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| Protocol Name: | Effect of ketamine on laboratory-induced stress in healthy subjects: A Proof-of-Concept Translational Study (IRB #17-01105; GCO #17-2440) |
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# Effect of Ketamine on Laboratory-Induced Stress in Healthy Subjects: A Proof-of-Concept Translational Study

**Clinical Trial Protocol**
Icahn School of Medicine at Mount Sinai

**Brief Summary of Research:**

Stress exposure is one of the greatest risk factors for psychiatric illnesses like major depressive disorder (MDD) and posttraumatic stress disorder (PTSD). Stress resilience is the ability to experience stress without developing psychopathology. Enhancing stress resilience in at-risk populations could potentially protect against the development of stress-induced psychiatric disorders. Despite this, no resilience-enhancing pharmaceuticals have been identified yet. Preclinical studies showed that the administration of the glutamate N-methyl-D-aspartate (NMDA) receptor antagonist ketamine one week before an acute stress prevents the developing of depressive-like behavior in animals. In this project we propose a pilot study to test if this stress prophylactic effect of ketamine applies also to humans. Ketamine will be compared to an active placebo control condition, the anesthetic midazolam, in a sample of healthy volunteers. The specific aims of this project are to test the effect of ketamine administered 1-week prior an acute stress [the Trier Social Stress Test (TSST)] (1) on the anxious-composed subscale score of the Profile of Mood States (POMS) – Bipolar and (2) on the hypothalamic–pituitary–adrenal axis (HPA axis), adrenaline-noradrenaline axis (ANS axis), and self-reports of anxiety. We expect that subjects treated with ketamine, compared to midazolam, will experience reduced symptoms of negative affect and anxiety, as quantified by higher score in the anxious-composed subscale score of the POMS-Bipolar, and a blunted hormonal response to an acute stress.

## 1) Objectives

*Research Question:*
The overall goal of this pilot study is to investigate the prophylactic effect of ketamine compared to the control condition midazolam on bio-behavioral readouts following the TSST when administered 1 week prior in a sample of healthy volunteers.

**Aims of the study.** Primary Behavioral Aim: To test the effect of ketamine compared to midazolam administered 1 week prior to the TSST on the anxious-composed subscale score of the Profile of Mood States (POMS) – Bipolar in healthy volunteers. *Primary Behavioral Hypothesis: Ketamine will be associated with a reduced change of symptoms of anxiety from pre-TSST to post-TSST compared to midazolam*. Secondary Behavioral Aim: To test the effect

of ketamine compared to midazolam administered 1 week prior to the TSST on the other subscale scores of the POMS-Bipolar and other self-reports. *Secondary Behavioral Hypothesis: Ketamine will be associated with a reduced change from pre-TSST to post-TSST as measured by the other subscale scores of the POMS-Bipolar and other self-reports compared to midazolam.* Primary Biological Aim: To test the effect of ketamine compared to midazolam administered 1 week prior to the TSST on the hypothalamic–pituitary–adrenal axis (HPA axis). *Primary Biological Hypothesis 2: Ketamine will be associated with a blunted response or faster recovery of the HPA axis as measured with salivary cortisol.* Secondary Biological Aim: To test the effect of ketamine compared to midazolam administered 1 week prior to the TSST on an attenuated ANS reactivity, as measured by salivary α-amylase. *Secondary Biological Hypothesis: Ketamine will be associated with a blunted response or faster recovery of ANS reactivity compared to midazolam.* Exploratory Aims: Additional exploratory analysis will be conducted on blood pressure, heart rate, and plasma samples collected prior to and following the TSST on hormones including ghrelin, NPY, testosterone, and DHEA comparing the ketamine group to the midazolam group.

## 2) Background

A well-validated laboratory paradigm to provoke anxiety: the Trier Social Stress Test (TSST). Social stressors are amongst the most reliable forms of stress in humans and other species (Dickerson & Kemeny, 2004). The TSST (Kirschbaum et al., 1993) is among the most popular acute stress protocols used to experimentally study the stress response in humans. The TSST employs a combination of elements (public speaking, mental arithmetic, anticipation, social evaluation) that produces moderate stress in a majority of participants and reliable response in the self-reports of negative and positive affect (Allen et al., 2014; Henze et al., 2017). Acute elevations of the hypothalamic pituitary adrenal (HPA) axis, sympathetic-adrenal-medullary (SAM) axis activity, and self-reports of anxiety have been shown. Responses to the TSST appear to be altered in cases of stress-related disorders like major depression or anxiety disorders and both pharmacological and psychotherapeutic interventions can moderate the TSST response (Allen et al., 2014). This paradigm is reported under the Domain of Negative Valence Systems primarily responsible for responses to aversive situations or context, such as fear, anxiety, and loss, within the Research Domain Criteria (RDoC) of Acute Threat ("Fear") by NIH/NIMH. Procedures: During the TSST, participants after a rest period on entering the laboratory are introduced to a role-playing scenario. They have to prepare a speech to convince a panel that they are the perfect candidate for a job, which they must present to a panel of assessors, followed by a mental arithmetic task (serial subtraction). Following these tasks participants rest and post-measures are taken in multiple time-points (Kirschbaum et al., 1993). Please, refer to section f "Procedures Involved in the Human Research" for more details.

Ketamine is prophylactic against stress-induced depression in animal models. Recent preclinical work provides evidence that treatment with ketamine administered one week prior to a stress blocks the development of depression-like behavior in rodent models (Brachman et al., 2016). These findings represent the first demonstration in the field that a pharmacological agent can provide long-term prophylactic protection against the induction of stress-induced depression, setting the stage for a major paradigm shift in therapeutic discovery for disorders such as major depressive disorder (MDD) and posttraumatic stress disorder (PTSD). In this study by Brachman et al. (Brachman et al., 2016), the animals were pre-treated with ketamine or saline one week prior to a chronic social defeat (SD) procedure and behavior was assessed 2 weeks later using the forced swim test (FST) and a social interaction test [Dominant interaction (DI)]. Ketamine-treated mice showed significantly reduced immobility time and significantly more time exploring a social target mouse compared to saline treated mice following SD, *consistent with a blockade of the pro-depressive effects of SD and enhancement of stress resilience*. The investigators confirmed the prophylactic effects of ketamine in two additional models in which (1) depressive and anxious behavior is induced by chronic elevation of glucocorticoids and (2) depressive and anxious behavior is induced by repeated, inescapable shocks (learned helplessness [LH]). Several other recent preclinical studies provide additional data in line with these findings (Amat et al., 2016; McGowan et al., 2017).

Translating ketamine's pro-resilience effect from model animals to humans. Capitalizing on our group's experience conducting human studies of ketamine in the context of depression and PTSD (Murrough et al., 2013; Feder et al., 2014), herein we aim to determine for the first time the stress prophylaxis effect of ketamine in humans when administered 1 week prior to an acute stressor in the form of the TSST (Kirschbaum et al., 1993). We will use the TSST applied to healthy volunteers in order to determine the effects of ketamine prophylaxis on behavioral, physiological, and endocrine readouts. Importantly for the current study, the TSST has been used also to investigate the effects of pharmacological agents under the conditions of acute stress in healthy volunteer (Makatsori et al., 2004; Fries et al., 2006) and, similar to the acute stress method used in animal model, the TSST involves a combination of social stressors elements, thus appearing particularly suitable to translate the ketamine pro-resilience effect into humans.

## 3) Setting of the Human Research

The human research will be conducted at the Depression and Anxiety Center, Icahn School of Medicine at Mount Sinai Hospital (Director: Dr. Murrough). Part of the Research will also be conducted at the Clinical Research Unit (CRU) and at the Infusion Suite of the Psychiatry Department of Icahn School of Medicine at Mount Sinai.

## 4) Resources Available to Conduct the Human Research

We will be recruiting from various sources in the community for this research study. They include online recruitment sources, community outreach and physician referrals. All staff members assisting with this trial are adequately informed about the protocol, the investigational products, and their trial-related duties and functions. An anesthesiologist or other appropriately trained licensed physician will administer the study drugs to subjects. Dr. Murrough, Principal Investigator on this project, will be responsible for all trial related medical decisions, included the drug related procedures. Please refer to the Mount Sinai Treatment Protocol for Ketamine Clinical Research (ver. Jan 27, 2017) for more details.

## 5) Study Design

### a) Recruitment Methods

Our program implements a robust workflow for identifying and screening candidates for clinical research that is conducted in our program. Subjects contact the program through self-referral or referral by their treating physician. We employ both direct-to-patient advertising through radio, newspaper, internet-based and other media outlets, as well as foster strong collaborations with treating physicians within the Mount Sinai Health System and across New York City more broadly. Another resource for referrals will be via media advertisement and internet postings on medical and mental health-related websites. An IRB approved recruitment flyer will be used for this study and will be placed on bulletin boards in the Mount Sinai Medical Center. Subjects may also be accepted as referrals from the Mood and Anxiety Disorders Program screening protocol (GCO# 06-0945). Advertisements include the link to an online pre-screening survey, which is IRB approved under our MAP Screening Protocol (GCO 06-0945: A Screening Protocol for Adult Patients with Mood and Anxiety Disorders, Chronic Medical Conditions, and Healthy Volunteers; PI: Murrough, MD). The pre-screener is not required for participation. The pre-screener is just one of a handful of ways for participants to find our program and therefore find this specific trial. It is not a mandatory part of the GCO 17-2440 study and not all subjects will complete it. Part of the screening measures (SCID-5, self-reports) may be completed under the MAP screening protocol as long as the assessments are completed within one month of the participant signing consent for the present protocol.

Participants make contact with the program via phone, email, or our website. Subsequent to contact, patients go through a phone screening process prior to scheduling an in-person evaluation. Our centralized recruitment and screening process ensures that subjects are efficiently matched with appropriate research studies.

Once a patient expresses interest in the study, a phone screen is scheduled by a study coordinator to provide more information to the candidate over the phone and an initial IRB-approved phone screen is completed to assess broadly for the presence of inclusion and

exclusion criteria. Potential subjects are advised that if they do not enroll in research, the information will be destroyed, and that if they do enroll, the information becomes part of their research chart. An initial phone screening is completed after they give verbal authorization. The study coordinator, who is trained by the PI to perform a cursory assessment of study eligibility criteria, completes the phone screen. In general, this process is referred to as "pre-screening." If a candidate appears eligible following the completion of the pre-screen, the individual is scheduled for an in-person or remote study screen.

The in-person or remote study screen begins with the informed consent process and a written informed consent will be obtained. If the study screen is preformed remotely, the subject will be sent a copy of the approved consent form, a study doctor will discuss the consent form with the potential participant over a tele-communication platform, and the signature will be obtained via REDCap where both the subject and the study doctor can sign the consent. This consent form provides the details of the time requirements, steps in the study, potential risks, confidentiality of information, and the rights of patients as research subjects. A copy of the consent form will be given to each participant. Individuals enrolled in the study will have a full discussion regarding the project, procedures, relative benefits and risks, and options other than participating in this project. Following informed consent, the candidate completes demographic, medical history and symptom questionnaire self-report forms. Subsequently, the candidate will meet with a clinical rater trained on the SCID-5 and on relevant clinical administered scales. This may be completed remotely via a tele-communication platform.

The subject may also decline to participate in this study and this will not affect eligibility to participate in a different/future study or ability to receive treatment at Mount Sinai or from a private referring physician. Additionally, participants in the study will be able to withdraw from the study at any point.

**b) Inclusion and Exclusion Criteria**

Inclusion Criteria

1. Males and females aged 18-45 years;
2. Does not meet for any current or past psychiatric diagnoses as defined by DSM-V criteria;
3. Participants must have a level of understanding of the English language sufficient to agree to all tests and examinations required by the study and must be able to participate fully in the informed consent process.

*Exclusion Criteria*

1. Any current or lifetime psychiatric disorder as determined by the Structured Clinical Interview for DSM-V Axis Disorders (SCID-5);
2. Concomitant use of any medication with central nervous system activity, including treatment with antidepressants (classified as SSRIs, SNRIs, Atypical Antidepressants, TCAs);
3. Any unstable medical illnesses including hepatic, renal impairment, gastroenterologic (including gastro-esophageal reflux disease), respiratory (including obstructive sleep apnea, or history of difficulty with airway management during previous anesthetics), cardiovascular (including ischemic heart disease and uncontrolled hypertension), endocrinologic, neurologic (including history of severe head injury), immunologic, or hematologic disease;
4. Hypertension (systolic BP >160 mm Hg or diastolic BP >90 mm Hg) at screening or immediately prior to treatment with ketamine/midazolam;
5. Clinically significant abnormal findings of laboratory parameters, physical examination, or ECG; clinically significant is defined by an abnormality that suggests a disease and/or organ toxicity that is new or has worsened from screening, or if the abnormality is of a degree that requires additional active management (e.g., further diagnostic investigation).
6. Patients who have a positive urine toxicology test for illicit substances at screening and on the treatment day.
7. Previous recreational use of PCP or ketamine.
8. Subjects who have received ketamine in the past.

The proposed study does not involve vulnerable populations, such as fetuses, neonates, pregnant women, children, prisoners, institutionalized individuals, or others who may be considered vulnerable populations. Non-English speaking subjects will also not be enrolled in this study. Our staff is primary English speaking and is trained to use standardized diagnostic and rating tools are in English. Although non-English speaking individuals may be consented with appropriate translation services, we would not be able to diagnose and rate such individuals using the same standards. All participants are adults who are able to freely consent to research.

**c) Number of Subjects**

Considering the pilot nature of the present study, the research plan calls for the randomization of n=24 healthy volunteer aged 18-45. This is a highly feasible goal based on current patient flow at the study sites, and our demonstrated track record of enrollment at similar rates in prior studies recruiting from similar populations. Approximately 60 subjects will be screened to achieve the target numbers, based on an estimated screen fail rate of 50%. Given an estimated attrition rate of 20%, we expect n=24 to comprise the complete sample.

**d) Study Timelines**

The complete proposed study is intended to be completed in twenty-four months and includes twenty months recruitment period that allows for 2 months of regulatory start-up and 2 months for data cleaning, analysis, manuscript preparation, and preparation of final reports during the total 24-month project period. We have ensured that there is sufficient time to account for regulatory activities, study staff training, and activities related to data management and statistical analyses. Due to a change in funding availability and the COVID-19 pandemic, the study began recruiting and enrollment in August 2019 and enrollment is expected to be completed in July 2021 until 24 participants have been included in the sample.

Based on past experience with similar studies, we believe that about 1.2 subject randomized per month during the study to result in the enrollment of n=24 subjects ages 18-45 over a 2-year period. Recruitment of patients will end when 24 participants have been included in the sample. Each individual subject will participate in the study for up to 8 weeks.

**e) Endpoints**

The *behavioral primary endpoint* is a change from pre-TSST time point -25 (Visit 2) to post-TSST (Visit 2; time point +0) on the anxious-composed subscale score of the POMS-Bipolar in the ketamine group (KET) compared to the midazolam group (MID) one week after the administration of the study drug. The *behavioral secondary endpoints* are changes from pre-TSST (Visit 2; time point -25) to post-TSST (Visit 2; time point +0) on the other subscale scores of the POMS-Bipolar and other self-reports in the ketamine group compared to midazolam. Self-report measures include:

- Visual Analog Scale – Anxiety (VAS – stressed)
- Positive and Negative Affect Schedule (PANAS)
- Beck Anxiety Inventory (BAI)

In addition, the effect of treatment at subsequent time points following the TSST on the outcomes described above will be explored.

The *biological primary endpoint* is a change in HPA activity, as measured by salivary concentration of the stress hormone cortisol in response to the TSST in the ketamine group compared to midazolam. The *biological secondary endpoint* is a change in ANS activity, as measured by salivary α-amylase in response to the TSST in subjects who received ketamine compared to midazolam one week prior.

Additional *exploratory analysis* will be conducted on blood pressure, heart rate, and plasma samples collected prior to and following the TSST on hormones including ghrelin, NPY, testosterone, and DHEA comparing the ketamine group to the midazolam group.

**f) Procedures Involved in the Human Research**

This is a phase IIa, parallel arm, double-blind, randomized, proof of concept study. Upon completing the screening procedures, volunteers will be treated with ketamine 0.5 mg/kg (KET; n=12) or midazolam 0.045 mg/kg (MID; n=12) under randomized, double blind conditions one week prior to undergoing the TSST. We hypothesize that ketamine compared to midazolam will result in an attenuated stress response. Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study. Study participants will undergo assessments on the screening day (V-1) and on four separate times: 1) V0 (Infusion day): on the day of the administration of the drug (ketamine or midazolam); 2) V1: 24 hours after the administration of drug (ketamine/midazolam); 3) V2 (assessment day/TSST): one week after the administration of drug (ketamine/midazolam); 4) V3 (follow-up & study exit): two weeks after the administration of drug (ketamine/midazolam) or at the end of V2. Study visits may occur within 3 days before or after the scheduled visit, except for V2 (Assessment day), that will occur after one week from V0 (Infusion day). Please, refer to Table 1 "Schedule of Assessments, Outcome Measures, Labs and Procedures" for more details.

**g) Blinding:** Due to the objectives of the study, the identity of test and control treatments will not be known to investigators, research staff, or patients. The following study procedures will be in place to ensure double-blind administration of study treatments.

- Access to the randomization code will be strictly controlled by Investigational Drug services.
- Packaging and labeling of test and control treatments will be identical to maintain the blind.

The study blind will be broken on completion of the clinical study and after the study database has been locked. During the study, the blind may be broken only in emergencies when knowledge of the patient's treatment group is necessary for further patient management. Investigational Drug Services is responsible for the formulation, packaging, and labeling of the ketamine and midazolam. Please see the Mount Sinai Ketamine Protocol for more details on dispensation and administration of the study drugs.

*Study Visit -1: Screening*

Summary of study procedures during this phase:

- Informed Consent

- Structured Psychiatric Interview (SCID)
- Physician Evaluation
- Medical History
- Adverse Events Documentation
- Concomitant Medication Documentation
- Vital Signs
- Routine Laboratory Tests
- EKG
- Pregnancy test (for females)
- Female Reproductive Lifecycle and Hormones Questionnaire (RLHQ).

After receiving complete disclosure about the research and opportunity to fully review the consent form, potential participants will be given the opportunity to ask questions. If they choose to take part in the study, then they will be asked to sign the written informed consent form. Psychiatric history will be obtained by a member of the research team using the SCID and the DSM-5 Diagnostic Criteria. A medical history and physical examination will be performed for each patient and basic laboratory tests will be ordered. This will include: EKG, vital signs, complete blood cell counts, electrolytes, thyroid functioning test, liver function tests, urinalysis, and toxicology screening. A urine pregnancy test will also be performed. Results of these tests will identify patients who should be included or excluded based on criteria stated earlier in this protocol.

*Note: The screening visit may occur on separate days with a combination of remote and in-person study activities in an effort to reduce face-to-face exposure during the COVID-19 pandemic. All procedures will be completed before the infusion day (study visit 0).*

*Study Visit 0: Infusion day*

Summary of study procedures during this phase:
- Cognitive Testing
- Vital Signs
- Urine Toxicology
- Pregnancy Test (for females)
- Adverse Events Documentation
- Concomitant Medication Documentation
- Study Drug Infusion
- Perform rating scales:
  - Clinician-Administered Dissociative States Scale (CADSS)

- Brief Psychiatric Rating Scale (BPRS)
- POMS - Bipolar
- BAI
- VAS-stressed

Study Visit 0 will occur up to six weeks after Study Visit -1. The protocol for the treatment day (Study Visit 0) is as follows. A urine toxicology screen and pregnancy test will be performed before the infusion. The urine toxicology must be negative for illicit substances and the pregnancy test must be negative in order to proceed with the infusion.

- Before their infusion, participants will complete up to 1 hour of computerized cognitive tasks to assess reward processing, motivation, agency, and executive attentional function.
- Representative cognitive tasks are listed below:

**The Incentive Flanker Task (IFT):** The incentive flanker task (IFT) is a variant of the monetary incentive delay task (MID) in which participants win or lose money based on their performance on flanker trials (stern et al., 2011). Cues presented prior to letter stimuli (2-6 s) designate the monetary value for each trial: (1) "gain" cues indicate that subjects earn 50 cents with a correct response; (2) "loss" cues indicate that subjects can avoid a loss of 50 cents with a correct response; (3) "neutral" cues indicate that no money is at stake. Two-thirds of all cues are followed by the letter stimuli (congruent or incongruent). Immediately after the response, outcome feedback is presented for 2 s, followed by a blank inter-trial interval (ITI) for 2–6s before the next trial begins with a new cue. One-third of cues are followed by a blank screen for 2s ("catch" trials), which will break colinearity between the cue and feedback. Trial types are presented in pseudorandom order and equally divided across runs.

**The Self-Agency Task (SAT):** The self-agency task (15 minutes) is cognitive task programmed in PsychoPy. Subjects undergo training on the task in a self-paced manner before starting the task. In baseline block 1, subjects click the left and right keyboard buttons quickly to move a visual bar up the computer screen to a target position for 6 trials. In baseline block 2 subjects repeat this but are informed and see that the computer is interfering with the bar as they move it up. After the baseline blocks, reaction times for each target position are computed and used in the main block. In the main block, the trial starts with an image of the target bar position and information on whether the trial is a win trial (for $0.50) or a loss trial (-$0.50). Subjects then make button presses move the bar up to the target position as quickly as possible. Subjects then receive feedback on whether they won or lost depending on their reaction times compared to their performance during the baseline blocks. On 75% of the trails, the computer ambiguously interferes with the movement of the bar. After the feedback, subjects report on a visual analogue scale whether they or the computer were more in control of the bar. There are 90 trials, 45 win and 45 loss.

**The Attention Network Task (ANT):** The Attention Network Task is a game wherein the participant is presented with a series of five arrows in each trial and must work through several distractors to indicate the orientation (left or right) of the center arrow. In each trial, the participant is first presented with a crosshair and after a random length of time (50-300ms) a cue is presented either above, below, or in place of the crosshair. Immediately following the cue, the participant is presented with the arrows either above or below the crosshair (which are either in place of the cue, or the cue was a distractor) and must indicate whether the center arrow is pointing left or right. The flanking arrows can also act distractors by either being congruent, incongruent or in random directions compared to the center arrow. It is theorized that the Attention Network Task gauges three distinct faculties of the Attention Network: 1) Alerting -- involving arousal precipitated by anticipation of some event that requires fast reactions. 2) Orienting – is a more selective allocation of attention resources to goal-related stimuli in an environment, and 3) Executive control – which is characterized by higher-level decision making and discernment, error detection, etc. Performance in the task is gauged by error rate and reaction time of each trial.

Patients will be assessed by qualified staff throughout the infusions. Prior to Infusion, an indwelling catheter will be placed in the antecubital veins of right or left arm, and pulse, blood pressure, pulse-oximetry, and an EKG strip will be placed for continuous monitoring. At Time 0, the infusion will begin through an infusion pump and will continue until completion (on average after 40 minutes from the beginning of the infusion). Clinical ratings (BPRS, CADSS, see below) will be collected before and after the infusion period by clinical raters. Psychometric tests on anxiety, and positive and negative affect (POMS-Bipolar, PANAS, BAI, VAS-stressed) will also be performed before and after the ketamine infusion A study physician with privileges to administer ketamine will be present at the infusion throughout the administration of ketamine or midazolam. For more details regarding the ketamine infusion procedures, please refer to the Mount Sinai Treatment Protocol for Ketamine Clinical Research (ver. Jan 27, 2017).

*Study Visits 1: +24 h follow visit*

Summary of study procedures during this phase:

- Adverse Events Documentation
- Concomitant Medication Documentation
- Perform self-administered rating scales:
    - POMS – Bipolar
    - PANAS
    - BAI
    - VAS – Stressed
- Cognitive Testing (see Study Visit 0 for detailed description) *

Study Visit 1 will occur 24 hours after Study Visit 0.

*Note: The 24-hour follow up visit may be performed remotely in order to reduce face-to-face exposure during the COVID-19 pandemic. A member of the study team will call the participant for AE documentation, and Con Med documentation. Participants will complete the REDCap rating scales at home.*

* In case of a remote visit, participants will not complete cognitive testing remotely.

*Study Visits 2: Assessment day*

Summary of study procedures during this phase:

- Stress task (Trier Social Stress Task)
- Vital Signs
- Urine Toxicology
- Clinical interview and history interval with Adverse Events Documentation
- Concomitant Medication Documentation
- Saliva and blood draws for biomarker collection and pharmacokinetics *
- Perform self-administered rating scales:
  - POMS-Bipolar
  - PANAS
  - BAI
  - VAS-Stressed

Study Visit 2 will occur one week after Study Visit 1.

A urine toxicology screen will be performed at the beginning of the assessment and must be negative for illicit substances in order to proceed with the visit. The baseline rest and stress procedures will be carried out in separate rooms (depicted as Room A and Room B). The baseline rest phase takes place in Room A. Upon arrival, a 60-90 min period is required to allow hypothalamic-pituitary-adrenal axis activity to normalize, as the process of attending the laboratory session itself will likely cause an increase in HPA-axis activity in many participants. During this time an indwelling catheter for blood collection will be placed by trained study personnel. Samples/measures will be collected immediately pre- and post-stress. Following task instructions, participants then complete the stress procedure in Room B. The procedure runs as follows: participants are introduced to a role-playing scenario. They have to prepare a speech to convince a panel that they are the perfect candidate for a job, which they must then present to a panel of assessors, followed by a mental arithmetic task (serial

subtraction). Following these tasks participants will return to Room A for the resting period and post-stressor measures will be taken. Repeated samples are collected at 15 min intervals. A recovery period of 60 minutes will be used. During the baseline rest phase, the psychological task and the resting period a number of physiological and psychological parameters will be measured throughout the procedure via saliva (cortisol, salivary α-amylase), physiological tests (heart rate, blood pressure), and psychometric tests on anxiety, and positive and negative affect (POMS-Bipolar, BAI, VAS-Stressed). Plasma and serum sampling will be collected to test for biomarker such as DHEA, s-DHEA, testosterone, NPY. Prior to the stress procedure (TSST) blood sample will be collected to assess the circulating levels of ketamine and midazolam. Please refer to Table 2. Assessment day (TSST) – Visit 2: Schedule of events and procedures for more details.

*Study Visit 3: Follow-up Assessment/Study exit and Early Discontinuation Visit*

Summary of study procedures during this phase:

- Clinical interview with history interval and Adverse Events documentation
- Concomitant Medication Documentation
- Physical Exam
- Vital Signs
- Routine Laboratory Tests
- Perform self-administered rating scales:
  - POMS
  - PANAS
  - BAI
  - VAS

A medical history and physical examination will be performed for each patient and basic laboratory tests ordered. This will include vital signs, complete blood cell counts, electrolytes, liver function tests. The Follow-up Assessment/Study exit visit may occur on up to two separate days. The subject will be considered exited from the study when all the procedures listed above are completed.

*Note: In an effort to limit face-to-face exposure during the COVID-19, the physical exam, vital signs, and routine lab tests may be completed at the end of the V2 visit. The clinical interview, AE documentation, Con Med documentation, and rating scales may be performed on the day of V2 or remotely on another day. If the visit is completed on the day of V2, rating scales will not be administered.*

**Description of Study Instruments**

**The Profile of Mood States – Bipolar Version (POMS - Bi)** scale measures moods and feelings primarily in clinical rather than nonclinical settings. It can help to determine an individual's psychiatric status for therapy, or be used to compare mood profiles associated with various personality disorders. It is also a useful instrument in identifying the effects of drug treatments (O'Halloran et al., 2004). This instrument will represent the primary outcome measure of the study.

The **Structured Clinical Interview for DSM-IV Axis I Disorders (SCID)** (First et al., 2006) is a semi-structured interview that provides probe questions as well as follow-up questions to be asked by the clinician to assist in diagnosis. It includes an overview to obtain information about demographics, work, chief complaint, history of present illness, past history, treatment history, and current functioning. The main body of SCID (patient edition) includes 9 modules that are designed to diagnose 51 mental illnesses in all.

The **Brief Psychiatric Rating Scale (BPRS)** (Overall & Gorham, 1962) is used to assess acute behavioral changes during the infusions. Four key BPRS items for the positive (+) symptoms of psychosis will be used: conceptual disorganization, hallucinatory behavior, suspiciousness, and unusual thought content. Three items representing the negative (-) symptoms of psychosis will also be used: blunted affect, emotional withdrawal, and motor retardation.

The **Clinician-Administered Dissociative States Scale (CADSS)** (Bremner et al., 1998) is used to measure dissociative effects during the infusions. The scale includes 19 questions and 8 observer ratings scored from 0 (not at all) to 4 (extremely). The CADSS measures impairment in body perception, environmental perception, time perception, memory impairment, and feelings of unreality.

The **Visual Analog Scales**: These scales are scored in millimeters from the left-hand side of a 100-mm line to a perpendicular mark made by the patient at a point corresponding to the apparent magnitude of the feeling state. Range: 0 ("not at all") to 100 ("most ever").

**Beck Anxiety Inventory (BAI)** (Beck et al., 1988). This is a 21-question multiple-choice self-report inventory that is used for measuring the severity of anxiety in adults. The questions used in this measure ask about common symptoms of anxiety (such as numbness and tingling, sweating not due to heat, and fear of the worst happening). It is designed for individuals who are of 17 years of age or older and takes 5 to 10 minutes to complete. Several studies have found the Beck Anxiety Inventory to be an accurate measure of anxiety symptoms in children and adults.

The **Reproductive Lifecycle and Hormone Questionnaire (RLHQ)** (Freeman et al., 2013) is a standard brief questionnaire implemented systematically in clinical trials for MDD to assess reproductive lifecycle status and the use of exogenous hormones. This study will use Modules 1 and 2 in all women, and add Module 3 (menstrual cycle tracking) in applicable women.

The **Behavioral Inhibition/Avoidance Scale (BIS/BAS)** (Carver & White, 1994) is a 24-item self-report questionnaire designed to measure two motivational systems: the behavioral inhibition system (BIS), which corresponds to motivation to avoid aversive outcomes, and the behavioral activation system (BAS), which corresponds to motivation to approach goal-oriented outcomes. Participants respond to each item using a 4-point Likert scale: 1 (very true for me), 2 (somewhat true for me), 3 (somewhat false for me), and 4 (very false for me). The scale has four subscales that were derived via factor analysis. One subscale corresponds to the BIS. Seven items contribute to this score (e.g., "Criticism or scolding hurts me quite a bit"). The remaining three subscales correspond to three components of BAS. BAS Drive measures the motivation to follow one's goals. Four items contribute to this score (e.g., "When I want something I usually go all-out to get it"). BAS Reward Responsiveness measures the sensitivity to pleasant reinforcers in the environment. Four items contribute to this score (e.g., "It would excite me to win a contest"). BAS Fun Seeking measures the motivation to find novel rewards spontaneously. Five items contribute to this score (e.g., "I crave excitement and new sensations").

The **Connor-Davidson Resilience Scale (CD-RISC)** (Connor & Davidson, 2003) is a 25-item self-report scale, each rated on a 5-point scale (0-4), with higher scores reflecting greater resilience.

The **Perceived Stress Scale (PSS)** (Cohen, S et al., 1983). A 10-item self-report scale that was developed to measure the degree to which situations in one's life are appraised as stressful.

**h) Specimen Banking**

Samples will be banked at the Center for Affective Neuroscience at the Mount Sinai School of Medicine (PI: Scott Russo) for the purpose of analysis as outlined in the current protocol. Individual aliquots will be stored and accessed by trained laboratory personnel at regular intervals until the end of the study.
Samples will be identified with the following: study number, sample type, date, visit number, and participant study ID number. Samples will also be labeled with a unique number and barcode assigned by the labeling computer system. The participant study ID number is linked to identifying information (e.g. name, date of birth) however, only approved members of the

DAC research team will have access to the code list which links that participant study ID number to health-protected information. Only trained personnel will have access to samples. Participants have the right to withdraw consent at any time by contacting the study principal investigator. At that time, samples that have not already been used for research will be promptly withdrawn from storage and destroyed by trained personnel.
Results will be published as group data without the use of characteristics that would identify individual subjects. We quote information only by number in conference discussions, scientific reports, or publications, in order to maintain anonymity.

**i) Data Management and Confidentiality**

All data will be kept confidential and utilized only for the purpose of research. Participants will be given coded identifiers in order to store data and link this data with each participant. All questionnaires, forms, and medical/laboratory tests will have the patient's ID number with initials on each page but no other identifying information will be included on these forms. The diagnostic interviews will also only include coded identifiers and initials on all notes and forms. The individual managing the data will be the research coordinator and will not be blinded to patient names, addresses, or other personal patient information. No participant's identifying data will be published. The PI is responsible for maintaining the integrity of data and data analysis. The research coordinator will be in charge of maintaining the database under PI supervision. All electronic records will be kept confidential to the extent permitted by law, stored in a file (including the linking file) on an electronically secure database in the Department of Psychiatry at MSSM maintained by Sinai's IT department. This database is password protected and only study personnel will be given the password. A backup of the database will be conducted each day and stored on the principal investigator's laptop computer. All files (including linking files) on the laptop containing identifiers will be encrypted using software packages supported by MSSM. Only study personnel will have access to the database and backup. Data will be kept up to 7 years.

The data from the GCO 06-0945 online Pre-screener is stored in a secure redcap online database. The database has been designed such that no one has access to the subjects' answers. The study team, including Dr. Murrough who is also the PI for GCO 06-0945, only has access to the subject's contact information along with an indication as to whether or not the participant appears initially eligible based on the parameters set up in the pre-screener.

***Data analysis***

Demographic and clinical characteristics, blood, salivary biomarkers, and behavioral measures will be summarized separately. Safety and tolerability data will be summarized by treatment group. Adverse events will be summarized by the Medical Dictionary for

Regulatory Activities (MedDRA) system organ class and preferred terms. Separate tabulations and listings will be produced for related adverse events, serious adverse events, discontinuation due to adverse events and events of at least grade 3 severities.

Continuous variables will be summarized using either means and 95% confidence intervals or medians and inter-quartile ranges. The association between ketamine and positive-negative affect (as measured by the POMS-Bipolar subscale scores) will be assessed by means of multiple linear regression. Additional predictors may be explored in the analysis, such as clinical and demographic variables, including age, gender, BMI, baseline anxiety. The influence of stress exposure and cortisol will also be examined. See below for details.

The *primary behavioral outcome* will be a change of the anxious-composed subscale score of the POMS-Bipolar at the +0 time point in response to an acute stress in the KET group compared to the MID group using linear mixed models with pre-TSST -25-minute POMS scores as a within-subjects factor and treatment group as a between-subjects factor. Similar analyses will be done with the other behavioral measures for the *secondary behavioral outcome* (other POMS-Bi subscale scores, PANAS, VAS, BAI). In addition, time points beyond the 0-minute time point will be explored (15-minute, 30-minute, 45-minute, and 60-minute). For the *primary and secondary biological outcomes*, similar analysis will apply to the HPA axis activity (measured with salivary cortisol) and the ANS activity (salivary α-amylase) at the 0-minute, 15-minute, 30-minute, 45-minute, and 60-minute time points. *Exploratory analysis* will be performed on blood samples, to test for hormones like testosterone, DHEA, s-DHEA, ghrelin, and NPY, and on heart rate and blood pressure measurements before and after the TSST.

**j) Provisions to Monitor the Data to Ensure the Safety of subjects**

All data will be obtained for research purposes only. We will collect information about participants' psychiatric history, including previous treatments, medical history, and family history. As part of the medical evaluation, we will collect blood and urine specimens for analysis, and perform an ECG. The outcome of these tests will be shared with the participant. In the event that we detect a medical condition that requires treatment, we will provide the participant with an appropriate referral.

**Data and Safety Monitoring Plan**

This study will rely on the Data and Safety Monitoring Board (DSMB) created for the ongoing ketamine studies at DAC as an independent body charged with ensuring that the safety of study subjects is protected and that the scientific goals of the study are being met.

To achieve this goal, the DSMB will perform an ongoing evaluation of the safety of patients participating in the clinical trial. This includes monitoring adverse events and overall clinical outcomes in the studies. Through this process, the board will evaluate the need for study termination and/or modification of study criteria for inclusion, exclusion, or discontinuation. In addition, the DSMB will be responsible for the evaluation of the adherence to written protocols regarding the protection of human subjects and the assurance of identity protection as specified in the Data Sharing Section. The DSMB will expeditiously review all serious adverse events and perform ongoing scheduled reviews of non-serious adverse events and study dropouts.

Investigators will submit biannual reports to the DSMB. The DSMB reports will include a summary of all safety findings, as well as an assessment of protocol compliance and data quality. Any recommendations to improve patient safety, protocol adherence, or data quality will be made in the biannual DSMB report. If at any time during the course of the study, the PI or DSMB believes that risk to subjects outweighs the potential benefits, the PI or DSMB shall have the discretion and responsibility to recommend that the study be terminated or altered.

Adverse Events (AE) will be summarized by the MedDRA system organ class and preferred terms. Grading of adverse events will follow standard FDA guidelines and will utilize the MSSM adverse events tracking form template. Specifically, all adverse events will be graded as either serious or non-serious. In addition, adverse events will be graded in terms of the outcome, duration, intensity, and causality.

The PI will monitor the accuracy and completeness of the data. The following elements will be monitored: recruitment procedures and pace; adherence to the exclusion/inclusion criteria; deviation from protocol; the timeliness of documentation and data entry; and the accuracy and confidentiality of all information both in study documents and study database. The PI will provide the DSMB with a report on data quality and completeness. At a minimum, this will include an overview of the progress of patient intake and retention; summary reports describing patient compliance with visits, evaluations and dosing as described in the protocol; and a summary of the completeness and quality of key data elements needed to characterize patients, their dosing, and their primary and secondary outcomes. These reports will be used by the DSMB to evaluate the capacity of data capture and processing to support scientifically valid analyses.

In the event of temporary or permanent suspension of the study the DSMB and the FDA will be notified. The IRB will also be notified should a temporary or permanent suspension occur.

**k) Withdrawal of Subjects**

Subjects may withdraw at any point in the study as long as they provide consent. The study doctor, the PI or the institution may stop involvement in this research study at any time without consent. This may be because the research study is being stopped, the instructions of the study team have not been followed, the investigators believe it is in the patient's best interest, or for any other reason. All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. Subjects who discontinue the study will be invited to complete a final safety check and study exit procedures as early discontinuation visit (vital signs, a physical exam, blood chemistries, and an EKG will be performed).

## 5) Risks to Subjects

Though we have made every effort to reduce the potential risks associated with study procedures, the following sources of potential discomfort or harm could not be eliminated:

Risk related to Ketamine

Ketamine is a sedative/analgesic and general anesthetic for human and veterinary use. It may produce mild to moderate increases in blood pressure, heart rate, and cardiac output due to its sympathomimetic effects and can provoke ischemia in patients with underlying coronary artery disease. Overall, however, ketamine has a favorable acute safety profile. Over the past several decades, ketamine has been administered as an anesthetic to several million adults and children.

The reported incidence of ketamine-induced perceptual disturbances varies from less than 5% to greater than 30% (Knox et al. 1970; White et al. 1980) and may manifest as vivid dreaming, visualization of psychedelic color, suspension in space, kaleidoscopic floating, and out-of-body experiences. Some patients describe these psychic experiences as bizarre or frightening, while others find them pleasurable, joyful, and fascinating. Indeed, ketamine is also known as "Special K" as a drug of abuse related to PCP. For that reason ketamine was placed in Schedule III of the Controlled Substance Act in 1999. Nonetheless, when perceptual disturbances occur, they are usually mild and of short duration (Green and Johnson 1990). Ketamine administration has transient effects on symptoms, usually lasting less than 60 minutes and rarely lasting longer than 2 hours (Carpenter 1999). The perceptual disturbances may be more common in those with preexisting psychosis (Lahti et al. 1995).

Several studies have addressed the question of prolonged psychological effects in the general population secondary to its anesthetic use and concluded that ketamine does not place patients at a greater risk than other anesthetics (Hersack 1994; Reich and Silvay 1989; Schorn and Witwam 1980). There is no evidence in these long-term studies that individuals exposed to ketamine are at risk of abusing it on follow-up.

Both us and others have safely used ketamine in a large number of psychophysiological studies in patients with psychiatric disorders as well as in healthy with doses similar to that of the present study. A meta-analysis of a number of these studies found no evidence of behavioral sensitization following repeated ketamine exposure (Cho et al. 2005).

Risk related to midazolam

Midazolam is a water-soluble benzodiazepine available for intravenous or intramuscular injection. Intravenous midazolam has been associated with respiratory depression and respiratory arrest, especially when used for sedation in noncritical care settings. In some cases, where this was not recognized promptly and treated effectively, death or hypoxic encephalopathy has resulted. To protect study participant from the following risk continuous monitoring of respiratory and cardiac function will be provided and resuscitative drugs and appropriate equipment for ventilation and intubation, and personnel trained in ACLS will be available. Reactions such as agitation, involuntary movements, hyperactivity and combativeness have also been reported in adult and pediatric patients. Other side effect related to the administration of midazolam are hiccoughs, nausea, vomiting coughing ‘‘over sedation’’ headache and drowsiness. Reactions at the site of the injection, as tenderness, pain, redness, phlebitis have been reported.”

Psychological Screening

Subjects that meet exclusion criteria may become distressed when informed. All information will be shared in a private office with the door closed and a trained study team member will be available to offer information, support, and referral for any services indicated.

Medical Screening

Participants may experience minor discomfort or pain when a needle is used to draw blood. Bruising, infection, and dizziness are also common side effects. Risks of venipuncture are minimized by having experienced nursing personnel who will perform the procedure. Infection is avoided by adequate cleansing of the skin prior to intravascular line insertion.

Privacy risks

There always exists the potential for loss of private information; however, there are procedures in place to minimize this risk. Please refer to sections h. “Data Management and Confidentiality” and i. “Provisions to Monitor the Data to Ensure the Safety of subjects” for additional information

**7) Provisions for Research Related Injury**

We have described above the potential risks of the research procedures and the safeguards that will be used to minimize risks. These include termination of research participation if it is believed that such participation endangers a patient’s welfare. Monitoring procedures are used to evaluate potential side effects of treatment or of research procedures. The protocol stipulates an extensive

medical and psychiatric evaluation of all patients as a condition for research participation. Patients are monitored for potential reactions during the clinical trial.

**8) Potential Benefits to Subjects**

The possible benefits to the patient and to others are reasonable in relation to the risks of this study. All study participants will receive without cost an extensive clinical evaluation. No other direct benefits result from study participation.

**9) Provisions to Protect the Privacy Interests of Subjects**

If an individual appears to meet enrollment criteria and is interested in participating, a face-to-face interview will be conducted by one of the project Investigators. The nature of the project, procedures, relative risks and benefits, and alternatives to participation in this project will be discussed with each subject. Following this discussion, the potential study participant will be given a copy of the consent form to review at their leisure, and any questions are answered. Study visits and procedures will take place at the MAP clinic.

If the individual decides not to participate in this study, a staff member provides reasonable and timely assistance in obtaining an alternative referral, if so desired. The decision not to participate does not affect eligibility to participate in future studies, to receive treatment at Mount Sinai, or to receive treatment on a private basis from a referring clinician. Subjects will be also given the opportunity to withdraw from the study prior to analysis of their data.

The consent process also includes documentation of permission to obtain previous medical records, including contact with previous physicians, pharmacies, and family members, if needed. The IRB-approved forms for informed consent are made part of the patient's permanent medical record, with a copy filed in the research chart.

**10) Economic Impact on Subjects**

Participants will incur no costs for their participation in the study. All costs associated with participation in the study will be covered by internal funds.

However, if a study doctor arranges a patient to have additional medical tests which are not part of the study but needed for regular health care, subjects may have to pay for these if they are not covered by health insurance.

**11) Payment to Subjects**

| Procedure | Subject Payment |
|---|---|
| Visit -1 (screening) | $ 25 |
| Visit 0 | $ 100 |
| Visit 1 | $ 25 |
| Cognitive Tasks (x2) | $60 |
| Visit 2 | $ 100 |
| Visit 3 | $ 25 |
| Total | $ 335 |

If subjects agree to take part in this research study, we will pay them up to $335.00 for their time and effort. Payment, made by check, will be prorated based on the actual procedures completed, as described in Table 1 "Schedule of Assessments, Outcome Measures, Labs and Procedures".

## 12) Consent Process

If an individual appears to meet enrollment criteria and is interested in participating, a face-to-face interview will be conducted by one of the project investigators. If the study screen is preformed remotely, the subject will be sent a copy of the approved consent form, a study doctor will discuss the consent form with the potential participant over a tele-communication platform, and the signature will be obtained via REDCap where both the subject and the study doctor can sign the consent. Referring clinicians will be asked to inform potential participants of the possible alternatives to participating in the study. A release of information will be obtained for review of any available historical and clinical data. A written authorization form is also obtained from each patient, permitting the research team to use, create, or disclose his or her PHI for research purposes. A qualified staff member will discuss the nature of the project, procedures, relative risks and benefits, and alternatives to participation in the project with each potential study participant. Following this discussion, the subject will be given a copy of the consent form to review at their leisure, and any questions are answered. Study visits and procedures will take place at the Depression and Anxiety Center or via HIPAA-compliant telecommunication platform. A study physician will seek written voluntary informed consent from all participants prior to study entry. The initial consent form describes the nature of the procedures and time requirements, potential risks, the confidentiality of information, and the rights of research participants. If the individual remains interested in the project, written informed consent is obtained by a study physician, and medical and psychiatric screening procedures are undertaken to confirm eligibility. A copy of the consent form is provided to all participants. If the individual decides not to participate in this study, a staff member provides reasonable and timely assistance in obtaining an alternative referral, if so desired. The decision not to participate does not affect eligibility to participate in future studies, to receive treatment at Mount Sinai, or to receive treatment on a private basis from a referring clinician. Participants will be also given the opportunity to withdraw from the study prior to

analysis of their data. The consent process also includes documentation of permission to obtain previous medical records, including contact with previous physicians, pharmacies, and family members. The study will be using the standard PPHS consent form. If the study screen is preformed remotely, the subject will be sent a copy of the approved consent form, a study doctor will discuss the consent form with the potential participant over a tele-communication platform, and the signature will be obtained via REDCap where both the subject and the study doctor can sign the consent.

## 13) Process to Document Consent in Writing

We will be using the standard PPHS consent template to document consent. If the study screen is preformed remotely, the signature will be obtained via REDCap where both the subject and the study doctor can sign the consent.

## 14) Vulnerable Populations

The proposed study does not involve vulnerable populations, such as fetuses, neonates, pregnant women, children, prisoners, institutionalized individuals, or others who may be considered vulnerable populations. Non-English speaking subjects will also not be enrolled in this study. Our staff is primary English speaking and is trained to use standardized diagnostic and rating tools are in English. Although non-English speaking individuals may be consented with appropriate translation services, we would not be able to diagnose and rate such individuals using the same standards. All participants are adults who are able to freely consent to research.

| Include | Exclude | Vulnerable Population Type |
|---|---|---|
| | X | Adults unable to consent |
| | X | Individuals who are not yet adults (e.g., infants, children, teenagers) |
| | X | Wards of the State (e.g., foster children) |
| | X | Pregnant women |
| | X | Prisoners |

## 17) Sharing of Results with Subjects

We don't have a plan to share the results of the study with the study subjects, however if the subjects are interested, the study team will be available to discuss the results with them after completion of the study. If any abnormal results emerge from the physical exam, labs, or EKG,

the results will be discussed with the participant by a study physician and the subjects will be provided with a copy of the results if appropriate.

## 19) Control of Drugs, Biologics, or Devices

The pharmacist prepares the IV medication (ketamine and midazolam) in a 100 ml NS bag, and qualified personnel (e.g., nurse from the CRU) come to retrieve it the morning of infusion, such that a fixed dose of ml per kg is administered. Ketamine and midazolam will be stored and dispensed by the research pharmacy according to the package inserts information and GCP standards. Ketamine / midazolam infusions may take place in the Psychiatry Infusion Suite which is located at 1425 Madison Ave. adjacent to the Madison 5 Inpatient Psychiatry Unite, 5th Floor. In the event of a respiratory or cardiac arrest, Team 7000 will be called. The Psychiatry Infusion Suite is equipped with monitoring devices for vitals and EKG. Additionally, the infusion is conducted under the constant supervision of a trained provider who can interrupt the infusion if needed. Due to the short half-lives of ketamine and midazolam, stopping of these drugs will quickly reverse excessive sedation. If needed, a crash cart is available in the Infusion Suite.

## 20) Adverse Experience Reporting and Documentation

### a) Adverse Events

An AE is any untoward medical occurrence in a study subject administered an investigational product and that does not necessarily have a causal relationship with this treatment.

An unexpected AE is any adverse drug event, the specificity or severity of which is not consistent with the current IB or prescribing information for a marketed compound. Also, reports which add significant information on specificity or severity of a known, already documented AE constitute unexpected AEs. For example, an event more specific or more severe than described in the IB would be considered “unexpected”.

An AE therefore can be any unfavorable and unintended sign (including laboratory finding), symptom or disease temporally associated with participation in an investigational study, whether or not considered drug-related. In addition to new events, any increase in the severity or frequency of a pre-existing condition that occurs after the subject signs a consent form for participation is considered an AE. This includes any side effect, injury, toxicity, or sensitivity reaction.

Any condition, laboratory abnormality, or physical finding with an onset date prior to the subject signing consent for study participation is considered to be pre-existing in nature and part of the subject’s medical history.

**AE Severity**

List criteria for AEs (*such as the National Cancer Institute's Common Terminology Criteria for Adverse Events, if used*) and the version should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

| **Severity (Toxicity Grade)** | **Description** |
|---|---|
| Mild (1) | Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well. |
| Moderate (2) | Mild to moderate limitation in activity, no or minimal medical intervention/therapy required. |
| Severe (3) | Marked limitation in activity, medical intervention/therapy required, hospitalizations possible. |
| Life-threatening (4) | The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe. |

**AE Relationship to Study Drug**

The relationship of an AE to the study drug should be assessed using the following guidelines

| **Relationship to Drug** | **Comment** |
|---|---|
| Definitely | Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis. |

| | |
|---|---|
| Probably | An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions. |
| Possibly | An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors. |
| Unlikely | An event that does not follow a reasonable temporal sequence from administration of the Investigational Product, and that could readily have been produced by a number of other factors. |
| Unrelated | An event that can be determined with certainty to have no relationship to the study drug. |

**b) Serious Adverse Experiences (SAE)**

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- Death
- Life threatening experience defined as any adverse experience that places the subject, in the view of the treating physician, at immediate risk of death at the time of occurrence; ie, it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires inpatient hospitalization or prolongation of an existing hospitalization (except scheduled hospitalizations for non-acute, unrelated cause such as an elective surgery)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect in the offspring of an exposed subject
- Important medical events that may not result in death, be life threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, it jeopardizes the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
- Any death occurring within 30 days of the subject receiving study drug, regardless of the subject having discontinued from the protocol, must be reported as an SAE.

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

**AE/SAE Reporting Procedures to Mount Sinai PPHS (IRB)**

AEs are reportable to the IRB within 5 business days (SAEs within 24 hours of knowledge of the event) *when they meet the following definition*:

Any 'harm' experienced by a subject or other individual that in the opinion of the investigator is *unexpected* AND *at least probably related* to the research

All AE/SAEs with an onset date after the subject signs consent for study participation must be reported to the IRB *at the time of annual renewal*. Details of the event must include severity, relationship to study drug, duration, action taken, and outcome.

All AE/SAEs that are considered related to study drug must be followed to resolution or stabilization if improvement is not expected. AE/SAEs that completely resolve and then recur should be recorded as a new AE/SAE. AE/SAEs continuing at 30 days post-last dose should have a comment in the source documents by the PI that the event has stabilized or is not expected to improve.

## 21) Protocol Violations

A protocol violation occurs when the subject, investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The investigator will determine if a protocol violation will result in withdrawal of a subject

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by the Investigator. A copy of the form will be filed in the site's regulatory binder.

## 22) Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators for resolution. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

## 23) Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database. At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

**24) Availability and Retention of Investigational Records**

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.
All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the *Sponsor* should be contacted prior to removing study records for any reason.

**25) Protocol Amendments**

Any amendment to the protocol will be written by *the Principal Investigator*. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

**26) Institutional Review Boards and Independent Ethics Committees**

The protocol and consent form will be reviewed and approved by the IRB prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB.

The IRB written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review. Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

## 27) Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

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## Table 1: Schedule of Assessments, Outcome Measures, Labs and Procedures

| Study Phase | Screening | Infusion Day | +24h Assessment | Assessment Day (TSST) | Follow-up & Study exit |
|---|---|---|---|---|---|
| **Study visit** | V-1 | V0 | V1 | V2 | V3 |
| **Study day** | Day -28 | Day 0 | Day 1 | Day 7 | Day 14 |
| ***Screening*** | | | | | |
| Informed Consent | X | | | | |
| Inclusion/Exclusion Criteria | X | | | | |
| Demographics | X | | | | |
| Medical History | X | | | | |
| SCID-5 | X | | | | |
| BIS/BAS | X | | | | |
| PSS | X | | | | |
| CD-RISC | X | | | | |
| RHLQ | X | | | | |
| ***Drug Administration*** | | | | | |
| Randomization | X | | | | |
| Ketamine/Midazolam | | X | | | |
| ***Cognitive Assessments*** | | | | | |
| Cognitive testing | | X | X | | |
| ***Safety Assessments*** | | | | | |
| Physical Exam | X | | | | X |
| Vital Signs | X | X | | X | X |
| Laboratory Tests [a] | X | | | | X |
| EKG | X | | | | X |
| U-Toxicology | X | X | | X | |
| Adverse Events | X | X | X | X | X |
| ***Efficacy Assessments*** | | | | | |
| POMS [b] | X | X | X | X | X |
| CGI-S | X | | | | |
| VAS-Stressed | X | X | X | X | X |
| BAI | X | X | X | X | X |
| Biomarkers [c] | | | | X | |

[a] Clinical laboratory tests include chemistry, electrolytes, complete blood count, liver function tests, thyroid-stimulating hormone (TSH) levels, and urinalysis; [b] POMS-Bipolar represents the primary outcome measure of the study; [c] Biomarkers: salivary and blood samples will be used for biomarker collections.

Abbreviations: BAI, Beck Anxiety Inventory; BIS/BAS, Behavioral Avoidance / Behavioral Inhibition Scales; CD-RISC, The Connor-Davidson Resilience Scale; CGI-S, Clinical Global Impression-Severity; ECG, Electrocardiogram; RHLQ, Female Reproductive Lifecycle and Hormones Questionnaire; POMS-Bipolar, Profile of Mood States – Bipolar Version; PSS, Perceived Stress Scale; VAS-Stressed, Visual Analog Scale

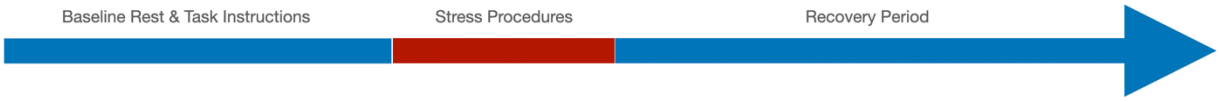
| | |
|---|---|
| Protocol Name: | Effect of ketamine on laboratory-induced stress in healthy subjects: A Proof-of-Concept Translational Study (IRB #17-01105; GCO #17-2440) |
| Principal Investigator: | James Murrough<br>212-585-4640<br>james.murrough@mssm.edu |

**Table 2: Assessment Day (TSST) – Visit 2: Schedule of events and procedures**

Room A.
Baseline Rest

Room B.
Stress Procedures
- 3 minute preparation
- 5 minute speech
- 5 minute mental arithmetic

Room A.
Recovery period
Biomarker collection




| MEASURES | TIME POINTS | | | | | | |
|---|---|---|---|---|---|---|---|
| | Baseline | Stress Procedure | Primary Outcome | Recovery Period | | | |
| | t-25 | t-10 | t0 | t+15 | t+30 | t+45 | t+60 |
| Behavioral: POMS, PANAS, BAI & VAS - Stressed | X | | X | X | X | X | X |
| HPA axis: salivary cortisol | X | | X | X | X | X | X |
| SAM axis: salivary α-amylase | X | | X | X | X | X | X |
| Blood Biomarkers | X | | X | X | X | X | X |
| Cardiovascular: HR & BP | X | | X | X | X | X | X |