Study Protocol with Statistical Analysis Plan

Study Title: Effects of Salvinorin A on Brain Function

Clinical Trials ID (NCT number): NCT03418714

Last Protocol Approval Date: October 5, 2018

This protocol will include two or more administrations of an inhaled dose of salvinorin A in experimental laboratory settings. The first experimental session will involve administration of a 15 µg/kg dose of salvinorin A without brain fMRI scanning. The first session will allow the participant to become familiar with the inhalation procedure as well as the dose of salvinorin A that they will encounter in the fMRI scanner. If the volunteer displays involuntary movement or no memory for the experience, we may repeat the drug administration at a lower dose (12 µg/kg). The final experimental session will involve inhalation of either 12 or 15 µg/kg salvinorin A during fMRI scanning. Our previous research has shown that both 12 and 15 µg/kg doses of salvinorin A are well tolerated in participants with history of hallucinogen use (Johnson et al., 2011; Johnson, MacLean, Caspers, Prisinzano, & Griffiths, 2016; MacLean, Johnson, Reissig, Prisinzano, & Griffiths, 2013; Maqueda et al., 2015).

The primary objective of this study is to investigate the effects of salvinorin A on human brain activity and connectivity. Secondary objectives include investigating the relationship between individual differences in subjective effects of salvinorin A and individual differences in brain response to salvinorin A.

Study Procedures

Recruitment and screening: Potential volunteers will learn of the protocol by posted, mailed, internet, newspaper, and newsletter advertisements, and word of mouth referral. The flyer and advertisements will seek individuals who have experience with psychedelics and a desire to participate in a scientific study of the effects of salvinorin A. Volunteers 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. Individuals will not sign consent before telephone screening, as it would not be practical to mail individuals a consent form for the telephone screen because we believe that individuals could find this inconvenient and would not respond, and also some individuals might be unwilling to provide their name and mailing address before knowing more about the study. At the end of the telephone screen we will obtain oral consent to retain PHI for the duration of the study and to contact the individual for the present study if the inclusion/exclusion criteria are changed in the future. If the individual does not provide oral consent, we will destroy all PHI collected during the telephone screen. We will retain the de-identified information because we want to be able to summarize the reasons that individuals failed screening or choose not to participate in this study.

Participants who do not fail telephone screening will be invited to the Behavioral Pharmacology Research Unit (BPRU) on the Johns Hopkins Bayview Campus for in-person screening. We will consent and screen up to 200 volunteers in order to provide 20 volunteers who complete all study procedures and provide complete data. Potential volunteers will be carefully screened to eliminate those with significant medical or psychiatric illnesses. Evaluation includes a history and physical examination, structured psychiatric diagnostic interview, urine drug test, and, for females, a urine pregnancy test. Volunteers will also complete baseline questionnaires assessing psychological traits and previous subjective experiences with hallucinogens. A blood sample (15 ml) will be drawn for medical screening (including CBC, CMP, and lipid and liver function panels). Volunteers will also be requested to refrain from illicit drug use (i.e., street drugs or non-prescribed use of prescription medication) during the course of the study and a urine test will be conducted prior to each drug session.

Drug sessions: After screening and study intake, participants will report to the Behavioral Pharmacology Research Unit (BPRU) at the Johns Hopkins Bayview Medical Campus, and then to the MRI Research Service Center at the Johns Hopkins Hospital for two experimental laboratory sessions lasting about two to three hours each. The first laboratory session will involve administration of salvinorin A without brain imaging, and the second laboratory session will involve administration of salvinorin A during functional Magnetic Resonance Imaging (MRI). A 15 µg/kg dose of salvinorin A will be inhaled for each drug administration. This dose has been shown in previous studies to be well-tolerated, and provide moderate to

strong drug effects (Johnson et al., 2011; Maqueda et al., 2015). If a given volunteer displays involuntary movement and/or no memory for the experience, we may repeat the first drug administration with a lower dose of salvinorin A (12 µg/kg). If the involuntary movements or lack of memory do not occur for that volunteer at the lower dose, we will administer 12 µg/kg in the subsequent MRI drug administration for that volunteer.

Volunteers will be instructed to consume a low-fat breakfast before reporting to the laboratory in the morning for the experimental sessions. Upon arrival for each session, the volunteer will provide a urine sample for a urine drug screen and, for females, for pregnancy testing. The urine sample will be tested for the presence of abused drugs (e.g., various opioids, stimulants and sedatives). If a test reveals evidence of drug use, the volunteer will not participate in test sessions that day, but may be rescheduled. If a continuing pattern of illicit abuse is suspected, that volunteer's participation in the protocol may be terminated. In addition, a urine pregnancy test will be conducted in females of child bearing potential. A negative test will be required in order to continue in the study.

First experimental session day: During the first drug administration session day, similar to our previous research with salvinorin A (Johnson et al., 2011; Johnson et al., 2016; MacLean et al., 2013), participants will be administered salvinorin A via inhalation while lying down. At least one staff member will be present in the room to directly monitor the participant for at least 45 minutes after each drug administration. Staff members will rate participant behavior and ask the participant to make verbal ratings of subjective effects at set time intervals throughout the drug administration session. In our previous research with salvinorin A, subjective effects resolved within 45 minutes (Johnson et al., 2011; Johnson et al., 2016; MacLean et al., 2013). Volunteers will complete several questionnaires and computer-based assessments approximately 45 minutes after each drug administration.

Study staff will record observations of subject movement during the acute effects of salvinorin A. Participants who are particularly sensitive to the effects of salvinorin A may exhibit involuntary movement of head, torso, or appendages, or resting or kinetic tremor. If a given participant exhibits gross involuntary movement or resting tremor after drug administration during the first experimental session, the study team will determine if this movement would invalidate MRI data collection. If the volunteer exhibits involuntary movement that would invalidate MRI data collection, or reports no memory for the experience, the session may be repeated with a 12 µg/kg dose of salvinorin A. Study volunteers will not advance to the MRI experimental session if it is determined that they have exhibited involuntary movement judged likely to invalidate MRI data collection at both 15 and 12 µg/kg.

Schedule of Events for First Experimental Session Day

Time Point (minutes from drug administration)	Procedure	Description
-30 (or more)	Intake procedures	Participant will complete drug and pregnancy urine tests, and complete a pre-session questionnaire to assess the psychological "set" of the volunteer
-10 (approximate)	Setup	The participant will be brought to the experimental session room, and the prepared salvinorin A dose for this participant will be brought to the session room from the BPRU pharmacy. All materials and equipment for drug delivery and study monitoring will be set up.
0	Drug administration	Participant will be administered salvinorin A via inhalation while lying down.
1	Post drug-administration measurement	Participant will rate overall drug strength. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant.
2	Post drug-administration measurement	Participant will rate overall drug strength. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant.

3	Post drug-administration measurement	Participant will rate overall drug strength. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant.
4	Post drug-administration measurement	Participant will rate overall drug strength. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant.
5	Post drug-administration measurement	Participant will rate overall drug strength. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant.
10	Post drug-administration measurement	Participant will rate overall drug strength. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant.
15	Post drug-administration measurement	Participant will rate overall drug strength. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant. Monitors will also complete the Fahn-Tolosa-Marin Tremor Rating Scale.
20	Post drug-administration measurement	Participant will rate overall drug strength. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant.
25	Post drug-administration measurement	Participant will rate overall drug strength. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant.
30	Post drug-administration measurement	Participant will rate overall drug strength. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant. Monitors will also complete the Fahn-Tolosa-Marin Tremor Rating Scale.
45	Final questionnaires	Participant will rate overall drug strength and complete questionnaires describing the subjective effects experienced during the acute effects of salvinorin A. Monitors will rate observed intensity of drug effect and amount of spontaneous speech and movement of the participant. Monitors will also complete a questionnaire to rate the overall qualitative nature of the participant's session.

The schedule of events for the first experimental session day is approximate, but the listed events will always occur in the same sequence.

Second experimental session day: During the second experimental session day, participants will be lying down in a supine position within an MRI scanner during administrations of placebo (inhaled air) and a 15 or 12 µg/kg dose of salvinorin A (whichever dose did not yield erratic movement during the first experimental session). Participants will be told that they are being continuously monitored by an intercom and can stop the scan at any time, but that it is best for them to remain still and refrain from any head, face or jaw movements. Participants will also be told that a study team member will remain in the scanner room with them throughout the procedures. The placebo condition (inhaled air) will always precede the salvinorin A condition. MRI scans will be used to obtain structural brain images, as well as functional data that permit the direct measurement of oxygenated blood (using blood-oxygenation level dependent [BOLD] techniques) to assess acute effects of salvinorin A on brain function. Heart rate (via pulse oximetry), respiration, and end-tidal carbon dioxide may also be collected in order to correct for artifacts in BOLD signal. BOLD signal data will be collected during two 20 minute scans (one scan for placebo and one scan for salvinorin A). During each scan, an inhalation period will begin 30 seconds after the beginning of BOLD data collection, and will be cued via audio stimulus using MR-safe headphones. A study team member will be present in the scanner room with the volunteer during scanning and inhalation procedures, to provide support to the participant during procedures. Participants will be told that one of the inhalations will be the dose of salvinorin A that they received during the first session, and the other inhalation will be either a placebo or a variable dose of salvinorin A, but no dose of salvinorin A higher than the dose they received during the first session will be administered.

All imaging procedures will be performed using 3T Siemens MRI scanner at the MRI Research Service Center at Johns Hopkins Hospital, by individuals specifically trained in these procedures. MRI assessments

involve standard methods and established pulse sequences utilized in research evaluations and do not involve the injection of any contrast or other invasive procedures. We will work closely with the neuroimaging experts in the MRI Research Service Center to ensure safe and effective implementation of the tasks conducted in the scanner. Dr. Frederick Barrett, the principle investigator on the team, has extensive experience with brain imaging and is currently leading MRI and PET neuroimaging studies of the acute effects of the classic hallucinogen psilocybin. Dr. Barrett will oversee the development and conduct of brain imaging procedures and analysis of the resulting data.

After completing the scanning procedures, the participant will complete brief (e.g., 10-15 minute) behavioral measures and/or questionnaires regarding the subjective effects that they experienced during the scanning session. These measures will be used along with ratings made in the scanner to interpret differences in brain activation between participants and across conditions. For example, changes in brain activation may be correlated with ratings of subjective effects (e.g. the Mystical Experience Questionnaire, or the Challenging Experience Questionnaire) experienced during the scanning procedures. Subjects will also be asked to write a narrative description of the experience of each salvinorin A session within about one week after a session.

Participants will undergo drug administration during collection of BOLD signal in the MRI. Real-time fMRI analysis techniques may be employed to monitor volunteer head motion as the scans are collected. If participants exhibit gross (> 1mm of movement in any plane) frequent (for more than 25% of the scan time) head motion, we may repeat the given drug administration and scan, since frequent gross motion may invalidate BOLD imaging data (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012; Power et al., 2014). We may repeat drug administration and scanning up to two times. No more than one salvinorin A administration will occur on a single day. Repeated administrations of salvinorin A may occur on consecutive days.

Schedule of Events for Second Experimental Session Day

Time Point (minutes from drug administration)	Procedure	Description
-65 (or more)	Intake procedures	Participant will complete drug and pregnancy urine tests, and complete a pre-session questionnaire to assess the psychological "set" of the volunteer (including mood state and cognitive state)
-40 (approximate)	Setup	The participant will be brought to the MRI suite. The prepared salvinorin A dose for this participant and session will be brought from the BPRU pharmacy to the MRI suite. All materials and equipment for drug delivery and study monitoring will be set up, including passing the non-ferromagnetic inhalation tube through the pass-through panel from the MRI control room to the MRI scanning room. The volunteer will be placed in a head-first supine position on the scanner bed, with the head placed within the MRI head coil. The volunteer will be provided with MR-safe earbud head phones that provide hearing protection, as well as additional padding to prevent head movement and further provide hearing attenuation, consistent with standard MRI procedures. The inhalation tubing will be taped on to the MRI head coil in a position that is accessible to the volunteer, and the volunteer will be moved into the magnet. A study team member will remain with the volunteer in the MRI scanning room throughout the procedures. Structural scanning procedures will then commence.
-20	Placebo scan	A 20-minute EPI scanning sequence will be started, and placebo inhalation will begin 30 seconds after the start of the EPI scan. The participant will be instructed via inserted MR-safe earbud headphones to begin the inhalation procedure.
0	Salvinorin A scan	After verbally confirming that the volunteer is comfortable and ready to continue, a 20-minute EPI scanning sequence will be started, and salvinorin A inhalation will begin 30 seconds after the start of the EPI

		scan. The participant will be instructed via inserted MR-safe earbud headphones to begin the inhalation procedure.
20	Scan end	After the salvinorin A scan has completed, the volunteer will be removed from the MRI and brought to a private adjacent room and verbally debriefed.
25	Final Questionnaires	The participant will be asked to complete brief (10-15 minute) behavioral measures and/or questionnaires regarding the subjective effects that they experienced while in the scanner.

The schedule of events for the second experimental session day is approximate, but the listed events will always occur in the same sequence.

Meeting(s) with study staff before and after experimental sessions: Before the first drug administration session, volunteers will meet with one or more study staff members for a minimum of 2 hours contact time. The main purpose of this meeting is to develop rapport and trust between the participant and study staff, which we believe helps minimize the risk of fear or anxiety reactions during sessions with salvinorin A. These rapport-building meetings will include discussions of the volunteer's childhood, romantic life, current relationships with family and friends, and the volunteer's philosophical and/or spiritual beliefs. Reviewing personal history and feelings may be important in establishing a significant level of trust. By the time of the first drug session day, the volunteer will ideally feel very comfortable with the study staff, reducing the likelihood of paranoia (e.g., feeling that the study staff are trying to control her or his mind, or have deceived the volunteers about the nature of the study). During the preparation meeting(s), study staff will describe the broad range of possible psychological experiences that are reported to occur during test sessions and techniques for handling difficult psychological experiences. Since many volunteers are eager to discuss personal topics and possible drug-induced experiences during this pre-drug contact time, and some may not have a substantial amount of personal information or experience to discuss, it is not necessary to mandate more than 2 hours as a minimum timeframe for discussion for all individuals, however some volunteers have far more information and experience to discuss than can reasonably fit within 2 hours. Additional meetings and contact hours (up to 10 hours) will be scheduled if it is judged necessary to establish rapport and trust. The volunteer's life history and current situation in life will be reviewed. Intentions and expectations for the test sessions will also be discussed.

After each experimental session, volunteers may have a follow-up telephone conversation or in-person meeting (up to 8 hours) with study staff to discuss the session and any thoughts and feelings that emerged from the experience. These follow-up conversations may also coincide with subsequent experimental session days (before drug administration). The general purpose of the meetings following sessions is to provide a context for discussing and interpreting experiences from the drug administration sessions. Volunteers will also complete follow-up questionnaires and a telephone call with study staff one week after the final drug administration session. Study staff contact and follow-up questionnaires will assess whether there are lasting psychological effects of salvinorin A.

Study duration and number of study visits required of research participants: Participants are expected to complete between 2 and 6 study visits. This will, at minimum, include 1 screening visit, a preparation meeting with the guides to review session procedures and establish rapport, and 2 drug administration sessions (approximately 2 to 3 hours each). The preparation meeting may coincide with the screening visit, or with the first experimental drug administration session. The first and second drug administration sessions may also occur on the same day. Also, as described above, we may repeat any drug administration up to two times if there was an experimental issue rendering the session invalid, and we may lower the dose of salvinorin A from 15 to 12 µg/kg if a given volunteer exhibited erratic or involuntary movements or reported no memory for the experience. Also, we may schedule additional pre-and post-session meetings if study staff judge that rapport and psychological stability has not been sufficiently well established to proceed, or if there is a need for discussion and interpretation of experiences from the drug administration sessions. The total study duration for each volunteer will be between 1 and 6 weeks. Each volunteer's total

study duration will be dependent upon schedules and availability of the volunteer as well as the experimental resources of the BPRU and MRI Research Service Center.

Schedule of Study Visits

Visit	Purpose	Description
	Phone Screening	An initial phone screening will determine whether an individual meets major inclusion/exclusion criteria, and thus whether they are eligible for in-person screening.
Visit 1	In-Person Screening	This evaluation includes a history and physical examination, structured psychiatric diagnostic interview, urine drug test, and, for females, a urine pregnancy test. A blood sample (15 ml) will be drawn for medical screening.
Visit 2	Preparation Meeting	The purpose of this meeting is to develop rapport and trust between the participant and study staff, which is understood to minimize the risk of fear or anxiety reactions during sessions with salvinorin A. The purpose of this meeting is also to prepare the volunteer for experiences they may have under the effects of salvinorin A.
Visit 3	First Experimental Session Day	Participants will be administered salvinorin A via inhalation while lying down. Volunteers will complete several assessments approximately 45 minutes after drug administration.
	Follow-up conversation (optional)	After the first experimental session day, volunteers may have a follow-up telephone conversation or in-person meeting (up to 8 hours) with study staff to discuss the session and any thoughts and feelings that emerged from the experience. This conversation after the first drug administration day may also coincide with the second experimental session day (before drug administration). The general purpose of follow-up conversations is to provide a context for discussing and interpreting experiences from the drug administration sessions.
Visit 4	Second Experimental Session Day	Participants will be lying down in a supine position within an MRI scanner during administration of placebo (inhaled air) and a 15 µg/kg dose of salvinorin A.
	Follow-up conversation (optional)	After the second experimental session day, volunteers may have a follow-up telephone conversation or in-person meeting (up to 8 hours) with study staff to discuss the session and any thoughts and feelings that emerged from the experience. The general purpose of this meeting is to provide a context for discussing and interpreting experiences from the drug administration sessions.
1 week after Visit 4	Final follow-up	One week after the final experimental session day, volunteers will have a telephone conversation with study staff to discuss any lasting psychological effects of salvinorin A, and they will complete online questionnaires.

Illustrative Timeline of Study Participation (*Study duration could vary between about 1 to 6 weeks, depending on timing and number of visits,)*:

Visit 1: Screening (~ 6 hours)
Visit 2: Preparation meeting (~ 2 hours; may coincide with Visit 1 or Visit 3)

Visit 3: First experimental session day (questionnaire completion and salvinorin A administration; ~ 2-3 hours total; no MRI is conducted during this session day)
Visit 4: Second drug administration day (placebo and drug administration and MRI scanning; up to 3 hours total)
1 week after Visit 4: Follow-up questionnaires and phone call (~1 hour)

Visit 2 may coincide with either Visit 1 or Visit 3. Visit 1 and Visit 3 will not occur on the same day, but may occur on subsequent days. Visit 3 and Visit 4 may occur on the same day. The timeline above is an illustration of a possible study timeline, although these are only estimates. Variables out of the study team's control (e.g. participant/monitor availability, university closings, availability of study rooms; illness) may necessitate deviations from the stated schedule. Additionally, on rare occasions we may ask participants to repeat a test session if data cannot be collected during the time period of drug activity (e.g., a dosing administration issue in which the volunteer may not have inhaled the entire dose, or an error with data collection equipment occurs). We will report any such deviations to the IRB in the Continuing Review.

Inclusion/Exclusion Criteria

Based on clinical interviews and a structured psychiatric diagnostic interview, volunteers will be eligible if they are judged to be psychologically stable and do not meet any of the medical or psychiatric exclusion criteria described below.

Inclusion criteria
- 21 to 65 years old
- Have given written informed consent
- Have a high school level of education
- Have a minimum of 10 lifetime uses of hallucinogens (e.g., LSD, psilocybin mushrooms, DMT, ayahuasca, mescaline, *Salvia divinorum,* ketamine, dextromethorphan, PCP) and recent (within the past year) experience with a hallucinogen.
- Recent experience (within the past year) with an inhaled psychoactive drug.
- 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 mornings of drug session days. If the volunteer does not usually consume caffeinated beverages, he or she must agree not to do so on session days
- Agree to refrain from using any psychoactive drugs, including alcoholic beverages and any tobacco product such as cigarettes or any other nicotine product such as e-cigarettes, within 24 hours of each drug administration. Exceptions include daily use of caffeine.
- Be healthy and psychologically stable as determined by screening for medical problems via a personal interview, a medical questionnaire, a physical examination, and routine medical blood and urinalysis laboratory tests
- Agree that for one week before each session, he/she will refrain from taking any nonprescription medication, nutritional supplement, or herbal supplement except if 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
- Agree not to take any PRN prescription medications on the mornings of the sessions

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, or TIA in the past year
- Seizure disorder or 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
- More than 25% outside the upper or lower range of ideal body weight
- Current or past history of meeting DSM-V criteria for schizophrenia, psychotic disorder (unless substance-induced or due to a medical condition), dissociative disorder, bipolar I or II disorder, an eating disorder
- Current or past history of substance-induced psychosis.
- Current or past history within the last 2 years of meeting DSM-5 criteria for moderate or severe alcohol or substance use disorder (excluding caffeine)
- Daily or more frequent tobacco or nicotine use
- Current severe obsessive-compulsive disorder, dysthymic disorder, or panic disorder.
- Current, severe, major depression
- Have a first or second degree relative with schizophrenia, psychotic disorder (unless substance induced or due to a medical condition), or bipolar I or II disorder
- Has a psychiatric condition judged to be incompatible with establishment of rapport or safe exposure to salvinorin A
- Head trauma, traumatic brain injury, or concussion with loss of consciousness for >2 minutes
- Claustrophobia incompatible with MRI scanning
- Medical device incompatible with MRI scanning (e.g. cardiac pacemaker**, i**mplanted 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
- Left-handedness (assessed by the Edinburgh Handedness Inventory)

Drugs/ Substances/ Devices

Dosing information and rationale: A 15 μg/kg dose of salvinorin A was well-tolerated and rated as a moderate drug strength in our previous research (Johnson et al., 2011; Johnson et al., 2016; MacLean et al., 2013), and this dose is between doses that elicited a moderate and strong drug effect in more recent research (Maqueda et al., 2015; Maqueda et al., 2016). This dose is substantially less than the maximum dose of salvinorin A that has been safely administered within our laboratory (21 μg/kg). Data from our prior studies (Johnson et al., 2011, 2016; MacLean et al., 2013) indicated that volunteers experienced strong drug effect but no involuntary movement or lack of memory for drug effects at 15 μg/kg. Two volunteers in a previous study within our laboratory experienced brief periods of involuntary movements and no memory for the experience at doses greater than 15 μg/kg (MacLean et al., 2013). The volunteers who experienced involuntary movements and no memory for drug effects in our previous study did not experience these states at lower doses of salvinorin A, suggesting that this may be a dose-dependent phenomenon. However it is theoretically possible that future volunteers may experience these effects at 15 μg/kg. In the case that a volunteer has experiences these effects during their first drug administration session, we may repeat this first session with a lower dose of salvinorin A (12 μg/kg). If that dose of salvinorin A is well-tolerated (e.g., no involuntary movements, memory for the experience, and the participant is willing to be re-administered that dose), the imaging session will be conducted with this dose (12 μg/kg).

Administration method: The inhalation route of administration was selected because inhalation via smoking/vaporization is the most common route for contemporary abuse of *Salvia divinorum* or salvinorin A (Baggott, M.J., Erowid, E., Erowid, F., Mendelson, J. E., 2004; Gonzalez et al., 2006), and this inhalation route has fast onset and short duration of effects, making it suitable for pharmaco-fMRI study

(Carhart-Harris et al., 2012; St Lawrence et al., 2003; Stein et al., 1998). The delivery device for salvinorin A will be identical to that used in our previous research with salvinorin A (Johnson et al., 2011; Johnson et al., 2016; MacLean et al., 2013), with one exception. A longer length of tubing (approximately 400 cm) will be used to permit inhaled administration while in the MRI scanner. The same length of tubing will be used for all inhaled administrations, including those not administered in the scanner, to keep the apparatus consistent across sessions.

Vaporization and inhalation procedures will be identical to those in our previous research with salvinorin A. Salvinorin A will be placed in a glass pipe. A member of the study staff will use a small butane micro torch lighter to heat the bottom of the bowl of the glass pipe. The participant will then inhale vaporized material through a length of non-ferromagnetic tubing with specified inhalation and inhalation hold-time instructions (e.g., 45 seconds). Inhalation of vaporized drug from glass pipes has been used in numerous human pharmacology studies to administer cocaine freebase (Foltin & Fischman, 1997; Haney, Hart, & Foltin, 2006; Hart, Haney, Vosburg, Rubin, & Foltin, 2007; Martinez et al., 2004) as well as dimethyltryptamine (Riba, McIlhenny, Bouso, & Barker, 2015). Previous studies have also administered inhaled salvinorin A (Johnson et al., 2011; Johnson et al., 2016; MacLean et al., 2013; Maqueda et al., 2015; Maqueda et al., 2016; Ott, 1995; Siebert, 1994). Specifying inhalation breath hold times with vaporized or smoked drugs has also been commonly done in human research with drugs of abuse, including cocaine (e.g. Dudish & Hatsukami, 1996), marijuana (e.g. Zacny & Chait, 1989) and tobacco (e.g. Johnson, Bickel, & Kirshenbaum, 2004). During the second experimental session, the inhalation tubing will be affixed to the head coil of the MRI scanner, available to the participant's mouth, and the tubing will be directed out of the MRI scanner room into the control room through a pass-through panel. Vaporization will occur outside of the scanner room, in the control room, while the participant is in the scanner. Instructions to inhale will be given using MR-safe headphones that can function while the scanner is operational.

Inhalation device: The inhalation delivery system (i.e. pipe) is made of glass. The part of the glass pipe which will hold the salvinorin A will be a 5 ml round bottom chemistry flask meeting American Society for Testing and Materials (ASTM) specification E438 for Type I, Class A borosilicate glass. The non-ferromagnetic tubing attached to the inhalation delivery system will be polytetrafluoroethylene (PFTE) tubing, which meets FDA standard for food contact, has a high maximum working temperature (260^{o} C, which is higher than the 244-245^{o} C vaporization point of salvinorin A), and does not thermally degrade until temperatures greater than 400^{o} C. The tubing will be 0.635 cm inner diameter, and up to 400 cm in length. The same tubing will be used for a given subject or a set of new tubing will be used between drug administrations. Tubing will not be cleaned with solvents.

A label will be prepared for each test dose and affixed to the neck of the 5mL round bottom flask containing the dose. This label will be removed immediately prior to heating the flask for drug administration. Each dose will be labeled with subject name, date, session number, pharmacy compounding number, dose of salvinorin A, and a statement that the drug is limited to investigational use only.

Study Statistics

Primary outcome variable: The primary outcome measures for this study will consist of changes in activity and connectivity of brain regions during the acute effects of salvinorin A, compared to the preceding period after inhalation of the placebo condition (inhaled air).

Secondary outcome measures: The secondary outcome measures will include a range of subjective measures described below.

> *Subject-rated overall drug strength*: Volunteers will be prompted to verbally rate the overall drug strength on an 11-point scale (0 – none, no effect to 10 – extreme, strongest imaginable) at various

time points (e.g., 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, and 45 minutes after each drug inhalation) during the first experimental drug session day.

Fahn-Tolosa-Marin Tremor Rating Scale (TRS; Fahn et al., 1993): The TRS, which was used to classify resting and kinetic tremor severity in our dose-effects study of salvinorin A (Johnson et al., 2011), will be assessed at 15 and 30 minutes after salvinorin A administration during the first experimental drug session day. At these time points, study staff will visually assess kinetic tremor intensity as the participant bends both of his or her extended arms to touch middle finger to nose. Study staff will also rate the magnitude of resting tremor during the time interval leading up to the assessments of kinetic tremor (i.e., 0-15 min,15-30 min) by closely observing the participant. Those who exhibit significant resting tremor during the first experimental session day that is determined to likely invalidate MRI data collection will not continue to the second experimental session day.

Statistical analysis plan

Sample size: Since no brain imaging data are available to directly assess the effects of salvinorin A on human brain activity, power analyses were calculated based on behavioral data from previous investigations in our laboratory that included a range of doses of the classic hallucinogen psilocybin (Griffiths et al., 2011), and a previous investigation in our laboratory that included a range of doses of salvinorin A (Johnson et al., 2011). Psilocybin dose produced large increases on drug intensity ratings (d = 0.89, where 0.2 represents a small effect, 0.5 represents a medium effect, and 0.8 represents a large effect), and salvinorin A dose (Johnson et al., 2011) exerted a very large effect on the area under the curve of rated drug strength (η^2 = 0.81, where 0.02 represents a small effect, 0.13 represents a medium effect, and 0.25 represents a large effect). However, these are behavioral, and not brain imaging, outcome measures.

Using the lower (more conservative) of the two effect sizes from our previous psilocybin and salvinorin A studies (d = 0.89), and an alpha of 0.05, 6 volunteers would be sufficient to detect effects on ratings of drug intensity with 95% power. Empirical and simulation studies have provided recommendations for sample size of imaging experiments (Zandbelt et al., 2008). For similar parameters, including an effect size of 0.89, alpha of 0.05, and 80% power, 21 participants are necessary to power an ROI-based analysis of effects on brain activity similar to behavioral effects of psilocybin. Far fewer participants would be necessary to detect effects similar to the behavioral effects of salvinorin A, though no specific sample size recommendations were made by Zandbelt and colleagues for such a large effect size (η^2 = 0.81, equivalent to $d > 4$). Thus, the proposed study of 20 participants would be powered to detect clinically significant differences in brain activity between placebo and salvinorin A conditions. Given that consent will be obtained at the initial in-person screening interview, we are requesting approval to consent up to a total of 200 volunteers.

MRI data analyses: MRI data will be preprocessed using standard methods. The time course of brain regions in standard brain atlases (e.g. Chen Atlas or Power Atlas) will be extracted and submitted to functional connectivity analysis.

Statistical analysis of behavioral data: Baseline demographic data will be summarized with descriptive statistics to describe the volunteer population. For measures assessed at multiple time-points, including subjective effects ratings, both time-course and peak-effect data will be examined. For outcomes assessed at screening and one week after the final salvinorin A session, a paired t-test will be used to assess change from baseline (screening). Behavioral measures (including monitor ratings, subjective effects ratings, and questionnaire data) will be correlated with brain-imaging outcome measures to investigate the relationship between individual differences in subjective drug response and individual differences in neural drug response.