connectivity change ($p < 0.05$) for a small observed effect size ($r=0.25$). The proposed study will provide data to directly estimate the effect of psilocybin on longer-term change in emotion and neural circuitry.