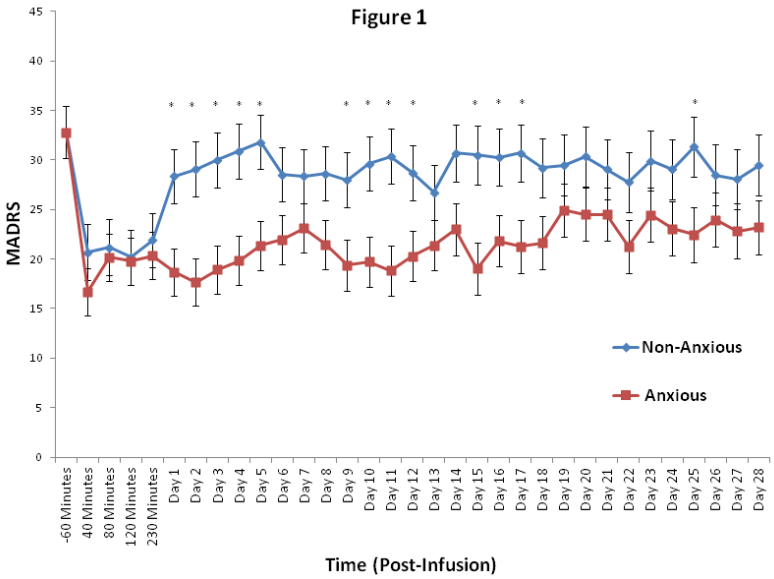
Detailed Protocol:

Baseline Insular Dysfunction as a Predictor of Ketamine's Antidepressant Effects in Anxious Depression

I. Background and Significance

Remission of major depressive disorder (MDD) often takes weeks to months to achieve on traditional monoamine-based antidepressants. Only about one-third of individuals with MDD remit on an initial course of treatment, with even lower rates experienced among individuals with particular subtypes, such as anxious depression. There is a compelling and widely acknowledged need for antidepressants that work more rapidly, that recruit novel/non-monoamine mechanisms, and that target subtypes of depression that respond poorly to current antidepressants. Towards this end, single subanesthetic infusions of ketamine (an antiglutamatergic) have recently been shown to improve the symptoms of depression in a rapid (within hours), robust (across many symptoms), and relatively sustained manner in patients with treatment-resistant depression (TRD).(1) Ketamine may have greater efficacy for forms of depression not well treated by current agents, including anxious depression.(2) However, knowledge concerning the mechanism of ketamine's antidepressant action (especially in anxious depression) is limited. Furthermore, predictors of response to ketamine are largely unknown. The current proposal is aimed at further delineating the neurobiology of ketamine's effects among individuals with treatment-resistant anxious depression. *A better understanding of the mechanisms of ketamine's profound and rapid impact on depressive symptoms and depression subtypes (such as anxious depression) will likely play a major role in antidepressant drug discovery while further elucidating the pathophysiology of depressive disorders.*

Preliminary Data and Rationale: Over the course of 28 days, **patients with anxious depression** (*n*=15) **have been shown to have significantly greater antidepressant responses to ketamine compared to patients with nonanxious depression** (*n*=11; Figure 1, * = $p<0.05$; largest effect at Day 2 (Cohen's *d*=0.76)), with longer median days-to-relapse (19.0±17.9 *vs* 1.0±0.0 days-to-relapse, respectively; *p*=0.002), and with no differences in differ in dissociative (*p*=0.62) or psychotomimetic (*p*=0.41) side effects.(2) These are clinically relevant findings, given that anxious depression is typically more difficult-to-treat than nonanxious depression,(3) and suggest that symptomatically-diagnosed anxious depression may, indeed, be a *clinical* predictor of ketamine response. However, the neurobiological mechanism of action by which ketamine exerts its superior antidepressant effects in anxious depression remains unknown.



One theory that may explain ketamine's superior antidepressant properties in anxious depression compared to nonanxious depression involves cortico-limbic brain regions, specifically, the insula. Recent resting state functional magnetic resonance imaging (fMRI) studies have found that patients with anxious depression had increases in the amplitude of low-frequency fluctuation (ALFF) and fractional ALFF (fALFF) in several brain regions, including the right dorsal anterior insular cortex, relative to patients with remitted depression and healthy controls, indicating abnormal spontaneous brain activity in the insula.(4) This increased ALFF in the dorsal anterior insula was also positively correlated with stronger anxiety in the anxious depression group. Furthermore, compared to healthy controls, patients with anxious depression had significantly reduced desynchronization (less activation), as measured by magnetoencephalography (MEG), in the left insula during the 2-back condition compared with the 1-back condition of the N-back working memory task—indicating less activation of these neural networks in patients with anxious depression during the condition with the highest level of task demands.(5) Furthermore, one structural analysis found that patients with anxious depression showed dysfunctional gray matter volume (GMV) compared to those with

nonanxious depression in several brain regions, including increased GMV in the insula.(6) Additional analyses of these data suggested that the left insula might be critically important for distinguishing anxious from nonanxious depressives. **Together, these data suggest that alterations in cortico-limbic brain regions—specifically, the insula—may play a role in the pathophysiology of anxious depression.** For the next step, the current study aims to determine the extent to which the insular neurocircuitry changes in patients with anxious depression following ketamine.

Anxious depression is associated with high rates of treatment resistance on monoamine-based antidepressants. A better understanding of the neurobiology of anxious depression, together with the unique efficacy of ketamine among individuals with anxious depression, will undoubtedly advance therapeutic discovery for challenging to treat forms of depression. Additionally, if the insula is found to mediate clinical changes with ketamine, then subsequent studies can explore the use of insula dysfunction for targeted personalized medicine in depression treatment. Data from this study may also be relevant to other disorders that have been successfully treated with low-doses of ketamine, including PTSD, bipolar depression, tinnitus, and migraines.

II. Specific Aims

The overall aims of this study are to assess insular involvement in predicting ketamine's antidepressant effects, using magnetic resonance imaging (MRI) scans. This study is an open-label trial.

1. To determine if anxious depression has more insula dysfunction compared to nonanxious depression. We propose to measure insula activity at resting-state and during affective processing tasks among patients with anxious depression, nonanxious depression, and healthy controls. We predict that, based on previous data, patients with anxious depression will demonstrate dysregulated insular activity—specifically, increased activity during resting state and decreased activity during the affective processing tasks.

2. To determine the effect of ketamine on insula activity. Specifically, we propose to measure insula activity at resting-state and during affective tasks before and after treatment with ketamine. We predict that insular dysfunction in anxious depression will resolve post-ketamine, and will more closely resembling insular activity in healthy controls pre-ketamine.

3. To explore if changes in insula activity mediate clinical effects. We will measure the relationship of changes in insula activity with changes in affective rating scales. We predict that changes in insula activation mediate change in clinical status post-ketamine.

III. Participant Selection

Inclusion Criteria: Patients

Patients will:

1) be 18-64 years old,
2) read, understand, and provide written informed consent in English,
3) meet criteria for a primary psychiatric diagnosis of major depressive disorder for ≥ 4 weeks, according to the Mini International Neuropsychiatric Interview (M.I.N.I.) and have a Hamilton Depression Rating Scale (HDRS-28) total score ≥ 20; depression may have started at any time point in their life, and certain co-morbid diagnoses (e.g., anxiety disorders) will be allowed,
4) have a history of ≥1 failed medication trial during the current episode (based upon the Massachusetts General Hospital Antidepressant Treatment History Questionnaire; MGH ATRQ),
5) be on a stable antidepressant and psychotherapy regimen for ≥28 days prior to Study Phase II,
6) maintain a treating doctor who is in agreement with study participation,
7) have a reliable chaperone accompany them home following the completion of Visit 2 (the ketamine infusion day),
8) be generally healthy, as assessed by medical history, physical examination (including vital signs), clinical laboratory evaluations, and electrocardiogram (EKG),
9) be of non-childbearing potential or use of an acceptable form of birth control (females only), and
10) be right handed.

Exclusion Criteria: Patients

Patients will be excluded if any of the following criteria are met:

1) delirium or dementia diagnosis,
2) unstable medical illness or clinically significant laboratory results,
3) history of clinically significant cardiovascular disease or electrocardiogram (EKG) findings, or medical conditions that put the patient at risk for possible cardiac side effects or alter brain morphology (e.g., recent head trauma, post intracranial surgery, intracranial mass or bleed, unstable sleep apnea), or a blood pressure >140/95 mmHg at Screening,
4) history of multiple adverse drug reactions, (e.g., history of hives or anaphylaxis in response to a medication, severe intolerance and/or severe side effects to a medication), including hypersensitivity to ketamine or rescue medications,
5) current/past history of psychotic disorders, history of out-of-body feelings or derealization,
6) current/past history of substance use disorders within the past 15 years (except nicotine and caffeine) or lifetime history of ketamine/PCP/LSD abuse (we will confirm this with collateral information from their primary care doctor),
7) requirement of excluded medications that may interact with ketamine (see exclusionary medications list),
8) weigh >250 lbs.,
9) pregnancy, breastfeeding, or unacceptable means of birth control (females only)
10) presence of MRI contraindications (e.g., presence of metallic (ferromagnetic) implants (e.g., heart pacemaker, aneurysm clips)),
11) current serious suicidal or homicidal risk,
12) concurrent participation in other research studies involving medications or treatments,
13) narrow angle glaucoma,
14) acute intermittent porphyria history
15) history of seizures in the past 6 months, regardless of seizure type
16) hyperthyroidism or untreated hypothyroidism, or
17) airway instability or pulmonary disease with hypercarbia.

Inclusion Criteria: Healthy Controls

Healthy Controls will:

1) be 18-64 years old,
2) read, understand, and provide written informed consent in English,
3) have a reliable chaperone accompany them home following the completion of Visit 2 (the ketamine infusion day),
4) be generally healthy, as assessed by medical history, physical examination (including vital signs), clinical laboratory evaluations, and electrocardiogram (EKG),
5) be of non-childbearing potential or use of an acceptable form of birth control (females only), and
6) be right handed.

Exclusion Criteria: Healthy Controls

Healthy controls will be excluded if any of the following criteria are met:

1) current or past psychiatric diagnosis (excluding phobias), including alcohol or substance abuse or dependence diagnosis (except for nicotine or caffeine; we will confirm this with collateral information from their primary care doctor),
2) presence of MRI contraindications (e.g., presence of metallic (ferromagnetic) implants (e.g., heart pacemaker, aneurysm clips)),
3) presence of medical illness likely to alter brain morphology and/or physiology (e.g., traumatic brain injury, unstable sleep apnea), or a blood pressure >140/95 mmHg at Screening,
4) requirement of excluded medications that may interact with ketamine (see exclusionary medications list),
5) presence of psychiatric disorders in first-degree relatives,
6) pregnancy, breastfeeding, or unacceptable means of birth control (females only), or

7) weight >250 lbs.
8) narrow angle glaucoma,
9) acute intermittent porphyria history,
10) history of seizures in the past 6 months, regardless of seizure type,
11) hyperthyroidism or untreated hypothyroidism, or
12) airway instability or pulmonary disease with hypercarbia, or
13) known hypersensitivity to ketamine or hypersensitivity to the rescue medications.

MRI Reasons for Exclusion for both Healthy Controls and Depressed Participants

High magnetic fields may pose a serious health hazard to participants with implanted ferromagnetic objects. Every participant in this study will be screened for implanted ferromagnetic objects before they are enrolled and will provide written responses to a questionnaire to screen for implanted ferromagnetic objects before entering the high magnetic field shielded room. Participants with the following conditions/diseases will be excluded from the study:

a) History of head trauma
b) Surgical aneurysm clips
c) Cardiac pacemaker
d) Prosthetic heart valve
e) Neurostimulator
f) Implanted pumps
g) Cochlear implants
h) Metal rods, plates
i) Screws
j) Intrauterine device
k) Hearing aid
l) Dentures (which might create artifacts)
m) Metal injury to eyes
n) Metallic tattoos anywhere on the body or tattoos near the eye
o) History of claustrophobia

IV. Participant Enrollment

We will enroll 42 participants into the study; 16 patients with anxious depression, 16 patients with nonanxious depression, and 10 healthy controls.

Methods

Recruitment: 32 outpatients with depression and 10 healthy volunteers will be enrolled via response to flyers, advertisements, e-mails, MGH Depression Clinical and Research Program (DCRP) past call logs of individuals willing to be recontacted for research, and letters to providers. Realistically, we will plan to recruit 52 participants to account for drop outs and screen fails. The DCRP has used these methods for over two decades with considerable success, receiving an average of over 35 phone calls per week. All patients 18-64 years old calling into the DCRP will be considered for this study.

Retention: All patients are eligible to receive a free consultation at the DCRP immediately following the completion of all study materials. This care is supplemental to their ongoing care with their treating psychiatrists. The treating psychiatrist will be informed before any medications changes are considered.

Remuneration: All participants will have their parking validated. They will also be given a snack 2 hours after the ketamine infusion at Visit 2.

Procedures for Informed Consent

Written informed consent will be obtained from all patients before protocol-specific procedures begin by a licensed physician. The investigator obtaining consent will explain in detail the protocol of the study, its purpose, and potential benefits to the society. Participants will be informed about minimal risks of routine high magnetic field and non-ionizing radiation involved in MRI. Participants will also be informed about small space within the magnet and noises made by switching gradients. Participants will be informed that if they feel uncomfortable with the study, they can choose to terminate the study at any time.

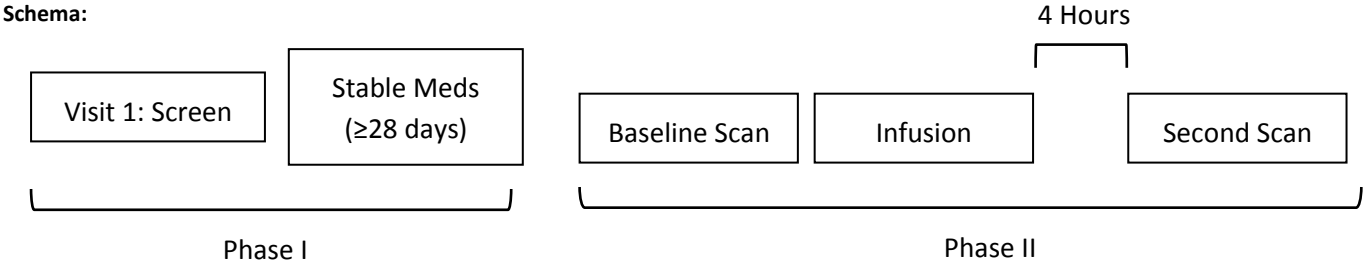
Treatment Assignment and Randomization (If Applicable)

N/A; All participants will be assigned to open-label ketamine.

V. Study Procedures

Research Design Summary: The proposed research (Aims 1-3) will begin with a screening visit (Visit 1) and a medication stabilization period of ≥28-days (Phase I). In Phase II, 32 patients (16 with anxious depression, 16 with non-anxious depression) and 10 healthy volunteers meeting research criteria (specified above) will undergo a baseline MRI scan, will receive an open-label infusion of subanesthetic intravenous ketamine (0.5mg/kg over 40 minutes), and will undergo a second MRI ("post-ketamine") scan approximately 4 hours later (see "Schema" for overall study outline):

Schema:



Visits and Parameters to be Measured/Data to be Collected and When the Data is Collected

Study Phase I: At Visit 1 ("Screening" visit), patients and healthy controls will undergo initial evaluation (i.e., physical and psychiatric examination, neuropsychological testing, basic laboratory and pregnancy/urine toxicology testing, blood genetics and biomarkers collection, and EKG) following informed consent to evaluate their eligibility based on inclusion/exclusion criteria. This will occur at the MGH DCRP. Prior to Study Phase II, patients will remain stable on their medications for ≥28 days. If their current medication regimen does not meet criteria for adequacy for "treatment-resistant depression," according to the MGH Antidepressant Treatment History Questionnaire, they will be referred back to their primary psychiatrist for optimization and re-screened after 28-days of stable medications. In order to ensure that participants do not have a history of substance abuse or dependence, we will perform a urine toxicology screen at the screening visit. Furthermore, we will obtain permission from both depressed and healthy participants to contact their primary care physician or other treaters, to obtain further collateral information about substance use problems.

Study Phase II: Ketamine Administration and MRI Testing: Phase II consists of a single experimental day ("Visit 2"). All female subjects will provide a urine sample upon arrival to the CNY campus to test for pregnancy. Subjects will fill out baseline SHAPS, CUDOS-A, GAD-7, and RRS assessments (see below), and the clinician will administer baseline HDRS, HAM-A, and YMRS questionnaires. Patients and healthy controls will then undergo a baseline ("pre-ketamine") 90 minute 3T magnetic resonance imaging (MRI) scan.

After the MRI scan, participants will receive a single intravenous infusion of subanesthetic ketamine (0.5mg/kg over 40 minutes). Intravenous ketamine will be administered at the Anesthesia Research Suite (White 5) by an anesthesiologist, with a psychiatrist present. Participants will be monitored for 3 hours after the start of the infusion by a physician and trained research staff. After the infusion the anesthesiologist will be in the building and available by pager. Vital signs (Temp, Pulse, Respiration Rate, BP, SpO2 and Sedation level) will be measured at +0 minutes, +5, +10, +15, +20, +30, +40, +60, +90, +120, +180 minutes in reference to the start of the ketamine infusion. An oxygen saturation probe is available.

At 4 hours post-infusion (+240 minutes), participants will answer questions on the neuropsychological assessments (on the same questionnaires that they did at baseline), have a post-ketamine blood sample drawn for genetics and biomarkers, and will receive a follow-up ("post-ketamine") MRI scan, with the same sequence as the initial scan in the morning. Afterwards, participants will be discharged home into the care of a responsible adult escort. The study will provide transportation from CNY after the first scan to the main hospital for the infusion and back to CNY for the second scan, with a staff member as a chaperone.

A follow-up phone call will be made by a study physician to all participants the day after completing the Study Phase II/Visit 2 to ensure that no worsening of their symptoms has occurred, as well as to administer final assessments. Patients are eligible to receive a free follow-up consultation at the DCRP. This care will be in the form of consultative care, as a supplement to their ongoing care with their treating psychiatrists. The treating psychiatrist will be informed before any medications changes are considered.

Neuropsychological Assessments: Self- and clinician-rated assessments will take place during the study. We will administer the following scales to all participants at both visits (Visit 1 and Visit 2): the Hamilton Depression Rating Scale (HDRS) is a 28-item validated clinician-administered scale that is widely used as an observational rating measure of depression presence and severity;(7) the Montgomery Asberg Depression Rating Scale (MADRS), a 15-item validated clinical-administered scale widely used for the assessment of depression(8); Hamilton Psychiatric Rating Scale for Anxiety (HAM-A) is a 14-item validated clinician-administered scale that is widely used as an observational rating measure of anxiety presence and severity;(9) Clinically Useful Depression Outcome Scale (for the DSM-5 Anxious Distress Specifier) (CUDOS-A) is a 22-item self-rated scale for the assessment of DSM-5 anxious distress;(10) Young Mania Rating Scale (YMRS) is an 11-item clinician-rated scale for the measurements of hypomanic/manic symptoms;(11) *Snaith-Hamilton Pleasure Scale* (SHAPS) is a 14-item self-report questionnaire for the assessment of anhedonia (please loss to previously pleasurable activities);(12) Generalized Anxiety Disorder 7-item Scale (GAD-7) is a 7-item self-rated scale for the assessment of anxiety symptoms;(13) the MGH Sexual Functioning Questionnaire (SFQ) is a 7-item self-rated scale for the assessment of sexual functioning; Ruminative Responses Scale (RRS) is a 22-item self-rated questionnaire that assesses rumination in depression.(14)

At both visits, we will also administer a computer based pattern separation task. The pattern separation task is a high throughput behavioral task that captures the input-output transformation function characteristic of pattern separation processes. (15) For example, if you park your car in the same lot everyday, but not the same space, pattern separation is thought to be involved in the process of you finding your car everyday despite being in a different space; this may be dysfunctional in people with depression. In this task, patients are shown a series of every-day objects (e.g., a car, garden tool, food, etc.) and are asked to identify the objects as being indoor or outdoor objects. Immediately after this, a second part of the task is started in which the patients are shown another series of objects. They are asked to call the objects as “old” if they have seen the objects before in the task, “new,” or “similar.” As previously done by Stark and colleagues,(15) a third of the objects in the testing phase are “old”, “similar” and “new”. Identifying a “similar’ object correctly conveys pattern separation, whereas, incorrectly identifying it as “old” conveys pattern completion. By plotting responses as a function of object similarity, we can generate an input-output transfer curve. This task will serve the purpose of rapid assessment of putative changes in pattern separation.

We will administer the following to all participants at Visit 1 only (screening): *Mini-International Neuropsychiatric interview (M.I.N.I.)* to assess for the presence of depression;(16) the MGH Antidepressant Treatment History Questionnaire (ATRQ), a clinician-rated scale used to determine treatment resistance in depression;(17) the Edinburgh Handedness Inventory (EHI), a self-rated scale used to determine hand dominance for the purpose of MRI analysis;(18) the *Early Trauma Inventory Self Report-Short Form* (ETISR-SF), a 29-item self-report item on early trauma history,(19) and a demographics form. All participants will also fill out an MRI safety form with the staff clinician at the screening visit.

We will administer the following scales to all participants during Visit 2 only: *Brief Psychiatric Rating Scale (BPRS)* is an 18-item clinician-administered scale for the assessment of psychotic symptom constructs;(20) *Clinician Administered Dissociative States Scale (CADSS)* is a 28-item clinician-administered scale for the assessment of dissociative symptoms.(21) BPRS and CADSS will be administered at baseline, +40 minutes, +80 minutes, and +120 minutes in reference to the start of the ketamine infusion at Visit 2.

We will administer a HDRS and a MADRS at the follow up phone call within 48 hours after the infusion.

Study clinicians at the DCRP will be responsible for administering all clinician-administered scales and assessments. Clinicians have been extensively trained in the use of the MADRS and $HDRS_{28}$ by videotapes and live patient interviews.

Peripheral Blood Sample Genetics and Biomarkers: At Screening, one 10 mL blood sample, one 8-10mL blood sample, one 6-8 mL blood sample, and two 10mL blood samples will be collected at Baseline, and

two 8-10mL blood samples will be collected and at Visit 2, to obtain DNA for possible pharmacogenetic and biomarker (e.g. cytokines, metabolites, etc.) studies. The PI will ensure that appropriate privacy and de-identification procedures are in place for the collection of biomaterials.

In order to collect iPS cells for future biomarkers and genetics studies, all subjects will be referred to participate in Protocol 2009P000238, "The Use of Human Skin Cells and Blood Derived Cells in the Creation of Cellular Models of Neuropsychiatric Disorders" (P.I.: Perlis)." Subjects' choice as whether or not to participate in 2009P000238 will have no bearing on their ability to participate in the current protocol.

Concomitant Medications and Adverse Events: Concomitant medications (dosage, start and stop dates) will be reviewed and recorded at both visits in the chart, and psychiatric medications should be identical for Visit 1 and 2. Adverse events will be recorded at Visit 2 on the Adverse Events Form.

Neuroimaging Procedures: Prior to scanning, participants will fill out a MRI safety checklist with a trained study staff member or MRI tech. Eligible participants will undergo neuroimaging using a 3 Tesla (3T) MRI scanner. Specifically, we will use a Siemens 3T whole-body scanner (Prisma-System). Neuroimaging will include a structural, a resting state, and a functional MRI (fMRI) scan. Head motion will be minimized during the image acquisition by use of a foam head holder. When applicable, the study participants will be instructed to lie still during the imaging protocol.

Structural Procedures: Both MRI sessions will begin with participants lying still in the scanner for a 15-minute structural scan.

Resting State Procedures: A 6-minute resting-state scan will be conducted.

Functional Procedures/IAPS: Finally, each scan will conclude with a 15-minute functional task-based scan, during which Blood Oxygenation Level Dependent (BOLD) data will be collected. Specifically, we will probe insula function with an affective rapid-event related task, which displays negative and neutral pictures from the International Affective Picture System (IAPS)(22). These expressive faces reliably activate anterior insula.(23)

Diffusion Tensor Imaging: A 10-minute DTI scan will be conducted.

Discharge: After the second MRI scan, if medically stable (as deemed by a board-certified physician study investigator), participants will be discharged home with a responsible family member or other adult caretaker. Discharge home will be based on criteria established by the MGH Department of Anesthesia practices for discharge from the hospital following ambulatory surgery. The participant must have stable vital signs, be able to respond appropriately to normal commands, be pain free, be free from any nausea and vomiting, and have no bleeding from the intravenous sites. Participants must be able to walk unassisted and have an accompanying adult to escort them home. Participants will be advised not to return to work and against driving or operating heavy equipment for 24 hours. A follow-up phone call will be made by a study physician to all participants the day after completing the Study Phase II/Visit 2 to ensure that no worsening of their symptoms has occurred (within 48 hours following infusion). Patients will also be asked to complete a MADRS and HDRS over the phone. Patients are eligible to receive a free follow-up consultation at the MGH DCRP at a later date.

Drug to be Used: Ketamine will be the only research drug used as part of the study.

Ketamine Administration and Monitoring: Ketamine is a glutamatergic receptor antagonist that has been administered to millions of patients worldwide as a general anesthetic, though its antidepressant mechanism of action (at sub-anesthestic doses) remains largely unknown. Although low-dose ketamine has been shown to have a favorable safety profile when given to depressed patients in several studies,(1, 24-27) the risk of adverse psychotomimetic, dissociative, and/or sympathomimetic events remains a risk. Similar to previous studies showing efficacy and safety of intravenous ketamine in adults with mood disorders,(1, 28, 29) ketamine will be administered at 0.5mg/kg over 40 minutes by an anesthesiologist

Patients will be monitored for changes in vital signs (i.e., heart rate, blood pressure, respiration) and for treatment-emergent side effects (i.e., as measured by the CADSS and BPRS) during administration and post-administration. The most common side effects during the low-dose ketamine infusion are increased heart rate and blood pressure, increased salivation, increased bronchial secretions, gastrointestinal distress, and

horizontal nystagmus. The most common neurologic and psychiatric side effects are increased anxiety, confusion, paranoid feelings, "out-of-body" feelings (or derealization). The side effects considered to be clinically significant will be managed by the study anesthesiologist and psychiatrist with ancillary medications.

Specifically, the psychiatrist will be in charge of managing psychotomimetic and dissociative side effects (i.e., lorazepam for severe agitation and anxiety; haloperidol for severe hallucinations, severe delusions, and severe agitation).

The anesthesiologist will be responsible for managing sympathomimetic side effects. Abnormally high blood pressures ≥160/100 for 3 readings in a row will be treated (i.e., labetalol), according to the anesthesiologist's best clinical judgment. Infusions will be stopped if BP > 160/100 persists for more than 20 minutes. Asymptomatic persistent heart rate >100 bpm for more than 20 minutes, regardless of blood pressure, will prompt an assessment by the anesthesiologist, with specific management decisions left to his /her clinical judgment. All symptomatic tachycardia and blood pressure elevations will be treated, based on clinical judgment. Any symptomatic tachycardia (>100 bpm) will be evaluated by the anesthesiologist with ECG, to assess for arrhythmias (PSVT, Afib/Aflutter, of VT). Other rescue medications, to be given by the anesthesiologist, include: glycopyrrolate for excessive secretions; ondansetron for severe nausea; acetaminophen and ibuprofen for headache; and oxygen for desaturation of <93%) and behavioral interventions (i.e., reorientation, reassurance). The anesthesiologist will stay for the duration of the infusion, or longer if clinically necessary. If an elevation of 160/100 mmHg is noted at anytime, monitoring will be a minimum of every 10 minutes.

In our previous studies evaluating the efficacy of ketamine as a rapid fast acting treatment for major depression (2014P000972, 2012P001042), as well as from the P.I.'s previous experience at the NIMH, the team has successfully treated over 50 patients with ketamine; in 2012P001042, several patients received up to 6 infusions of ketamine (per the protocol). The cardiovascular and respiratory stability of ketamine at the dosing scheme we have employed is evidenced by the fact that only no patients have received labetalol for hypertension or aberrations in heart rate. Also, we have found that ketamine-induced neuro-psychiatric side-effects are well addressed by reassurance. Furthermore, our colleagues at Mt. Sinai School of Medicine and Baylor University have published data on ketamine's favorable safety and tolerability profile in clinical trials for depression.(30) Thus, based on our expertise, as well as the expertise of Wan and colleagues,(30) low-dose ketamine for the study of depression has little risk.

Research staff will be responsible for administering scales and for monitoring side effects. In order to minimize external stimuli, ketamine administration will occur in a room with low lighting and minimal noise throughout the administration, as well as during the monitoring period. Participants will be given a small lunch and snack 2 hours after the end of the ketamine infusion.

Medication Transport and Storage

Consistent with other medication studies conducted at CNY, a dedicated research assistant for 2015P001912 will pick up the medications from the MGH Research Pharmacy on the morning of the scanning visit at CNY. The RA will be responsible for bringing all of the medications to White 5. Unused ketamine will be wasted by the anesthesia staff.

Devices to be Used

MRI data will be acquired using a Siemens 3.0-T whole-body scanner (Prisma-System).

Procedures/Surgical Intervention

N/A

VI. Biostatistical Analysis

Specific Data Variables to be Collected

As described above, we will administer the following scales at <u>both visits (Visit 1 and Visit 2)</u>: HDRS, HAM-A, CUDOS-A, YMRS, SHAPS, GAD-7, MGH SFQ, and RRS.

We will administer the following at <u>Visit 1 only</u> (screening): M.I.N.I., ATRQ, EHI, and ETISR-SF.

We will administer the following scales during <u>Visit 2 only</u>: BPRS and CADSS on Visit 2 at baseline, +40 minutes, +80 minutes, and +120 minutes, in relationship to the time of ketamine infusion.

We will administer the MADRS, HDRS, and MGH SFQ at the follow-up phone call, within 48 hours after the infusion.

Vital Signs will be recorded once at Visit 1 (the screening visit), and again at Visit 2 at baseline, +0 minutes, +5, +10, +15, +20, +30, +40, +60, +90, +120, and +180 in relationship to the time of ketamine infusion.

Data will be analyzed and compared between baseline and post-ketamine; regression analysis will be conducted between groups. Insula functioning during fMRI data will be analyzed using SPM8 (spatial preprocessing, first and second level analysis). Correlations to clinical measures (i.e., questionnaires) will also be examined.

Study Endpoint

Primary clinical endpoint is change in the $HDRS_{28}$ total score. Scores that decrease by ≥ 50% from baseline will be considered "response." We will compare MRI data between healthy controls, responders and nonresponders, as well as anxious depression status.

Statistical Methods

For Aim 1: Groups will be compared using a flexible factorial Model for both resting state and task-based activations (for the contrast of interest: negative - neutral) with group as the between subjects factor and task condition as the within subjects factor followed by pairwise comparisons.

For Aims 2 and 3, time point (before and after ketamine infusion) will be included as an additional within subjects factor. Mediation effects (changes in insula activity mediating changes in clinical state) will be tested using linear regression approaches developed by Kraemer et al., 2002. As recommended by Kraemer, these analyses will focus on effect sizes rather than significance testing.

Power Analysis

Sample Size Determination: Previous ketamine research for depression have observed moderate-to-large effect sizes (d=0.72) at 1 day after a single intravenous infusion of ketamine in anxious vs. nonanxious depression.(2) In that study, there were a total of 26 patients (15 with anxious depression, 11 with nonanxious depression) but no control groups. The data collected from this proposed study uses similar sample sizes (16 anxious vs. 16 nonanxious), and 10 healthy controls for a comparison group. These numbers will allow for detection of an effect size >0.7.

VII: Risks and Discomforts

Complications of Procedures

Safety: At any time during the study, participants, family members, and treating psychiatrist will be encouraged to contact the principal investigator via phone or pager in the case of adverse events or worsening symptoms. The treating psychiatrist will be immediately notified of any concerns.

Drug Side Effects and Toxicities

Ketamine: Ketamine is a relatively commonly used anesthetic in both veterinary and human medicine. Although it has a good safety profile overall, ketamine has documented sympathomimetic activity that may result in mild to moderate increases in heart rate, blood pressure, and cardiac output, though this activity is generally short-lived.(31) Findings from previous research on single- and repeated-dose intravenous ketamine for treating depression have provided evidence that the autonomic changes that may occur during the active administration (i.e., elevated blood pressure, pulse) return to normal shortly after the infusion stops, with no clinically significant sequelae.(32, 33) Other possible side effects reported during ketamine infusion include arrhythmia, increased salivation, increased bronchial secretions, horizontal nystagmus, euphoria and hallucinations. Nystagmus may persist for a period after the ketamine infusion has terminated.

Rare side effects are allergic reactions (skin rash), pain at site of injection, increased intraocular pressure, ulcerations and inflammation in the bladder (reported in ketamine abusers). Ketamine is a controlled substance and has the potential for abuse and dependence, in particular in participants with history of drug abuse. Participants with a current/past history of substance abuse disorders within the past 15 years (except nicotine and caffeine) or lifetime history of ketamine/PCP/LSD abuse will be excluded.

In light of ketamine's relatively good safety profile, all participants will be medically screened (including EKG and vital signs) prior to entering the active treatment portion of the study, to ensure healthy baseline general and cardiac functioning. In addition, as ketamine is an anesthetic that may result in respiratory depression, participants will be excluded from the study if they require sedatives or opiates. During the administration, an anesthesiologist and a psychiatrist will sit in the room with a research nurse, the research assistant, and the patient, and vital signs will be monitored every 5-10 minutes during the 40 minutes of administration. Any concerning changes will be managed by the physicians. The anesthesiologist may choose to stop the infusion at any time if he/she believes it is in the best interest of the participant, based upon his/her clinical judgment.

Escalation of care to the inpatient hospital will occur, if necessary. Participants will stay at the MGH Main Campus or the MGH DCRP for 3 hours following the start of the administration, and will be monitored for psychotomimetic, dissociative, and sympathomimetic side effects during this time. If stable, patients will be taken to have the second ("post-infusion") MRI.

Afterwards, they will be discharged home in the care of a responsible adult family member or caretaker after completing rating scales with the study physicians. If participants experience adverse events that emerge at home, they will be instructed to immediately go to the closest emergency room. These instructions will also be provided to the responsible adult escort prior to discharge. The participant's individual insurance plan will be responsible for covering this visit.

Prior to informed consent, potential patients will be informed of alternatives to research, and will be provided with the option to seek further psychopharmacological or psychotherapy-based interventions.

Safety and Monitoring: Special Case of Sertraline and the Urine Drug Screen

Sertraline (Zoloft) is known to cause frequent false positives on urine drug screens for benzodiazepines (see https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2728940/). Over the past several years, the DCRP has experienced an increase in false positive urine drug screens for benzos while patients were taking sertraline. In the case when a patient (who is taking sertraline for their depression) is screened for the study and tests positive for benzodiazepines on urine drug screen (despite denial of benzo use), the following precautions will be taken: 1. The patient's physician will be contacted for collateral information pertaining to alcohol and substance abuse, including benzodiazepine use; 2. The patient will be checked in the Mass PAT system for active benzodiazepine prescriptions within the past year from the screening date. If the patient's physician confirms that they are not taking benzos, and there is no evidence of benzo use in Mass PAT, the patient will be deemed eligible for the study for this particular criteria. As a precaution, the patient will be informed that if they are, in fact, taking benzodiazepines, they may interfere with ketamine's mechanism of antidepressant/antisuicidal action, rendering ketamine less effective. Furthermore, the concomitant use of benzos and ketamine may lead to more severe side effects (such as increased somnolence) when combined.

Device/Radiation Side Effects

MRI risks: Risks already established for MRI include: 1) claustrophobia due to confinement of the patient in the system, 2) malfunction of electromagnetic implants caused by interaction with the magnetic fields, 3) projectiles and tissue burns caused by metallic tattoos or implants, 4) risks to the fetus of a pregnant patient, 5) surface burns due to interaction of metallic system components or surface adhesives with the patients skin, 6) slight hearing impairment due to high acoustic noise levels generated by the system, and 7) slight neuromuscular twitching (for the higher field strength systems). However, system safeguards through MGH have been designed and operating guidelines have been provided to minimize any of the aforementioned risks.

Psychosocial (non-medical) Risks

Privacy: As per standard DCRP procedures, study data is recorded using standard forms. All data will be stored in locked cabinets. For statistical analysis, only study IDs will be used as identifiers. Separate folders with unblinded information (e.g., patient name) will be kept in a locked cabinet in a separate office to ensure the blind.

Safety Risk

Suicidal ideation will be assessed via phone screen and via screening visit (Visit 1). If participant indicated "Yes" to having suicidal ideations and a plan, they will speak to a clinician who will conduct safety planning and, if deemed necessary, assist in emergency room procedures. Suicidal ideation will be assessed at screening via HDRS, CUDOS-A, MINI, and MADRS. All events of suicidal ideation and behavior will be carefully monitored throughout the study. Patients with severe suicidal ideation, as deemed by the assessments and clinician judgement, will be excluded from the study at screening and safety planning will occur. If necessary, the investigator will contact the treating physician. Though patients with severe suicidal ideation will be excluded from the study at screening, any patient who, based on the investigator's judgment, poses an imminent risk of suicide will be discontinued from the study and referred to a local emergency room for further evaluation. All efforts will be taken to minimize the risk of suicide and the investigator will carefully monitor the patient for the full duration of the study (including the follow-up phone call). The treating physician will be notified if participant describes severe suicidal ideation and behavior after the phone-call and safety planning with the participant will occur accordingly.

VIII: Potential Benefits

To Participating Individuals and Society

The patients participating to the study may feel better, if for only a brief period of time. Some may not receive any direct benefit. Healthy controls may have no benefits. The results of this study may lead to improved understanding of the neurobiology of anxious depression, as well as the mechanism of ketamine's antidepressant effects. Furthermore, the results from this study may lay the groundwork for future studies into the mechanism of action of ketamine's antidepressant effects, as well as using neurobiology to subtype depression.

IX: Monitoring and Quality Assurance

The PI and Co-Is will be responsible for the monitoring and quality assurance of the study and the data. Weekly meetings have been established to discuss study patients, data and safety issues, and overall study procedures.

Serious Adverse Events: Expedited review will occur for all events meeting the FDA definition of SAEs – i.e., any fatal event, immediately life-threatening event, permanently or substantially disabling event, event requiring or prolonging inpatient hospitalization, or any congenital anomaly. This also includes any event that a study investigator(s) judges to impose a significant hazard, contraindication, side effect, or precaution. For purposes of this study, all SAEs will be required to be reported to the IRB, regardless of any judgment of their relatedness to the study drug. All relevant information will be reported for each SAE including information about the event and its outcome, dosing history of all study drugs, concomitant medications, the participant's medical history and current conditions, and all relevant laboratory data. Unanticipated problems involving risks to participants or others including adverse events will be reported to the Partners Human Research Committee (PHRC) in accordance with PHRC unanticipated problems including adverse events reporting guidelines. Information will be reviewed and a determination made of whether there was any possible relevance to the study drug.

Study Stopping Rules: If at any time during the course of the study, the study investigators(s) judge that risk to participants outweighs the potential benefits, they shall have the discretion and responsibility to recommend that the study be terminated.

AE Reporting Guidelines

Concomitant Medications and Adverse Events: Concomitant medications (dosage, start and stop dates) will be reviewed and recorded at each visit. The study doctor will document and side effect or adverse event during Phase II.

In case of serious adverse cognitive side effects (delirium or confusion of clinical concern, with or without other symptoms like hallucinations, paranoia) or other serious side effects (e.g., cardiac, neurologic), patients will be discontinued from the study and immediately treated with the appropriate pharmacologic management (e.g., haloperidol, as needed, for delirium and psychotic symptoms).

MRI Monitoring and Safety

Safety monitoring will include an immediately available cardiopulmonary resuscitation cart, oxygen, ambu bag, and defibrillator for use in need of an emergency. A fully functional MRI compatible physiological monitoring system and a fully stocked ACLS cart with equipment for airway management will always be readily available. In addition to an ACLS cart, the MR suite also has an urgent care cart and an allergic reaction case cart.

The Siemens system has a built in self-monitoring system that automatically shuts off if parameters exceed safe levels. For backup protection, the study stuff will constantly be in contact with the participant during the scan. Participant monitoring will be performed using the 2-way intercom system between the scanner operator and participant and by visual monitoring of the participant through the window into the scan room or monitoring cameras (the participant is visible to the operator at all times). Quality assurance of the scanner's performance is obtained by a daily quality assurance protocol. More extensive quality assurance protocols are performed monthly under the commercial service contract with Siemens Healthcare.

Monitoring of the study data itself will largely be performed by the investigators in conjunction with other faculty members in the Martinos Center. All data will be available for review with deletion of participant identifying material. The research scan will not become part of the hospital's medical records.

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