

**Psychophysiological and Cognitive Biomarkers of Ketamine's Antidepressant Effects
(Protocol 2015P002397)**

NCT02669043

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Detailed Protocol:

I. Background and Significance

Establishing 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 of remission among individuals with certain depressive 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 ant glutamatergic medication) have 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—especially *anxious depression*.(2) Low dose parenteral ketamine has now been studied for difficult to treat depressive disorders by numerous research groups and off label administration of ketamine is increasingly available in some clinical settings.

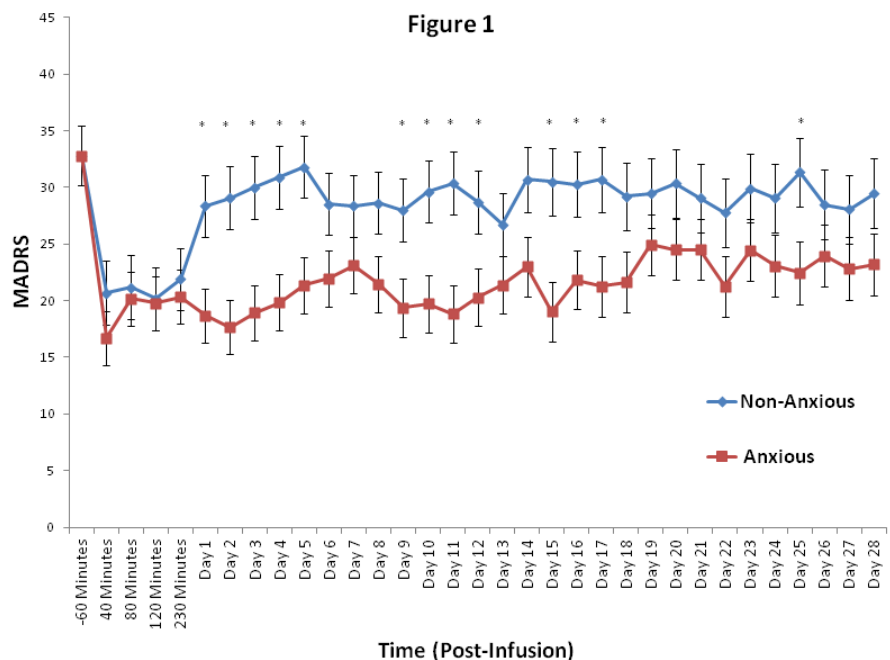
There is a widely recognized need for developing biomarkers that may predict response to rapidly acting antidepressant agents such as ketamine. The identification of biomarkers of treatment response to rapidly-acting therapeutics for depression is likely to advance targeted drug development and the goal of personalizing depression treatment. The current protocol is aimed at further delineating potential biomarkers of ketamine's effects among individuals with treatment-resistant anxious depression, as well as using putative biomarkers to diagnose depression subtypes (i.e., anxious depression).

Background and Preliminary

Results: Ketamine and Anxious Depression.

This protocol stems from earlier work by the PI with colleagues at the NIH/NIMH on the definition,(3) neurobiology,(4) and treatment(5) of anxious depression. Because anxious depression is a common depressive subtype, we sought to examine the extent to which symptomatically-diagnosed anxious depression predicted response to ketamine. Specifically, in our *post hoc* analysis, we found that patients with anxious depression ($n=15$) had significantly greater antidepressant responses (defined as a $\geq 50\%$ improvement in Montgomery-Asberg Depression Rating Scale (MADRS) scores from baseline) to ketamine compared to patients with nonanxious depression ($n=11$).(6) This

effect was observed over the course of 28 days (see Figure 1; $* = p < 0.05$), with the largest effect at Day 2 (Cohen's $d=0.76$)—a clinically relevant finding, given that anxious depression is typically more difficult-to-treat than nonanxious depression.(5) In addition, patients with anxious depression had significantly longer median days-to-relapse compared to nonanxious depression patients (19.0 ± 17.9 vs 1.0 ± 0.0 days-to-relapse, respectively; $p=0.002$). The two groups did not differ in their experiences with the dissociative ($p=0.62$) or psychotomimetic ($p=0.41$) side effects of ketamine. These results suggested that symptomatically-diagnosed anxious depression may be a clinical predictor of ketamine response, a particularly intriguing finding in the context of the majority of clinical trials involving standard antidepressants in which anxious depression is related to poorer antidepressant response.



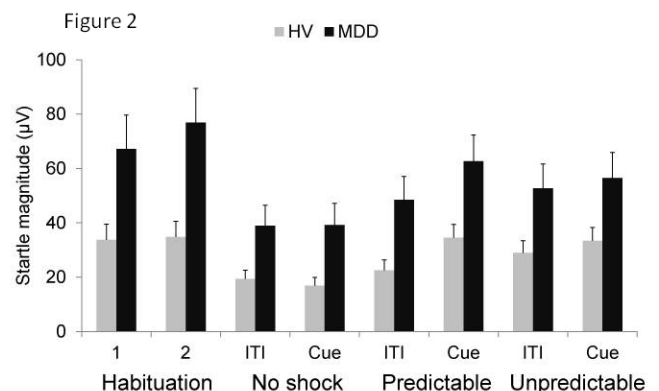
Though symptomatically-diagnosed anxious depression appears to predict a better antidepressant treatment response to ketamine compared with depression without anxious features, several important aspects remain to be elucidated include: 1) the neural mechanisms through which ketamine exerts its superior antidepressant effects in anxious depression, 2) the relationship of objective and subjective measures of anxiety within mood dysregulated patients (Aim #1), and 3) the extent to which *objective* physiological measures of anxiety might predict patients' response to ketamine (Aim #2). **One theory that may help to explain ketamine's superior antidepressant properties in anxious depression compared to nonanxious depression involves the reward/motivation brain circuit.** The combination of reward pathway dysfunction and hyperactive threat responses has been suggested as a mechanism for the pathophysiology of anxious depression.(7, 8) Indeed, anxiety, depression, and dysfunctional reward processing are highly comorbid.(9) Furthermore, a recent review of the literature(8) highlighted that chronic stress (a model of anxiety), aversive stimuli, and punishment all lead to "stress-induced" clinical symptoms of anhedonia, low motivation, and depression through indirect inhibition of the reward circuitry.

The reward circuitry receives both excitatory (glutamatergic) and inhibitory (GABAergic) inputs from the bed nucleus of the stria terminalis (an area of the brain involved in hypervigilant threat monitoring and anxiety).(10, 11) Preclinical research has suggested that during aversive events, the reward circuitry is indirectly inhibited by increased excitatory (glutamatergic) and decreased inhibitory (GABAergic) neuronal activity from the bed nucleus of the stria terminalis.(12) Conversely, a reduction in anxiety-related behaviors and an increase in reward-motivated behaviors are observed when this inhibitory tone is increased.(11) Altogether, these findings suggest that the bed nucleus of the stria terminalis may have a unique role in integrating stress information with reward responses.(13)

Ketamine, an N-methyl-D-aspartate (NMDA) receptor glutamatergic antagonist, may work in the treatment of anxious depression by blocking actions from the excitatory (glutamatergic) neurons in the bed nucleus of the stria terminalis, thereby blocking the "stress-induced" inhibition of the reward circuitry. In depressed patients, this action may reinstate more normal functioning of the reward circuit. Indeed, in one recent mouse study, it was shown that knocking out the glutamatergic receptors within the bed nucleus of the stria terminalis mimicked the antidepressant effects of systemic ketamine administration, further implicating the bed nucleus' relationship with ketamine's antidepressant effects.(14) We propose several noninvasive experiments to interrogate these pathways.

Background and Preliminary Results: Psychophysiology and Cognitive Testing. In this protocol, several psychophysiological and cognitive experiments will be used to objectively measure anxiety for the prediction of ketamine's antidepressant effects. The startle eye-blink reflex is a well-documented primitive defensive/protective reaction that occurs when an individual is exposed to an unexpected, mildly aversive stimulus such as a highly annoying but not painful electric shock administered (15). In the literature on the startle reflex, a distinction has been made between fear and anxiety. Fear is thought to be associated with predictable and phasic administration of an aversive stimulus while anxiety is thought to be associated with an unpredictable and sustained aversive stimulus. Despite their clinical similarities, assessing *both* fear- and anxiety-potentiated startle response is critical, as a growing literature suggests that their pathophysiology involves distinct brain circuits—specifically, phasic fear-potentiated startle is mediated by the amygdala, whereas sustained anxiety-potentiated startle is mediated by the amygdala *and* the bed nucleus of the stria terminalis.(15, 16)

In previous work, we used the NPU-threat test (**N**o shock, **P**redictable shock, and **U**npredictable shock) to examine whether baseline fear- and anxiety-potentiated startle (as elicited by electric shocks) differed between a *heterogeneously diagnosed* group of patients with depression ($n=28$) and healthy volunteers (HV; $n=28$).(17) Briefly, participants intermittently experienced abrupt bilateral auditory stimuli (40-ms duration, 103-dB noise burst) through headphones, eliciting a startle response. These startle responses were then measured during experiments of predictable (signaled by a cue) and unpredictable (unsignaled) shock, related to fear and anxiety, respectively. Baseline startle responses revealed



significantly greater startle magnitude in the depressed group compared to the HV group ($p < 0.02$; Figure 2), extending both to fear- and anxiety-related potentiation. Furthermore, the depressed group exhibited significant increases in startle upon the *mere placement* of the shock electrodes (reflecting *contextual* anxiety; $p < 0.005$) compared to a period prior to shock electrode placement—a difference that was not seen in the HV group.

In addition to eye-blink startle, we will collect other physiologic measures, including autonomic responses to threat stimuli that have a demonstrated link to pathological anxiety in other studies. Specifically, a larger heart rate response to loud tones (a threat stimulus) has been shown to be one of the most robust physiological findings in patients with current PTSD, and may reflect increased defensive responding to threats.(18-21) This increased reactivity appears to be acquired *after* the onset of the disorder, rather than pre-existing, further suggesting that this physiological marker is an accurate indicator of disease.(18) Heightened heart rate activity has also been observed in patients with obsessive compulsive disorder.(22) It is our hypothesis that autonomic responses to threat stimuli will be similarly found as indicators of anxiety within depression.

As described earlier, dysfunction in reward processing may be critical in the pathophysiology of anxious depression (7,8). In order to examine the spectrum of reward dysfunction in depressed patients, as well as to test the extent to which cognitive deficits in reward processing predict ketamine's antidepressant effects, we will conduct a pre-treatment probabilistic reward task, an objective measurement of participants' ability to modulate behavior as a function of rewards. Clinically, depressed patients have a deficit in reward learning (a construct of the so-called RDoC [Research Domain Criteria] positive valence domain), as they have difficulty modifying their behavior despite positive reinforcement.(8) In one previous probabilistic reward task study, pre-treatment inability to modify reward learning in depressed patients predicted continued depressive symptoms at 8-weeks after treatment was initiated.(23) In view of ketamine's effect on the NMDA receptor and its relationship to reward processing, we hypothesize that greater reward dysfunction will predict response to ketamine.

In summary, we found that subjectively-diagnosed anxious depression predicts a greater antidepressant response to ketamine compared to nonanxious depression. Aberrant brain circuitry between the bed nucleus of the stria terminalis and the reward circuitry may contribute to the psychopathology observed in the anxious-depression phenotype, and might be the targets that account for ketamine's superior antidepressant efficacy among individuals with this depressive subtype. Therefore, higher pre-treatment psychophysiological (i.e., anxiety-potentiated startle magnitude) and cognitive (i.e., reward dysfunction) measures of anxiety in depressed patients may serve as biomarkers that can clinically predict better treatment response to ketamine and that reflect the activity of specific circuits that could be targeted for future drug development.

II. Specific Aims

The overall aims of this protocol are to study biomarkers for diagnosis and treatment response in mood disorders. Specifically:

Specific Aim #1: Determine the extent to which objective measures of anxiety are related to subjective measures of anxiety in patients with mood dysregulation. Our *working hypothesis* is that patients with clinically-diagnosed anxious depression will have higher objective measures of anxiety (anxiety-potentiated startle responses and lower reward responsiveness) compared to those with nonanxious depression.

Specific Aim #2: Determine the extent to which these biomarkers predict response to ketamine. Our *working hypothesis*, based on pilot data and previous work, is that higher pre-treatment psychophysiological measures of anxiety-potentiated startle and lower reward responsiveness on cognitive measures will correlate with a better antidepressant response to ketamine.

Specific Aim #3: Determine the effect of ketamine on the biomarkers. Our *working hypothesis* is that greater reductions in anxiety-potentiated startle magnitude, and greater improvements in measures of reward responsiveness, will be found among ketamine responders compared to nonresponders.

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 (MDD) 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 ≥ 1 failed medication trial during the current depressive episode (per the MGH Antidepressant Treatment History Questionnaire),
- 5) be on a stable adequate dose of an FDA-approved antidepressant medication for ≥ 28 days prior to Study Phase II (see medication inclusion/exclusion list),
- 6) maintain a treating doctor who is in agreement with study participation,
- 7) have a reliable chaperone to accompany them home following the completion of Visit 3 (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).

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 (e.g., requirement of cardiac pacemaker) or alter brain morphology (e.g., recent head trauma, post intracranial surgery, intracranial mass or bleed or 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,
- 5) current/past history of psychotic disorders, history of out-of-body feelings or derealization,
- 6) active substance use disorders (except nicotine and caffeine) within the past six months or past history of ketamine/PCP abuse (we will confirm this with collateral information from their doctor if necessary),
- 7) requirement of excluded medications that may interact with ketamine (see exclusionary medications list),
- 8) caffeine or nicotine use within 1 hour of psychophysiology testing, or alcohol use within 1 day of testing,
- 9) pregnancy, breastfeeding, or unacceptable means of birth control (females only)
- 10) clinically significant hearing impairment,
- 11) current serious suicidal or homicidal risk,
- 12) concurrent participation in other research studies involving medications or other 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,
- 17) airway instability or pulmonary disease with hypercarbia, or
- 18) current or past cubital or carpal tunnel syndrome.

IV. Participant Enrollment

We will enroll 58 depressed patients outpatients into the study.

Recruitment: 58 outpatients will be enrolled via response to flyers, advertisements, e-mails, participation in previous studies with an interest in participating in further research, and letters to providers. The DCRP has used these methods for over two decades with considerable success, receiving an average of over 35 phone calls/week.

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 psychiatrist/doctor. The treating psychiatrist will be informed before any medications changes are considered.

Renumeration: All participants who complete all parts of the study will be paid \$200, and parking will be validated for the study visits. For patients that come in for the screening visit only, they will be renumerated \$20; parking will also be validated. The payment schedule for the other three visits is \$60 each.

Note, the probabilistic reward task is a monetary reward task. Patients will be told that their payment for this task, based on the money they win, will be included in their total payment for study participation.

Procedures for Informed Consent

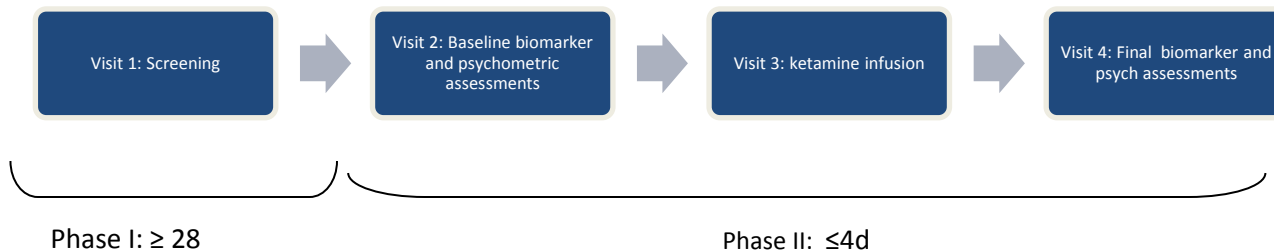
Written informed consent will be obtained by a licensed physician from all patients before protocol-specific procedures begin. The investigator obtaining consent will explain in detail the protocol of the study, its purpose, and potential benefits to the society. Participants will be informed that they can choose to terminate the study at any time, for any reason.

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, 58 depressed patients meeting research criteria (specified above) will undergo baseline biomarker and psychometric assessments (Visit 2). One-to-two days later, they will receive an open-label infusion of subanesthetic intravenous ketamine (0.5mg/kg over 40 minutes), and will be monitored for 4 hours in the MGH Clinical Research Center (Visit 3). Visit 4, which will occur on the day after the ketamine infusion, will consist of the same biomarker and psychometric assessments from Visit 2 (see “Schema” for overall study outline):



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

Study Phase I: Screening: At Visit 1 (“Screening” visit), patients will undergo initial evaluation (i.e., physical and psychiatric examination, vital signs, blood genetics and biomarkers collection, neuropsychological testing, basic laboratory and pregnancy/urine toxicology testing, EKG, hearing assessed for the ability to detect a white noise stimulus at 25dB) 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 antidepressant dose for ≥ 28 days.

In order to ensure that participants do not have a history of substance abuse or dependence, we will perform a structured clinical interview, as well as urine toxicology screen at Visit 1. Furthermore, if necessary, we will obtain permission from participants to contact their primary physician or other treaters, to obtain further collateral information about substance use problems.

Study Phase II: Testing and Treatment: Study Phase II consists of three visits (Visits 2-4) over four days. At Visit 2, participants will have pre-treatment psychophysiological and cognitive testing assessments in the morning. Visit 3 will occur ideally 24 hours after Visit 2, (but within 1-2 days), and will consist of an infusion of ketamine (0.5mg/kg over 40 minutes). Prior to ketamine administration, participants will have a urine test for pregnancy (females only). Intravenous ketamine will be administered at the Clinical Research Center by a medical doctor. Participants will be monitored for 4 hours after the start of the infusion at the CRC trained

research nursing staff. 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 relationship to the time of ketamine infusion. At the +240 minute mark, participants will have a post-ketamine blood sample drawn for genetics and biomarkers. Afterwards, participants will be discharged home into the care of a responsible adult escort. At Visit 4 (which is 24 hours after Visit 3), participants will undergo final psychophysiology and cognitive testing, identical to those from Visit 2, as well as have a blood sample drawn for genetics and biomarkers. All visits in Phase I and II will include psychometric assessments.

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.

Special Case of Medical Marijuana Use

Ketamine and Cannabis: Marijuana was recently passed for legal medicinal use in the Commonwealth of Massachusetts. As a result of this law, the occurrence of medical marijuana prescriptions is likely to increase. Subjects who indicate use of medical marijuana will be reviewed on a case-by-case basis by the PI, and will not be immediately excluded from the study. The PI will speak with the participant's physician and confirm that the medical marijuana is prescribed for medical purposes; furthermore, the physician will be asked to confirm that the subject does not have a history of substance or alcohol misuse. This exception will only be given to participants who are currently prescribed marijuana by a medical professional and will not apply to those who use marijuana recreationally.

MONITOR: Central nervous system- and/or respiratory-depressant effects may be additively or synergistically increased in patients taking multiple drugs that cause these effects, especially in elderly or debilitated patients.

MANAGEMENT: During concomitant use of these drugs, patients should be monitored for potentially excessive or prolonged CNS and respiratory depression. Patients will be counseled to avoid hazardous activities requiring mental alertness and motor coordination until they know how these agents affect them, and to notify their physician if they experience excessive or prolonged CNS effects that interfere with their normal activities.

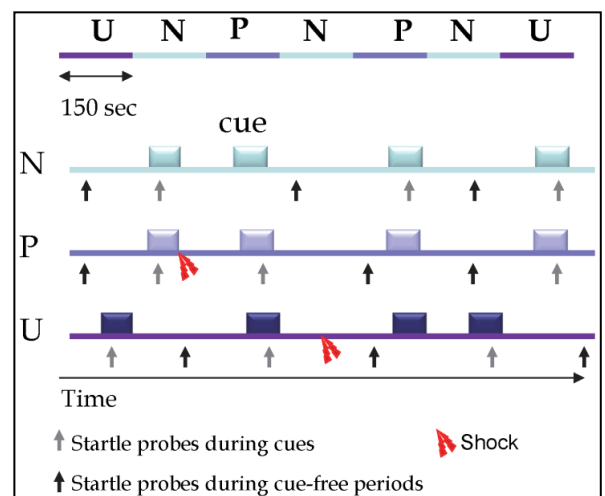
Ketamine Administration and Monitoring: A single, open-label infusion of ketamine at a subanesthetic dose of 0.5mg/kg over 40 minutes will be administered on the morning of Visit 3 at the MGH Clinical Research Center (CRC) by a BLS and ACLS-certified licensed physician, as this dose and procedure has been previously used in depression research, (1, 24, 25) including studies completed at the MGH CRC (e.g., Protocol 2012P001042). Prior to the infusion, all patients will be fasting since midnight, except for required morning medications. Monitoring of oximetry, pulse rate, and blood pressure will be done before, during, and after the infusion. Although subanesthetic ketamine has been shown to have a favorable safety profile when given to depressed patients in research studies, (1, 24-27) there is a potential risk of adverse psychotomimetic, dissociative, and/or sympathomimetic events. Patients will be discontinued from the study if they experience serious adverse events during or after the infusion. See below for details on monitoring and rescue medications.

Psychophysiology Testing: At Visits 2 (“pre-ketamine”) and 4 (“post-ketamine”), psychophysiological testing will be used to measure variations in startle responses (elicited by auditory white noise stimuli) during three conditions: no-shock (**N**), predictable (**P**) shock, and unpredictable (**U**) shock (“**NPU**-threat test”).(28) The NPU threat test has been approved for the use in several protocols by the Human Subject Committee at the National Institute of Mental Health/National Institute of Health. Similar shock experiments have been approved by the Partners IRB (e.g., “The Psychophysiology of Delayed Extinction and Reconsolidation in Humans,” PI Scott Orr, PhD). First, patients will be seated in the testing room. Second, electromyogram (EMG) of the obicularis oculi muscle will be recorded using a BioPac EMG amplifier via two electrodes placed under the left eye to assess blink magnitude of the startle response; this is an established reliable measure of startle magnitude in humans.(29) Third, skin conductance will be measured directly from the non-dominant hand using a BioPac EDA amplifier with a constant value of 0.5 V. Fourth, heart rate will be recorded using standard limb EKG leads connecting to a BioPac EKG amplifier. Interbeat interval will be measured from the EKG and then converted to heart rate. Next, nine 103-dB auditory white noise stimuli will be delivered biaurally via headphones every 25-30 sec for the purpose of habituating the study patient to startle response (Habituation 1). Following habituation, two disc electrodes will be placed on the left wrist for the purpose of administering the shocks. Shocks are an aversive stimulus and are intended to be highly annoying, but not painful, with intensity not in excess of 80 mA and of 500ms duration. Prior to starting the NPU-threat test, patients will experience up to fifteen “sample” shocks for the purposes of 1) choosing an appropriate subjective level that is “highly annoying but not painful” (the “shock work-up”), and 2) allowing for withdraw from the study if the stimuli are perceived as excessively unpleasant.

Prior to testing, (females only) will receive a pregnancy screen. For the NPU-threat test, patients will be seated at a computer. Text will appear continuously on the computer screen to indicate the cue for the upcoming testing condition as follows: “no shock” (N), “shock only during cue” (P), or “shock at any time” (U). The cue (represented by a geometric shape) will then be displayed during the testing condition, with cues differing in color between N, P, and U, though they are meaningless in the N and U conditions. Each testing condition will last for 150 sec, with four cues presented during each condition (See Figure). Cues will be displayed for 8 sec each time. Patients will be presented with two blocks of conditions: 1) P-N-U-N-U-N-P and 2) U-N-P-N-P-N-U. Two shocks (the aversive stimulus) will be administered during each of the P and U conditions, for a total of 8 shocks throughout the entire block. Specifically, shocks will be delivered at the end of the cue in the P condition and in the absence of a cue in the U condition. No shocks will be delivered during the N condition.

Each individual condition (N, P, U) will have one auditory startle stimulus during three of the four cues (1-4 sec following cue onset), and three auditory startle stimuli during cue-free periods (i.e., the intertrial interval, or ITI), for a total of six startle stimuli per condition. The mean intertrial interval will range from 25-30 sec, and we plan to minimize short-term sensitization of startle by delivering the auditory startle stimulus (no less than 8 sec after the aversive shock stimulus). Following each block, patients will subjectively rate their anxiety levels during the cue and intertrial interval for each condition (N, P, U) on a written scale of 0 (not anxious) to 10 (extremely anxious). Patients will also rate their anxiety on a scale from 0 to 10 during the task, using the computer keyboard. Throughout the experiment heart rate and skin conductance will be continuously recorded with the BioPac systems during administration of the NPU-threat test at Visits 2 and 4. The data will be preprocessed and scored using AcqKnowledge software.

Cognitive Testing: Cognitive testing will be carried out to measure behavior and self-reports of anhedonia, reward processing and pattern separation. Regarding behavior, we will use a validated 25-minute computer-based Probabilistic Reward Task(30) at Visits 2 (“pre-ketamine”) and again at Visit 4 (“post-ketamine”). Patients will be given verbal instructions for the task in that the objective is to win as much money as possible, which will be handed out in cash at the end of the task. The task will consist of 3200 trials, divided into 2 blocks of 100 trials, with



blocks separated by a 30-sec break. Each trial will start with the presentation of an asterisk for 500 msec in the center of the computer screen to serve as a fixation point. Then, a mouthless cartoon face will appear in the center of the screen. After a delay of 500 msec, either a short mouth (11.5mm) or a long (13mm) mouth will briefly appear on the face for 100 msec, and then will disappear. The mouthless face will remain on the screen until the patient chooses a computer key to identify which mouth (long or short) was presented. For each block, both stimuli will be shown an equal number of times. Correct responses will be reinforced with monetary reward feedback that will be given as follows: "Correct! You won 20 cents." In order to induce a response bias toward one of the mouth lengths, an asymmetrical reinforcer schedule will be used; for example, correct responses for 1 mouth (e.g., the long mouth) will be rewarded three times more frequently than correct responses for the other mouth (e.g., the short mouth). This designation will be randomized across patients. Because of this unequal frequency of reward feedback, patients with high reward responsiveness are predicted to favor the stimulus with more reinforced positive feedback, whereas patients with low reward responsiveness are expected to have a lower bias, or none at all, towards the positive feedback. As in published work(30-32) for the task, the main dependent variables will be response bias across blocks as well as Reward Learning [= Response Bias (Block 3) - Response Bias (Block 1)].

In addition, we will include a computer based pattern separation task at Visits 2 and 4. The pattern separation task is a high throughput behavioral task that captures the input-output transformation function characteristic of pattern separation processes. (33) 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,(33) 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.

Psychometric Assessments: Self- and clinician-rated assessments will take place during the study. We will administer the following scales to all participants at all visits (Visits 1-4): the Hamilton Depression Rating Scale (HDRS) (the primary dependent outcome measure) is a 28-item validated clinician-administered scale that is widely used as an observational rating measure of depression presence and severity;(34) the Montgomery Asberg Depression Rating Scale (MADRS), a 15-item validated clinical-administered scale widely used for the assessment of depression (35); 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;(36) 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;(37) Young Mania Rating Scale (YMRS) is an 11-item clinician-rated scale for the measurements of hypomanic/manic symptoms;(38) MGH Sexual Functioning Questionnaire (SFQ) is a 7-item self-rated scale for the assessment of sexual functioning; *Snaith-Hamilton Pleasure Scale* (SHAPS) is a 14-item self-report questionnaire for the assessment of anhedonia (please loss to previously pleasurable activities);(39)

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;(40) the MGH Antidepressant Treatment History Questionnaire (ATRQ), a clinician-rated scale used to determine treatment resistance in depression;(41) the *Early Trauma Inventory Self Report-Short Form* (ETISR-SF), a 29-item self-report item on early trauma history,(42) the EHI (a self-reported measure of dominant handedness), and a demographics form.

We will administer the following scales to all participants during Visit 3 (ketamine administration) only: *Brief Psychiatric Rating Scale (BPRS)* is an 18-item clinician-administered scale for the assessment of psychotic symptom constructs;(43) *Clinician Administered Dissociative States Scale (CADSS)* is a 28-item clinician-administered scale for the assessment of dissociative symptoms.(44) BPRS and CADSS will be administered at baseline, +40 minutes, +80 minutes, and +120 minutes in reference to the start of the ketamine 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 HDRS₂₈ by videotapes and live patient interviews.

Concomitant Medications and Adverse Events: Concomitant medications (dosage, start and stop dates) will be reviewed and recorded at all visits in the chart. Adverse events will be recorded at all visits on the Adverse Events Form.

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; two 8-10mL blood samples will be collected and at Visits 3 and 4, 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.

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-anesthetic 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) there is a potential risk of adverse psychotomimetic, dissociative, and/or sympathomimetic events. Similar to previous studies showing efficacy and safety of intravenous ketamine in adults with mood disorders,(1, 45, 46) including studies completed at the MGH CRC (e.g., Protocol 2012P001042), ketamine will be administered at 0.5mg/kg over 40 minutes by a BLS and ACLS-certified, licensed physician at the MGH General Clinical Research Center. Patients will be monitored at the General Clinical Research Center by a medical doctor and trained nurse for changes in vital signs (i.e., heart rate, blood pressure, respiration, temperature, pulse ox, and sedation level) and for treatment-emergent side effects (i.e., as measured by the CADSS and BPRS) during administration and post-administration. Rescue medications, including intravenous labetalol, lorazepam, ondansetron, and haloperidol will be available in the event that unwanted side effects occur; participants requiring such interventions will be withdrawn from the study. PO ibuprofen and acetaminophen will also be available. The following parameters are specified in the order set for the CRC:

1. Oxygen 4 to 6 liters/min via face mask or nasal cannula PRN, SpO₂ <93% at bedside
2. Labetolol 2.5 mg IV push over 2 mins PRN BP ≥3 readings in a row of BP ≥ 160/100; may repeat every 2 to 5 mins X5. Given by MD/NP. Infusions will be stopped if BP ≥160/00 persists for more than 20 minutes. PRN for tachycardia >100 bpm; symptomatic tachycardia >100 bpm requires EKG.
3. Lorazepam 2mg IV push PRN severe agitation, attempting to pull out IV; May repeat every 2-5 minutes X 3. Given by MD/NP
4. Haloperidol 1-2mg IV push PRN delirium (not oriented to space and time, hallucinations, attempting to pull IV); May repeat every 10 minutes X 5. Given by MD/NP
5. Glycopyrrolate 0.1mg IV push PRN excessive salivary secretion; May repeat every 2-5 minutes X 3. Given by MD/NP
6. Ondansetron 4mg IV PRN severe nausea, may repeat x1 after 30 min if no effect.
7. Acetaminophen 325mg tablets, take 1 - 2 tablets PO PRN headache, may repeat x1 after 30 min
8. Ibuprofen 200mg tablets, take 2 tablets PO PRN headache, may repeat x1 after 30 min

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 snack 2 hours after the end of the ketamine infusion.

Discharge: After the ketamine infusion, 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.

Medication Transport and Storage

The MGH Research Pharmacy and MGH CRC will store medications, including ancillary medications.

Devices to be Used

N/A

Procedures/Surgical Intervention

N/A

VI. Biostatistical Analysis

Specific Data Variables to be Collected

As described above, we will administer the following scales at all visits (Visits 1-4): HDRS, MADRS, HAM-A, CUDOS-A, YMRS, SFQ, and SHAPS.

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

We will administer the following scales during Visit 3 only (ketamine): BPRS and CADSS at baseline, +40 minutes, +80 minutes, and +120 minutes in relationship to the time of ketamine infusion.

Vital Signs will be recorded once at Visit 1 (the screening visit), and again at Visit 3 at baseline, +0 minutes, +5, +10, +15, +20, +30, +40, +60, +90, +120, +180 minutes in relationship to the time of ketamine infusion.

Study Endpoint

Primary clinical endpoint is change in the HDRS₂₈ total score. Scores that decrease by $\geq 50\%$ from baseline will be considered "response."

Specific Statistical Methods

Testing of Hypotheses: Statistics and Data Analysis

Specific Aim #1: Objective and subjective measures of anxiety will be positively correlated with each other. A primary analysis will examine Pearson correlations between subjective measures of anxiety (i.e., the HDRS Anxiety-Somatization Factor Scale, HAM-A, and CUDOS-A) and eye-blink startle response. Similarly, we will examine correlations between subjective anxiety and reward responsiveness. We will analyze the data to ensure that linearity exists. If linearity does not exist, we will conduct comprehensive sub-analyses to find the best way to characterize the relationship of all data. For example, we will use covariates (e.g., clinical descriptors such as treatment failures) to determine their role in creating non-linearity.

Specific Aim #2: Greater psychophysiological and cognitive objective measures of anxiety will predict better antidepressant response to ketamine, regardless of subjective depression symptoms. We will use a nonlongitudinal linear regression model to assess the extent to which the combination of pre-treatment psychophysiological (e.g., anxiety-potentiated eye-blink startle) and cognitive reward processing (high versus low reward responsiveness) predict the antidepressant treatment response to ketamine (Visits 3 and 4). Antidepressant response to ketamine will be defined as a $\geq 50\%$ improvement in HDRS scores (primary dependent outcome measure).

Specific Aim #3: In ketamine responders, psychophysiological measurements will show a significantly larger decreases in magnitude compared to ketamine nonresponders; greater improvements will be seen in cognitive reward processing measures in ketamine responders. We will use longitudinal analyses (linear mixed effects random regression models) to analyze the effect of ketamine on

the biomarkers. These models account for the correlation of observations from the same individual. For the change in startle magnitude post-ketamine, the model will include the change in startle magnitude as the outcome, and time (Visit 2 and 4) as the predictor. For the change in reward processing post-ketamine, the model will include the change in reward processing as the outcome, and time (Visit 2 and 4) as the predictor. The coefficient for the correlations in this model quantifies the rate of change in the psychophysiological and cognitive measures associated with a change in depressive symptoms. A coefficient significantly different from zero indicates that changes in the clinical measures of depression are associated with changes in psychophysiological and/or cognitive measures. We will also perform between-group comparisons of measurements for responders vs. nonresponders. All models will be fit using SAS software, $\alpha=0.05$, two-tailed.

Sample Size Determination:

The sample size for Aims #1 and #2 was calculated using the G*Power Sample Size calculator (Version 3.9.1.2; downloaded from <http://www.gpower.hhu.de>) and was based on findings from a *post-hoc* analysis of the antidepressant treatment response to ketamine in anxious versus nonanxious depressed patients, where anxious depression predicted a better response to open-labeled ketamine.(6) The anxious depression effect size (Cohen's *d*) reached its maximum of $d=0.76$ on Day 2 post-infusion. With this, for Aims #1 and #2, we estimate requiring 58 patients with power >0.8 , $r=0.35$ and an $\alpha=0.05$, two-tailed, in order to reject the null hypothesis that there will be no correlations with baseline psychophysiological and cognitive measures between ketamine responders and nonresponders. For Aim #3, a sample size of 58 would allow us to detect a moderately-large effect size (Cohen's $d\geq 0.75$) for between-group comparisons. In order to account for potential drop-outs, we will recruit 70.

Expected Outcomes/Results: We expect that subjective and objective measures of anxiety will be at least moderately correlated in patients with mood dysregulation. Further, we predict that patients with higher pre-treatment levels of objective anxiety, as measured by psychophysiological and cognitive assessments, will have significantly greater antidepressant responses to ketamine, compared to patients with lower pre-treatment objective anxiety. These results will significantly contribute to the paucity of information regarding biomarkers of diagnosis and antidepressant response and provide insight into the anxiety and reward neurocircuitry that could contribute to the pathophysiology of anxious depression.

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.(47) 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.(48, 49) 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 history of substance abuse or dependence in the previous year 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, a medical doctor will remain in the room with a research nurse, the research assistant, and the patient, and vital signs will be monitored every 5 minutes during the 40 minutes of administration. Any concerning changes will be managed by the physicians. Criteria for discontinuation include ≥ 3 readings in a row of systolic BP ≥ 180 mmHg or diastolic BP ≥ 110 mmHg, HR ≥ 110 bpm, or symptomatic elevations in blood pressure (i.e., new onset headache, chest pain, or shortness of breath). Furthermore, the physician 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.

Participants will stay at the General Clinical Research Center (GCRC) for up to 4 hours following the start of the administration, and will be monitored for psychotomimetic, dissociative, and sympathomimetic side effects during this time. 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 at home, they will be referred for evaluation at the nearest emergency department. 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 participants will be informed of alternatives to research, and will be provided with the option to seek further psychopharmacological or psychotherapy-based interventions.

Electric Shocks

The electric shocks that participants receive will be self-selected to be highly annoying, but not painful. Subjects may stop the electric shocks at any time. The stimulator is manufactured by Digitimer. It is inspected annually by biomedical engineering under the auspices of Partners Human Research Committee (PHRC), who have affirmed compliance with safety regulation. The stimulator has been used in similar experiments across institutions, including the National Institute of Mental Health (NIMH), and has been approved by their IRB for use in humans.

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

Though patients with severe suicidal ideation will be excluded from the study at screening, any patient who, based on the investigator's judgment, is judged to present with an imminent risk of suicide will be discontinued from the study and referred to a local emergency room for further evaluation.

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. The results of this study may lead to improved understanding of the neurobiology of depression, as well as the biomarkers for diagnosis and treating depression. 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

Because this study is a physiological study of biomarkers, as opposed to a clinical trial, the principal investigator and co-investigators will be responsible for monitoring and quality assurance of the study. In conjunction with the research assistants, weekly meetings will be held and documented. Current participants in the study will be discussed at each meeting, and charts will be reviewed for completeness.

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 judges to impose a significant hazard, contraindication, side effect, or precaution. Reporting to the IRB will be done within 24 hours of the SAE.

Study Stopping Rules: If at any time during the course of the study, the PI or CO-Is judge that risk to participants outweighs the potential benefits, the PI/CO-Is 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).

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