YALE UNIVERSITY
HUMAN INVESTIGATION COMMITTEE

Application to Involve Human Subjects in Biomedical Research
100 FR1 (2013-1)

Please refer to the HIC website for application instructions and information required to complete this application. The Instructions are available at http://www.yale.edu/hrpp/forms-templates/biomedical.html Submit the original application and one (1) copy of all materials including relevant sections of the grant which funds this project (if applicable) to the HIC.	**HIC OFFICE USE ONLY**	

SECTION I: ADMINISTRATIVE INFORMATION

Title of Research Project: The Sustained Effects of Ketamine			
Principal Investigator: Chadi G. Abdallah, M.D.		**Yale Academic Appointment:** Assistant Professor	
Department: Psychiatry			
Campus Address: Yale Depression Research Program (YDRP), 100 York Street, Suite 2J, New Haven, CT 06511			
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Campus Phone: 203-932-5711 x5667	**Fax:**	**E-mail:** prerana.purohit@yale.edu	
Yale Cancer Center CTO Protocol Correspondent Name & Address (*if applicable*)**:**			
Campus Phone:	**Fax:**	**E-mail:**	
Business Manager:			
Campus Phone :	**Fax :**	**E-mail**	

Faculty Advisor:(required if PI is a student, resident, fellow or other trainee) ☐ **NA**	**Yale Academic Appointment:**

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Campus Address:			
Campus Phone:	**Fax:**	**Pager:**	**E-mail:**

Investigator Interests:

Does the principal investigator, or do any research personnel who are responsible for the design, conduct or reporting of this project or any of their family members (spouse or dependent child) have an incentive or interest, financial or otherwise, that may affect the protection of the human subjects involved in this project, the scientific objectivity of the research or its integrity? Note: The Principal Investigator (Project Director), upon consideration of the individual's role and degree of independence in carrying out the work, will determine who is responsible for the design, conduct, or reporting of the research.

See Disclosures and Management of Personal Interests in Human Research
http://www.yale.edu/hrpp/policies/index.html#COI
4Yes oNo

Do you or does anyone on the research team who is determined by you to be responsible for the design, conduct or reporting of this research have any patent (sole right to make, use or sell an invention) or copyright (exclusive rights to an original work) interests related to this research protocol?
4Yes oNo

If yes to either question above, list names of the investigator or responsible person:

The Yale University Principal Investigator, all Yale University co-investigators, and all Yale University individuals who are responsible for the design, conduct or reporting of research must have a current financial disclosure form on file with the University's Conflict of Interest Office. Yale New Haven Hospital personnel who are listed as con-investigators on a protocol with a Yale University Principal Investigator must also have a current financial disclosure form on file with the University's Conflict of Interest Office. If this has not been done, the individual(s) should follow this link to the COI Office Website to complete the form: http://www.yale.edu/coi/

NOTE: The requirement for maintaining a current disclosure form on file with the University's Conflict of Interest Office extends primarily to Yale University and Yale-New Haven Hospital personnel. **Whether or not they are required to maintain a disclosure form with the University's Conflict of Interest Office, all investigators and individuals deemed otherwise responsible by the PI who are listed on the protocol are required to disclose to the PI any interests that are specific to this protocol.**

SECTION II: GENERAL INFORMATION

1. **Performing Organizations:** Identify the hospital, in-patient or outpatient facility, school or other agency that will serve as the location of the research. Choose all that apply:

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a. Internal Location[s] of the Study:

☒ Magnetic Resonance Research Center (MR-TAC)
☐ Yale Cancer Center/Clinical Trials Office (CTO)
☐ Yale Cancer Center/Smilow
☐ Yale-New Haven Hospital
☐ Yale University PET Center
☐ YCCI/Church Street Research Unit (CSRU)
☒ YCCI/Hospital Research Unit (HRU)
☐ YCCI/Keck Laboratories
☐ Yale-New Haven Hospital—Saint Raphael Campus
☐ Cancer Data Repository/Tumor Registry
☒ Specify Other Yale Location: Yale Depression Research Program (YDRP)

b. External Location[s]:

☐ APT Foundation, Inc.
☒ Connecticut Mental Health Center
☒ Clinical Neuroscience Research Unit (CNRU)
☐ Other Locations, Specify:
☐ Haskins Laboratories
☐ John B. Pierce Laboratory, Inc.
☐ Veterans Affairs Hospital, West Haven
☐ International Research Site (Specify location(s)):

c. Additional Required Documents *(check all that apply)*: ☐ N/A

☒ *YCCI-Scientific and Safety Committee (YCCI-SSC) Approval Date:
☐ *Pediatric Protocol Review Committee (PPRC) Approval Date:
☐ *YCC Protocol Review Committee (YRC-PRC) Approval Date:
☐ *Dept. of Veterans Affairs, West Haven VA HSS Approval Date:
☐ *Radioactive Drug Research Committee (RDRC) Approval Date:
☐ YNHH-Radiation Safety Committee (YNHH-RSC) Approval Date:
☒ Magnetic Resonance Research Center PRC (MRRC-PRC) Approval Date:
☐ YSM/YNHH Cancer Data Repository (CaDR) Approval Date:
☐ Dept. of Lab Medicine request for services or specimens form
☐ Imaging on YNHH Diagnostic Radiology equipment request form (YDRCTO request) found at http://radiology.yale.edu/research/ClinTrials.aspx)

Approval from these committees is required before final HIC approval is granted. See instructions for documents required for initial submission and approval of the protocol. Allow sufficient time for these requests. Check with the oversight body for their time requirements.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities. **It is expected this project will last for 5 years.**

3. **Research Type/Phase: (Check all that apply)**

a. **Study Type**

☒ Single Center Study
☐ Multi-Center Study

Does the Yale PI serve as the PI of the multi-site study? Yes ☐ No ☒

☐ Coordinating Center/Data Management
☐ Other:

b. **Study Phase** ☐ **N/A**

☒ Pilot ☐ Phase I ☐ Phase II ☐ Phase III ☐ Phase IV

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☐ Other (*Specify)*

4. **Area of Research: (Check all that apply)** Note that these are overlapping definitions and more than one category may apply to your research protocol. Definitions for the following can be found in the instructions section 4c:

☐ Clinical Research: Patient-Oriented
☐ Clinical Research: Epidemiologic and Behavioral
☒ Translational Research #1 ("Bench-to-Bedside")
☐ Translational Research #2 ("Bedside-to-Community")
☐ Clinical Research: Outcomes and Health Services
☐ Interdisciplinary Research
☐ Community-Based Research

5. Is this study a clinical trial? Yes ☒ No ☐

NOTE the current ICMJE (International Committee of Medical Journal Editors) definition of a clinical trial: "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes." Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example, drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events"

If yes, where is it registered?

Clinical Trials.gov registry ☒
Other (*Specify*)

*Registration of clinical trials **at their initiation** is required by the FDA, NIH and by the ICMJE.*

If this study is registered on clinicaltrials.gov, there is new language in the consent form and compound authorization that should be used.

For more information on registering clinical trials, including whether your trial must be registered, see the YCCI webpage, http://ycci.yale.edu/researchers/ors/registerstudy.aspx or contact YCCI at 203.785.3482)

6. Does the Clinical Trials Agreement (CTA) require compliance with ICH GCP (E6)?
Yes ☐ No☒

7. Will this study have a billable service? A Billable Service is defined as a service or procedure that will be ordered, performed or result in charging in EPIC for individuals who are enrolled in a clinical research study, regardless if the charge is intended to be paid by the subject/their insurance or the research study.

Yes ☐ No☒

If you answered "yes", this study will need to be set up in OnCore Support
http://medicine.yale.edu/ymg/systems/ppm/index.aspx

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8. Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes _4__ No ___ *If Yes, please answer questions a through c and note instructions below. If No, proceed to Section III.*

a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform?

Yes.

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure?

No.

c. Will a novel approach using existing equipment be applied?

No.

If you answered "no" to question 7a, or "yes" to question 7b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

SECTION IV: PRINCIPAL INVESTIGATOR/FACULTY ADVISOR/ DEPARTMENT CHAIR AGREEMENT

As the **principal investigator** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- I assume full responsibility for the protection of human subjects and the proper conduct of the research.
- Subject safety will be of paramount concern, and every effort will be made to protect subjects' rights and welfare.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- All members of the research team will be kept apprised of research goals.
- I will obtain approval for this research study and any subsequent revisions prior to my initiating the study or any change and I will obtain continuing approval of this study prior to the expiration date of any approval period.
- I will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set by the University and qualify to serve as the principal investigator of this project or have acquired the appropriate approval from the Dean's Office or Office of the Provost, or the Human Subject Protection Administrator at Yale-New Haven Hospital, or have a faculty advisor.
- I will identify a qualified successor should I cease my role as principal investigator and facilitate a smooth transfer of investigator responsibilities.

_____ _____

PI Name (PRINT) and Signature Date

As the **faculty advisor** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- This project has scientific value and merit and that the student or trainee investigator has the necessary resources to complete the project and achieve the aims.
- I will train the student investigator in matters of appropriate research compliance, protection of human subjects and proper conduct of research.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- The student investigator will obtain approval for this research study and any subsequent revisions Prior to initiating the study or revision and will obtain continuing approval prior to the expiration of any approval period.
- The student investigator will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set forth by the University and qualify to serve as the faculty advisor of this project.

_____ _____

Advisor Name (PRINT) and Signature Date

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC.)
☐ No

As Chair, do you have any real or apparent protocol-specific conflict of interest between yourself and the sponsor of the research project, or its competitor or any interest in any intervention and/or method tested in the project that might compromise this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC)
☐ No

I assure the HIC that the principal investigator and all members of the research team are qualified by education, training, licensure and/or experience to assume participation in the conduct of this research trial. I also assure that the principal investigator has departmental support and sufficient resources to conduct this trial appropriately.

_____ _____

Chair Name (PRINT) and Signature Date

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Department

YNHH Human Subjects Protection Administrator Assurance Statement

Required when the study is conducted solely at YNHH by YNHH health care providers.

As Human Subject Protection Administrator (HSPA) for YNHH, I certify that:

- I have read a copy of the protocol and approve it being conducted at YNHH.
- I agree to notify the IRB if I am aware of any real or apparent institutional conflict of interest.
- The principal investigator of this study is qualified to serve as P.I. and has the support of the hospital for this research project.

YNHH HSPA Name (PRINT) and Signature _____ Date _____

SECTION V: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.

   Ketamine is an anesthetic agent, recently found to exert a rapid and potent antidepressant effect in severely depressed patients. Mounting evidence indicates that a ketamine-induced surge in glutamate neurotransmission, particularly in the frontal cortex, plays a crucial role in the antidepressant and psychotomimetic effects of the drug. Our preliminary data also suggests ketamine has a normalizing effect on long-range neural dysconnectivity in Major Depressive Disorder (MDD), and that these changes in functional connectivity, as measured by resting state functional MRI (rs-fMRI), are related to the antidepressant effect of the drug.

   A better understanding of the mechanism of neuropsychiatric effects of ketamine are critically important, as pharmacological modulation of these effects can assist in the development of new medications for the treatment of multiple psychiatric disorders including depression and schizophrenia. The aims of the current proposal are to provide insight into (a) the impact of ketamine on functional connectivity as measured by rs-fMRI; (b) the relationship between rs-fMRI alterations and ketamine-induced neurochemical changes in glutamate; and (c) to investigate the influence of a glutamate release inhibitor (i.e., lamotrigine) on these ketamine-induced neural alterations in healthy volunteers using safe, minimally invasive, state-of-the-art multi-modal neuroimaging and behavioral assessment. Further examination of the effects of ketamine-induced changes in neural connectivity as well as the impact of a pre-treatment dose of lamotrigine can provide key insight into the role of glutamate and the mechanism of ketamine's rapid antidepressant and perceptual effects and its influence on neural connectivity.

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- **Aim 1:** To characterize the effects of a single, sub-anesthetic dose of ketamine in rs-fMRI in healthy subjects.
  Design: Twenty-four healthy participants will be randomized into one of two parallel groups—ketamine+lamotrigine or ketamine+placebo—and will complete pre- and post-ketamine rs-fMRI.
  Hypothesis 1: Post-ketamine rs-fMRI data will demonstrate a pattern of increased global brain connectivity (GBC) in fronto-temporal cortex.

- **Aim 2:** To examine the relationship between ketamine-induced glutamate alterations and changes in rs-fMRI of healthy subjects.
  Design: Using MRS we will measure alterations in glutamate levels during ketamine infusion and correlate this data to rs-fMRI changes.
  Hypothesis 2: Ketamine-induced glutamate alterations will positively correlate with changes in GBC.

- **Aim 3:** To examine the effect of administering a glutamate release inhibitor, lamotrigine, prior to ketamine infusion on neural network configuration and connectivity.
  Design: Participants will be randomized to receive 300 mg oral lamotrigine or a matched-placebo medication 2-hours prior to ketamine infusion.
  Hypothesis 3: Lamotrigine will attenuate the effect of ketamine-induced GBC enhancement in the neural network.

2. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.

Ketamine

Major Depressive Disorder (MDD) is a leading cause of disability worldwide [1], with a 21-fold increase of suicide attempts during major depressive episodes [2]. In the United States, the estimated lifetime prevalence is approximately seventeen percent [3]. While more than 20 antidepressant medications are currently available, the efficacy of these medications is limited [4-7]. Moreover, traditional antidepressants, all of which target the monoaminergic system, take weeks to months to reach their full clinical effects [8]. Hence, there is an urgent need for a novel class of antidepressants that can offer (1) rapid acting effects and (2) efficacy in patients not achieving adequate benefit from existing medications. Over the last decade, accumulating evidence has shown that low dose of ketamine, a *N*-methyl-D-aspartate (NMDA) receptor antagonist, may possess both of these properties [9].

NMDA receptor dysfunction has been implicated in the pathophysiologic processes of depression, schizophrenia, and other psychiatric illnesses [10,11]. Ketamine is now the prototype for a new generation of antidepressants that rapidly alleviates depression and demonstrates efficacy even in patients with symptoms that are refractory to currently available treatments. The association between glutamate alterations and psychotomimetic effects of ketamine in human has been suggested

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by studies showing that glutamate release inhibitors, such as lamotrigine (an anticonvulsant drug) or group II metabotropic agonists, attenuate these behavioral symptoms [12-15].

To date, more than 15 studies have investigated the efficacy of 0.5 mg/kg ketamine infusion in more than 300 depressed patients [16-31]. These studies consistently showed a rapid antidepressant action within 4-hours of ketamine's administration, with a short-term response rate ranging from 43% to 90% [25,32]. This antidepressant effect was sustained for 7-28 days after a single ketamine infusion [19,29], and for an average of 19-days after repeated dosing [32]. These rapid and potent antidepressant effects were also demonstrated in patient groups known to respond poorly to current antidepressants, such as patients diagnosed with bipolar disorder and patients with depressive symptoms that did not respond to electroconvulsive therapy (ECT) [17,22].

Animal Studies: The antidepressant effects of ketamine appear unique in the sense that the time of peak response, 4 to 72 hours post administration, is extremely rapid but is generally believed to be well after the drug has been metabolized and cleared from the body ($t_{1/2}$ 4 minutes; terminal plasma $t_{1/2}$ 2.5 hours). Although the exact neurobiological mechanisms associated with ketamine's rapid and sustained antidepressant effect remain largely unknown, recent animal studies have begun to elucidate downstream effects of ketamine that may underlie the beneficial effects in depressed patients. In brief, convergent evidence suggests that at subanesthetic doses, ketamine's antagonism of the glutamatergic NMDA receptor is the first step in a cascade of events that includes rapid increases in presynaptic glutamate release, enhanced regional activity in excitatory networks and ultimately marked changes in synaptic plasticity and connectivity. Recent animal studies have begun to elucidate downstream effects of ketamine that may underlie its beneficial effects in depressed patients. In brief, convergent evidence suggests that, at subanesthetic doses, ketamine's antagonism of NMDA receptors rapidly triggers 3 consecutive events: 1st a presynaptic disinhibition of glutamatergic neurons leading to a synaptic glutamate surge, 2nd an increased activation of AMPA receptors, and 3rd a post-synaptic activation of neuroplasticity-related signaling pathways resulting in overall synaptogenesis and synaptic potentiation (for a recent review see Ref. [33]). These acute effects of ketamine treatment rapidly oppose the stress induced prefrontal neuronal atrophy and synaptic dysconnectivity [34].

In rodents, microdialysis and electrophysiological studies consistently indicate that low doses of ketamine and other NMDA receptor antagonists induce a "glutamate surge" in the prefrontal cortex [35-43]. This glutamate surge has been confirmed by carbon-13 (^{13}C) Magnetic Resonance Spectroscopy (MRS) studies suggesting an increase in glutamate/glutamine cycling, as reflected by the ^{13}C incorporation into glutamate (enrichment) following injection of subanesthetic dose of ketamine [44]. At higher doses of ketamine, there are no changes or even a decrease in extracellular glutamate and in glutamate cycling [37,44]. Of interest, synaptogenesis and the antidepressant effects of ketamine are also limited to subanesthetic doses [45]. Hence, there is a dose-response parallel between the glutamate surge, synaptogenesis, and the antidepressant effects of ketamine. Further evidence supporting the crucial role

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of glutamate neurotransmission in ketamine's effect comes from a well-replicated finding in rodents demonstrating that AMPA receptor activation is required for the antidepressant effect of ketamine [45-48]. Consistent with this hypothesis, Dr. Duman's group (at Yale University) recently showed that blocking group II metabotropic receptors – receptors known to inhibit glutamate release – exerts a mTOR-dependent rapid antidepressant-like effect and synaptogenesis in a fashion similar to ketamine [49]. In addition to the antidepressant effect, the psychotomimetic effect of ketamine appears to be affected by glutamate neurotransmission. In that regard, glutamate release inhibitors has been repeatedly shown to block many of the physiological and behavioral effects of ketamine and other NMDA receptor antagonists in animal models of psychosis [38,41,50-55].

Human Studies: As noted, the association between glutamate alterations and psychotomimetic effects of ketamine in humans has been suggested by studies showing that glutamate release inhibitors, such as lamotrigine or group II metabotropic agonists, attenuate these behavioral symptoms [12-15]. Yet, a major challenge to the glutamate surge hypothesis is to quantitatively demonstrate the glutamate neurotransmission changes *in vivo* in human. Glutamate and glutamine are two abundant brain metabolites that can be detected using conventional ^{1}H-MRS. However, ^{1}H-MRS measures total (intra & extracellular) glutamate level in a brain region of interest, which may reflect many processes other than glutamatergic neurotransmission. In addition, at low- and mid-field strengths (1.5 – 4.7 Tesla) the ^{1}H spectroscopy signal from glutamate is difficult to distinguish from glutamine [56], which affects the reliability of these measures. An early 4T ^{1}H-MRS study in healthy subjects reported a transient increase in glutamine levels, but not glutamate, in the anterior cingulate following ketamine administration [57]. A recent 3T ^{1}H -MRS study – using comparable doses, timing, and region of interest – reported increased glutamate levels, but not Glx (glutamate + glutamine), during ketamine infusion [58]. Other ^{1}H-MRS studies reported no changes in Glx or glutamate levels during or following infusion of ketamine [18,59]. Thus, overall ^{1}H-MRS studies show alterations in the glutamatergic system induced by ketamine but fail to provide consistent results. Our studies using ^{13}C-MRS to measure ketamine's effect on glutamatergic neurotransmission are currently underway (HIC#1305011972).

Plasticity and Connectivity: The term "synaptic plasticity" applies to the mechanisms through which neural circuits regulate their excitability and connectivity, particularly in the context of adaptation, i.e., phenomena including development, learning, coping with stress, and aging [60]. These phenomena are accomplished by regulating synaptic strength (e.g. changing the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors) and synaptic number (e.g. altering dendritic spine density and shape). Of note, synaptic and extrasynaptic NMDA receptors have opposing effects on synaptic plasticity, promoting or reducing synaptic strength respectively [61]. Synaptic plasticity could be "local" such as long-term potentiation (LTP) or "global" such as homeostatic plasticity. The latter is of particular relevance to clinical depression, which is associated with reduced prefrontal synaptic connectivity (see below). A major form of homeostatic plasticity is "synaptic scaling", that regulates the overall strength of neuronal synaptic

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connectivity. For example, a prolonged increase in neuronal activities produces a downscaling in overall synaptic strength [62]. Synaptic scaling is regulated by inflammatory cytokines (e.g. Tumor Necrosis Factor) [63] and by neurotrophins (e.g. brain-derived neurotrophic factor (BDNF)) [64], alteration in both factors have been associated with depression [60,65].

Over the past two decades, convergent evidence highlighted the role of synaptic homeostasis in the pathophysiology and treatment of depression [66,67]. In particular, prolonged stress and depression have been associated with neuronal atrophy and overall synaptic depression in the prefrontal cortex (PFC) and the hippocampus [68-70], while other brain regions such as the amygdala and nucleus accumbens showed changes consistent with neuronal hypertrophy and synaptic potentiation [71,72]. These synaptic changes are believed to be the result of stress-induced altered glutamate release and astroglial loss leading to sustained increase in extracellular glutamate which precipitates excitotoxicity, altered synaptic strength, reduced dendritic spine density, dendritic retraction, and reduced dendritic branching in the PFC [67,73]. It has been proposed that down-regulation of the PFC activity leads to gain of function in other brain regions negatively controlled by the PFC such as the amygdala, a brain region associated with increased anxiety and hypothalamic-pituitary-adrenal (HPA) axis reactivity [74].

In this model, prefrontal synaptic deficits and the subsequent neuronal dysconnectivity are critical to the progress and treatment of depression. Molecular studies have started to identify signaling pathways implicated in the observed stress-related synaptic dysfunction. It has been found that synaptic deficits are precipitated by reduction in neurotrophins such as BDNF [60], and by inhibition of the mammalian target of rapamycin (mTOR) signaling pathway [75]. Inhibition of mTOR signaling or reduction of BDNF leads to depressive-like behavior and blocks the effect of antidepressants in animal models of depression [60,75]. Enhancing mTOR signaling or increasing BDNF produces antidepressant effects in preclinical studies [60,75]. In humans, reduced central and peripheral BDNF levels were found in depressed patients [60,76] and a functional variant of BDNF polymorphism (Val66Met) has been related to depression, especially in males [77]. Together these data posit that enhancing DBNF and mTOR signaling leading to prefrontal synaptic formation (synaptogenesis), and reversal of stress/depression-induced neuronal atrophy and synaptic dysconnectivity is a required step for efficacious antidepressant treatment. Traditional antidepressants, targeting the monoaminergic system, were found to increase BDNF and synaptogenesis [60,67]. However, these effects were only evident following chronic treatment, which is in line with the delayed antidepressant response to these drugs in humans. Therefore, it is proposed that rapid acting antidepressants would need to directly target the induction of mTOR signaling, the increase of BDNF levels, and the ultimate enhancement of prefrontal synaptogenesis.

Lamotrigine

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As noted above, lamotrigine is an anticonvulsant drug. It is also used in the treatment of bipolar disorder, specifically in the treatment and prevention of bipolar depressive episodes [78]. It is a use-dependent sodium channel blocker and phenyltriazine derivative, which has been demonstrated to possess multiple mechanisms of action [79,80]. Briefly, these include the selective blockade of the N- and P-type calcium channels in focal brain regions, and the voltage-dependent blockade of sodium channels via its action on the slow inactivation state that occurs when sodium channels are over-activated. This drug has also been shown to inhibit the release of excitatory amino acids such as glutamate and aspartate, and may have some agonistic effects on γ-aminobutyric acid (GABA). It selectively suppresses neuronal over-activities without affecting the basal neurophysiological state, which has clear implications in neuronal stabilization, and may also be a plausible explanation of its action in bipolar disorder, even though the pathophysiology of this condition is less clear [79]. Lamotrigine is also believed to act on serotonin reuptake, which may contribute to its antidepressant effects [79,81]. There is evidence of peripheral glutamate dysregulation in bipolar disorder [82], and the glutamatergic activity of lamotrigine may also be implicated in its therapeutic and neuroprotective effects. Lamotrigine levels have been shown to peak at 1 to 4 hours after oral administration, with a mean of 2 hours.

Several studies have found that lamotrigine reduced the neuropsychiatric effects of ketamine in healthy subjects [12] as well as preventing some BOLD signal changes in a neuroimaging study [13]. In a pioneering Yale study exploring the impact of lamotrigine on ketamine's influence on glutamate, healthy subjects were given 300 mg of lamotrigine approximately two hours prior to a ketamine infusion (0.25 mg/kg by intravenous bolus and 0.65 mg/kg per hour by intravenous infusion). Those subjects who received lamotrigine demonstrated significantly decreased ketamine-induced perceptual disturbances as assessed by the Clinician Administered Dissociative States Scale, as well as reduced positive and negative psychotomimetic symptoms as assessed by the Brief Psychotic Symptoms Scale, and reductions in learning and memory impairment. Of note, those participants who received lamotrigine showed increases in the rapid mood enhancing effects of ketamine [12]. In another study hoping to identify the mechanisms of action of ketamine in inducing symptoms and to determine the role of increased glutamate release in these alterations, participants were pretreated with lamotrigine, prior to a ketamine infusion to examine which effects of ketamine appear to be mediated by increased glutamate release [13]. They found that ketamine administration induced a rapid and focal decrease in the ventromedial frontal cortex, including the orbitofrontal cortex and subgenual cingulate, which strongly predicted its dissociative effects. They also noted increased activity in the mid-posterior cingulate, thalamus, and temporal cortical regions. They found that lamotrigine pre-treatment prevented many of the BOLD signal changes [13]. In the current proposal, we want to explore further the impact of lamotrigine on ketamine-induced changes in rs-fMRI and the relationship between alterations in glutamate levels and rs-fMRI changes.

Resting State fMRI

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Resting state fMRI studies in MDD have revealed increased functional connectivity in multiple brain regions [83-89], perhaps most notably the dorsal nexus, in the DMPFC region, with considerable overlapping connections across networks [89]. Studies characterizing the effects of ketamine on connectivity in MDD are lacking. Ketamine has been shown to induce greatest antidepressant response 24-hours after administration; therefore, we believe it is imperative to examine connectivity at this time point in order to determine whether there are changes in functional connectivity. These data are of utmost importance to guide development of novel medications that would induce a similar response in the human brain. Examination of functional connectivity at rest, as examined by rs-fMRI can provide fundamental insights regarding the underlying functional neural architecture [90]. Chronic treatment with SSRIs appears to reduce this abnormally heightened connectivity [91,92]. Interestingly, ketamine was found to rapidly reduce (i.e., normalize) functional connectivity in the dorsal nexus region of healthy controls 24-hours post-infusion [88].

Our pilot data collected from three healthy subjects who were administered intravenous ketamine (0.23 mg/kg over 1 minute followed by 0.58 mg/kg/h over 75) suggests a pattern of increased Global Brain Connectivity (GBC, as measured by Cole and colleagues [93]) in the fronto-temporal cortex, but reduced GBC in the occipito-parietal regions. The GBC value of each voxel is the average of its time series correlation with all other voxels, providing a measure of nodal strength in a graph-based network. We used voxel-wise paired t-tests pre-/post-ketamine and a p value of 0.05 uncorrected and cluster threshold at 100 voxels.

Similarly, in collaboration with James Murrough at Mount Sinai, we have recently identified an abnormal pattern of GBC in MDD, where we found an anteroposterior dissociation of reduced prefrontal cortex (PFC) GBC but increased parietal-occipital-cerebellar GBC. A set of complementary analyses has shown that these brain network alterations were largely driven by increased connectivity within the PFC, but reduced long-range connectivity between the PFC and other brain regions. Following ketamine treatment, we found clusters of increased GBC in the dorsomedial PFC (DMPFC), dorsolateral PFC (DLPFC), the insula, and the caudate (corrected $p < 0.05$). In contrast, there was a reduction in GBC in the posterior cingulate, precuneus, medial and lateral occipital, fusiform, and cerebellum (corrected $p < 0.05$). Reduction in depression scores correlated with the increase in GBC in the caudate and PFC (uncorrected $p < 0.01$). Overall, the complementary analyses showed reduction in connectivity within the PFC, but increased connectivity between the PFC and other brain regions following ketamine treatment.

Summary

While these preliminary data strongly support the efficacy of ketamine, there are several limitations that hinder its widespread use including its acute psychotomimetic and dissociative effects [94]. Convergent evidence indicates that a ketamine-induced glutamate neurotransmission surge in frontal brain regions plays a crucial role in the antidepressant and psychotomimetic effect of the drug. However, this glutamate surge has been difficult to demonstrate in humans. In the current

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proposal, we will implement state-of-the-art multi-modal neuroimaging methods to examine the role of ketamine-induced glutamate alterations on connectivity in rs-fMRI, the relationship between the alterations in connectivity and glutamate changes, and the impact of lamotrigine, a glutamate inhibitor. This will provide critical information concerning the impact of ketamine on neural connectivity and use of lamotrigine will allow us to confirm target engagement and the impact of blocking the glutamate system, furthering our understanding of the mechanisms of action and the role of glutamate in ketamine's effects. Moreover, given the well-characterized similarity between ketamine's acute psychotomimetic effects and psychosis [10], our ongoing studies are highly significant since the specific data generated with ketamine can equally contribute to the understanding and drug development of mood disorder and schizophrenia, devastating mental illnesses affecting millions of patients around the world [1].

3. **Research Plan:** Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths. Describe the setting in which the research will take place.

A. Overview: In this study, 32 healthy participants will be randomized into one of two parallel groups: (a) open-label ketamine and active lamotrigine, or (b) open-label ketamine with a matched-placebo control (i.e., sugar pill). All participants will complete a baseline rs-fMRI approximately 1-week prior to the ketamine infusion and a follow-up rs-fMRI 24-hours post-infusion. The subanesthetic dose of ketamine (0.23mg/kg bolus followed by 0.58mg/kg infusion over approximately 60 minutes) will be administered via intravenous infusion to occur during the MRS scan. Participants will be administered lamotrigine (300 mg oral dose) or the matched-placebo control about 2-hours prior to the start of the infusion.

B. Experimental Subjects: Twenty-four healthy subjects between the ages of 21-65 years will be recruited for this study through advertising and referral from clinics in the community. Subjects will complete an informed consent process and will be thoroughly screened for inclusion and exclusion criteria as described below.

B.1. Restrictions

Subjects will be advised not to drive or operate heavy machinery for at least 24-hours after completing the infusion.

Subjects will be asked to refrain from alcohol use for one-week prior to each MRS session.

Subjects must agree to use a barrier method (e.g., condom) as contraception for a 2-week period following the study drug.

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Subjects will be advised to refrain from undergoing any elective surgeries, including dental work, for a 2-week period following the study drug.

B.2. Discontinuation of subjects

Criteria for discontinuation

Subjects may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a subject from this study are:

- Voluntary discontinuation by the subject, who is at any time free to discontinue his/her participation in the study without prejudice to further treatment.
- Adverse reaction to ketamine or lamotrigine.
- Inability to complete MR studies due to claustrophobia or other.
- Stopping birth control or positive pregnancy test.
- Safety reasons as judged by the investigator.
- Severe non-compliance to protocol as judged by the investigator.
- Incorrect enrollment (i.e., the subject does not meet the required inclusion/exclusion criteria for the study).

Subject lost to follow-up.

A subject that discontinues will always be asked about the reason(s) for discontinuation and the presence of any adverse events. If possible, they will be seen and assessed by the investigator. Adverse events will be followed up as medically necessary.

C. Pre-Study Screening Procedure and Initial Assessments: After an initial phone screen to determine obvious exclusions from the study protocol, potential subjects will be invited for a clinic pre-screen and personally interviewed. All subjects will have an initial screening assessment that includes a complete medical history, physical examination, laboratory testing, HIV and Hep B & C testing, psychiatric history, and standardized psychiatric and cognitive assessments.

C.1. Historical and Demographic Information: Each subject will complete a demographic form containing commonly information that is routinely assessed in biomedical research protocols. Specifically, this document will elicit information about the subjects' demographics, including education level, socioeconomic status, race and ethnicity. In addition, it will also request information on the subject's family history of mental illness.

C.2. Screening Physical Exam and Laboratories: All subjects will have a standard physical examination at the time of the initial screening. Routine laboratory studies including a complete blood count (CBC) w/ differential, a comprehensive

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Table 1. Baseline measures utilized in this study

Baseline Assessments	Medical Assessments	Rating Scales
Demographic Data	Vital signs	BPRS
Psychiatric History	Physical examination	CADSS
Medical History	Mental status exam	PANSS
SCID	Laboratory Assessment	PSQI
MMSE	ECG	PSWQ
		VAS
		Cogstate Cognitive Tasks

Abbreviations: Structured Clinical Interview for DSM IV (SCID), Mini Mental Status Exam (MMSE), Electrocardiogram (ECG), Brief Psychiatric Rating Scale (BPRS), Clinician-Administered Dissociative States Scale (CADSS), Positive and Negative Syndrome Scale (PANSS), Pittsburgh Sleep Quality Index (PSQI), Penn State Worry Questionnaire (PSWQ), VAS (Visual Analog Scale of Mood States).

metabolic panel (e.g., BUN/creatinine, glucose, sodium, potassium, chloride, carbon dioxide, calcium, AST, ALT, fT4, TSH, bilirubin, total protein, VDRL, vitamin B12, folate, etc.), a complete lipid panel, electrolytes, HIV, hepatitis B and C. A urine sample will be taken and used for a urinalysis drug panel and pregnancy testing. All subjects will also complete an ECG. Care will be taken to exclude pregnant women. All female subjects will have a pregnancy test prior to participating in each MR study.

C.3. Screening Psychiatric Assessments: All subjects will have a psychiatric interview. In addition to the standard psychiatric and mental status examination, subjects will receive a structured diagnostic interview—the Structured Clinical Interview for DSM-IV Disorders (SCID). Baseline and follow-up ratings will also be obtained via assessment measures of depression, anxiety, and adverse effects (see Table 1).

D. Ongoing Clinical Assessments:

D.1. Medical Assessments: Physical examination (including vital sign determination) and clinical laboratories (discussed in greater detail previously) will be completed at the first visit. Urine toxicology screen and breathalyzer will be performed on the morning of each testing day, and the results will be determined before proceeding with the infusion. At the discretion of the study physician, the subject will be disqualified if urine toxicology results are positive for any substances of abuse or if his/her breathalyzer reveals recent alcohol intake. A pregnancy test will also be administered to all reproductive age females enrolled in the study prior to participation. Pregnant subjects will be excluded. In addition, subjects who test positive for HIV or viral hepatitis (hepatitis B and/or C) will be excluded as a means to protect the subjects and research staff from the increased potential risks blood borne pathogen transmission and ketamine infusion.

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D.2. Psychiatric Assessments: Ratings are performed by trained research personnel, whose performance is evaluated routinely. The primary investigator supervises the administration of clinical measures. Each of the psychiatric assessment instruments is briefly described below.

1. *Alcohol and Consumption Habits:* This is a brief measure that documents alcohol, caffeine, and nicotine habits.
2. *Behavioral Inhibition & Activation Scales (BIS/BAS):* The BIS/BAS is a self-report scale designed to assess dispositional sensitivity to the behavioral inhibition system (BIS) and the behavioral activation or behavioral approach system (BAS).
3. *Big Five Inventory (BFI):* The BFI measures an individual on the Big Five Factors (dimensions) of personality (Goldberg, 1993). Each of the factors is then further divided into personality facets.
4. *Brief Psychotic Rating Scale (BPRS):* The BPRS is a standardized instrument that contains scales assessing psychotic symptoms including positive and negative symptoms, activation and emotional distress.
5. *Clinician-Administered Dissociative States Scale (CADSS):* The CADSS has self and interviewer-administered items including 5 subscales, generated a priori, evaluating dissociation including altered environmental perception, time perception, spatial/body perception, derealization and memory impairment.
6. *Clinical Global Impressions Scale (CGI)* and the *Patient Global Impressions Scale (PGI):* The CGI and PGI are widely used instruments, which assess overall severity of illness on a 1 to 7 point scale with 1 indicating "normal, not at all ill" and 7 indicating "among the most extremely ill patients." These instruments also assess global improvement on a 1-to-7 point scale with 1 indicating "very much improved," 4 indicating "no change" and 7 indicating "very much worse."
7. *Clinician Administered Dissociative States Scale (CADSS):* The CADSS has self and interviewer-administered items including 5 subscales, generated a priori, evaluating dissociation including altered environmental perception, time perception, spatial/body perception, derealization and memory impairment.
8. *Cognitive tasks:* This will include Cogstate, a well-validated short computerized cognitive tasks. These short computerized tasks along with a brief neuropsychological battery will assess cognitive functions.
9. *Columbia-Suicide Severity Rating Scale (C-SSRS):* The C-SSRS is a brief clinician administered and standardized measure that uniquely assesses essential information about suicide behavior, ideation, lethality and severity, and distinguishes between suicidal occurrences and non-suicidal self-injury.
10. *Early Trauma Inventory (ETI-SR):* The ETI-SR is a self-report instrument to assess childhood trauma and includes physical, emotional and sexual abuse as well as general traumas.
11. *Global Perceived Early Life Stress (GPELS):* The GPELS is a self-report of perceived stress during childhood.
12. *Hamilton Anxiety Rating Scale (HAM-A):* The HAM-A is a standardized clinician-rated instrument to evaluate the severity of anxiety symptoms.
13. *Klein Loss Scale (KLS):* The KLS is a self-report of parental loss or separation during childhood.

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14. *Life Events Checklist (LEC):* The LEC is a self-report instrument that measures reports of traumatic life events.
15. *Massachusetts General Hospital Antidepressant Treatment History Questionnaire (MGH-ATRQ)*-This is a self-rated questionnaire used to determine treatment resistance in major depressive disorder.
16. *Mini Mental Status Exam (MMSE):* is a brief, clinician-administered instrument used to measure cognitive status and mental orientation. It will be given only at baseline.
17. *Modified Military Acute Concussion Evaluation (MACE)* - The MACE is a concussion screening tool for the acute assessment of service members involved in a potentially concussive event.
18. *Montgomery-Asberg Depression Rating Scale (MADRS):* The MADRS is a standardized instrument to ascertain depressed mood and neurovegetative signs and symptoms of depression.
19. *Penn State Worry Questionnaire (PSWQ):* The PSWQ is a self-report questionnaire to assess for 'worry' symptoms that are typical of generalized anxiety.
20. *Pittsburgh Sleep Quality Index (PSQI):* The PSQI is a self-report questionnaire to assess sleep quality and sleep disturbance.
21. *Positive and Negative Symptom Scale (PANSS):* The PANSS is commonly used to measure the severity of symptoms in psychotic disorders. It is a clinician-administered scale and includes three categories of symptoms: (1) positive symptoms, such as hallucination and delusion; (2) negative symptoms, such as flat affect and difficulty in abstract thinking; (3) general psychopathology, such as mannerisms and posturing.
22. *PTSD Checklist (PCL):* The PCL is used to measure PTSD symptoms and is a self-report questionnaire that has high reliability.
23. *Quick Inventory of Depressive Symptoms – Self-Report (QIDS-SR):* The QIDS-SR is a patient-rated depression instrument.
24. *Sheehan Disability Scale (SDS):* The SDS is a brief self-rated measure of disability and impairment.
25. *Beck Suicide Ideation Scale (BSI): The BSI is a 21-item self-report questionnaire that may be used to identify the presence and severity of suicidal ideation. Items on this measure also assess the respondent's suicidal plans, deterrents to suicide, and the level of openness to revealing suicidal thoughts.*
26. *Systematic Assessment for Treatment Emergent Events (SAFTEE):* The SAFTEE is a commonly used instrument originally developed by NIMH and adapted into a self-report instrument. It examines, in systematic fashion, possible treatment-emergent side effects and probes for specific adverse symptoms, including suicidal thoughts and behaviors, and self-injurious behavior.
27. *Structured Clinical Interview for DSM Disorders (SCID)* will be used to provide current and past diagnoses of psychiatric disorders, notably the inclusion/exclusion criteria of a current major depressive episode.
28. *Socio-demographic/General Information:* At intake, demographic data and medical history will be assessed with interviews and self-report forms that provide data on age, race, socioeconomic status, marital status, educational and

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occupational levels, and significant medical history. These are adapted from previous diagnostic and clinical studies at this center.

D.3. Biochemical Analyses: 10 mL of blood are drawn for the analysis of ketamine levels. Plasma ketamine levels are ascertained via a gas chromatography-mass spectroscopy assay developed by Edward Domino M.D. [95].

E. Lamotrigine Administration: Subjects randomized to the active lamotrigine group will receive a 300 mg oral dose of the drug 2-hours prior to the start of the ketamine-infusion. Lamotrigine levels will be measured using commercially available radioimmunoassay kits.

A physician who is trained and has experience with lamotrigine and possible allergic and other adverse events will remain in attendance at all sessions that involve drug administration.

F. Ketamine Administration: Subjects will complete one test day. Following an overnight fast, IV lines will be inserted into the antecubital vein. They will receive ketamine (0.23mg/kg bolus followed by 0.58mg/kg infusion over approximately 60 minutes).

A physician who is trained and has significant experience with both ketamine and infusion studies will remain in attendance at all sessions that involve ketamine infusion. The subjects will also be attended by a nurse who will accompany them through the study sessions, from the insertion of bilateral cannulae for drug infusion and blood sampling, through to recovery following ketamine infusion.

Baseline Test Session (Test Session 1 Day-0^{-})

- T -15 min: Urine toxicology; Urine pregnancy test (women only); Breathalyzer; MRI Safety Data Sheet; Metal Detector
- T -0 min: rs-fMRI (and other imaging protocols on the 3T scanner)
- Post fMRI Brief neuropsychological battery

Test session 1 (Day #1)

- T -120 min: Vital signs *; Urine toxicology; Urine pregnancy test (women only); Breathalyzer; PSWQ, PSQI, MRI Safety Data Sheet; Metal Detector; Lamotrigine or matched-placebo control administration
- T-90 min: Intravenous lines started
- T -45 min: MRS setup and baseline scan
- T-0 min: Ketamine infusion begins (0.23mg/kg bolus followed by 0.58mg/kg infused over 60 minutes)
- T 5-75: MRS acquisition continues

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Blood samples for ketamine, norketamine and lamotrigine levels will be repeated at 15, 30, 60, and 75 minutes **

- T 90: Vital signs, eat a meal, VAS, BPRS, CADSS, PANSS
- T 300: Participants may be discharged home after being cleared by a study physician.

*Blood pressure, heart rate, and oxygen saturation will be recorded every 10-20 minutes during infusion (T0-75 minutes).

Follow-up After Test Infusion (Day #2)

- T -15 min: Urine toxicology; Urine pregnancy test (women only); Breathalyzer; MRI Safety Data Sheet; Metal Detector
- T -0 min: rs-fMRI (and other imaging protocols on the 3T scanner)
- Post fMRI Brief neuropsychological battery

Follow-up (1-week, 4-weeks, 8-weeks):

All participants will be contacted by telephone 1-week, 4-weeks, and 8-weeks post-infusion and lamotrigine administration to assess for residual effects of ketamine or lamotrigine and other adverse events (AEs).

Table 2. Schedule of Study Events

Visit	***1- Baseline***	***2-fMRI***	***3-MRS / Infusion***	***4-24-hr fMRI***
Alcohol & Consumption	*x*			
BFI	*x*			
Blood draw/labs	*x*			
BIS/BAS	*x*			
BSI		*x*	*x*	*x*
Breathalyzer	*x*	*x*	*x*	*x*
CADSS			*x*	
CGI-S/I and PGI-S/I	*x*			
Collect AEs and SAEs		*x*	*x*	*x*
Cognitive Tasks	*Practice*	*x*	*x*	*x*
Concomitant Meds	*x*	*x*	*x*	*x*
C-SSRS	*x*			
Demographics	*x*			
ECG	*x*			
ETI-SR	*x*			
GPELS	*x*			
HAM-A	*x*	*x*	*x*	*x*
*Informed Consent	*x*			
Ketamine Infusion			*x*	

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KLS	*x*			
LEC	*x*			
MADRS	*x*	*x*	*x*	*x*
M-MACE	*x*			
MGH-ATRQ	*x*			
Medical Evaluation	*x*			
PANSS			*x*	
PCL	*x*	*x*	*x*	*x*
PSQI	*x*	*x*	*x*	*x*
PSWQ	*x*	*x*	*x*	*x*
QIDS-SR	*x*	*x*	*x*	*x*
Lamotrigine/Placebo Dose			*x*	
SAFTEE			*x*	
SCID-IV	*x*			
SDS	*x*			
Serum Pregnancy	*x*			
Urinalysis	*x*	*x*		
Urine Pregnancy		*x*	*x*	*x*
Vital Signs	*x*		*x*	

*Informed consent will be obtained prior to any study procedure.

Abbreviations: Big Five Inventory (BFI), Behavioral Inhibition & Activation Scales (BIS/BAS), Clinician Administered Dissociative States Scale (CADSS), Clinical Global Impression Severity/Improvement (CGI-S/I), Patient Global Impression-Severity/Improvement (PGI-S/I), Cogstate and brief neuropsychological battery comprises the cognitive testing portion, Columbia-Suicide Severity Rating Scale (C-SSRS), electrocardiogram (ECG), Early Trauma Inventory – Self Report (ETI-SR), Global Perceived Early-Life Stress (GPELS), Hamilton Anxiety Rating Scale (HAM-A), Klein Loss Scale (KLS), Life Events Checklist (LEC), Montgomery-Asberg Depression Rating Scale (MADRS), Modified Military Acute Concussion Evaluation (M-MACE), MGH Antidepressant Treatment Response Questionnaire (MGH-ATRP), Positive and Negative Symptom Scale (PANSS), Pittsburgh Sleep Quality Index (PSQI), Penn State Worry Questionnaire (PSWQ), Quick Inventory of Depressive Symptomatology – Self Report (QIDS-SR), Systematic Assessment for Treatment Emergent Events (SAFTEE), Structured Clinical Interview for DSM IV (SCID), Sheehan Disability Scale (SDS).

^{1}H-MRS Methods: ^{1}H-MRS will be used to measure glutamate concentrations in the frontal cortex using tissue segmentation processing to control for difference in tissue composition between subjects. Participants may be asked to return for one repeat session due to inadequate image acquisition or time constraints. This repeat session would be scheduled a minimum of 4-weeks after the original session to minimize carry-over effects and to minimize any potential risk from repeat dosing of ketamine or lamotrigine. No more than one repeat session would be conducted. A brief description of the methods is provided below.

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An 8-channel RF array (7.0 T magnet) will be used for RF transmission and signal reception. In the frontal cortex, the volume of primarily gray matter will be selected as a rectangular box placed across the anterior cingulate. The frontal white matter voxel will have identical dimensions offset from the midline. The sequence will be applied in pairs [96] to yield sub-spectra that contain either macromolecules alone or macromolecules combined with the metabolites of interest. Briefly, one sub-spectrum is acquired with a hyperbolic secant inversion pulse applied across the spectral bandwidth, followed by an inversion-recovery delay to null the metabolites, and one sub-spectrum is acquired without the inversion pulse. The macromolecules will be measured directly from the metabolite-nulled sub-spectrum. The macromolecule sub-spectrum will be subtracted from the other sub-spectrum to obtain a spectrum of metabolites alone. The data will be acquired in interleaved fashion, toggling between individual inverted and uninverted acquisitions, in short blocks. Each block will be stored, and the sequence will run for about 20-minutes to yield pairs of sub-spectra. Experience has shown that over 90% of subjects are able to lie still and tolerate this duration of scan. A spectrum of the water in the voxel will be obtained using the same pulse sequence and TE with the water suppression disabled. Spectral fitting will be performed to quantify the metabolites, and all metabolite measurements will be reported not only based on comparisons to creatine but as ratios to the tissue water peak.

Magnetic *Resonance Imaging:* Scans will be collected on a Siemens TIM Trio 3.0 Tesla MRI scanner located at the Yale Magnetic Resonance Research Center (MRRC), using standard MRRC sequences. The scan protocol consists of a 3-plane localizer collected from three cardinal planes. These images serve as a localizer series for the other acquisitions. The remaining multi-modal magnetic resonance pulse sequences may include: two high resolution T1-weighted imaging (MPRAGE or FLASH); high resolution T2-weighted imaging (SPACE); three-dimensional chemical shift imaging (CSI) provides high signal to noise 1H spectra; BOLD gradient echo planar imaging at rest and/or during FMRI tasks (e.g. trauma-related cues); high angular diffusion weighted imaging, arterial spin labeling (ASL), susceptibility weighted imaging, and ultra-high resolution T1 anatomical imaging (400 micron x 400 micron x 800 micron). FMRI tasks will be back-projected onto a screen by a high contrast projector connected to a laptop computer. Behavioral responses will be recorded during FMRI tasks with an optical response system. During the MRI scan, participants will be asked to perform certain tasks. They will be given detailed instructions by the experimenter explaining the task instructions. During some of the cognitive tasks they may see various distracting images some of which will be photographs. These photographs that they will see may cover a broad range of contents, including slides of flowers, children, families, people's faces, insects, animals, sports, and graphic slides similar to those that might be seen in a newscast or documentary of war footage (including mutilated individuals and dead bodies). Many people experience some degree of distress when viewing the most graphic pictures. If they will be shown these graphic images they will have an opportunity to view some sample pictures to make certain that they understand the types of slides they will see during the study and the level of intensity that the pictures may contain. They will be able to choose if they are comfortable with each level of intensity. If they agree to look at the pictures and become upset during the scanning they may indicate their distress and the pictures will be stopped. We will remind them that they may ask to stop

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viewing the pictures at any time during the testing and still continue in the study. Participants may be asked to return for a repeat session due to inadequate image acquisition or time constraints. No more than three repeat sessions will be attempted.

4. Genetic Testing **N/A** ☒

A. Describe

i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned
ii. the plan for the collection of material or the conditions under which material will be received
iii. the types of information about the donor/individual contributors that will be entered into a database
iv. the methods to uphold confidentiality

B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects?
C. Is widespread sharing of materials planned?
D. When and under what conditions will materials be stripped of all identifiers?
E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials?
    i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)?

F. Describe the provisions for protection of participant privacy
G. Describe the methods for the security of storage and sharing of materials

5. **Subject Population:** Provide a detailed description of the types of human subjects who will be recruited into this study.

Twenty-four healthy subjects, between the ages of 21-65 will be recruited. Subjects will be accepted into the protocol after an opportunity to review and provide voluntary written informed consent (see attached) and completion of an extensive medical and psychiatric history, physical examination, mental status examination and routine laboratory assessments. Participants will be enrolled if they meet the inclusion criteria mentioned below (see section 7). Additional demographic, diagnostic and other clinical data, including family psychiatric and substance misuse history and treatment history will be obtained during the face-to-face clinical assessment. All such data will be recorded in written and/or electronic format(s).

6. Check off all classifications of subjects that will be <u>specifically recruited for enrollment</u> in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

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☐ Children ☒ Healthy ☐ Fetal material, placenta, or dead fetus
☐ Non-English Speaking ☐ Prisoners ☐ Economically disadvantaged persons
☐ Decisionally Impaired ☐ Employees ☐ Pregnant women and/or fetuses
☒ Yale Students ☒ Females of childbearing potential

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects? ☐ Yes ☒ No (If yes, see Instructions section VII #4 for further requirements).

For the current study, healthy subjects are needed to investigate the effect of ketamine on functional connectivity and the impact of lamotrigine. An extensive medical and psychiatric assessment, as described above, will be conducted to exclude subjects who are at increased risk. Yale students are eligible for this study if they meet the inclusion and exclusion criteria. Yale students who are supervised/mentored by members of the research team will not be enrolled.

Females of childbearing potential will likely be enrolled, and, as mentioned above, safeguards will be instituted, e.g. serum pregnancy test and reproductive counseling, to prevent pregnant patients from enrolling or subjects conceiving, respectively, during this investigation.

7. What are the criteria used to determine subject inclusion or exclusion?

Inclusion Criteria:

- Male or female between the ages of 21-65 years. Females will be included if they are not pregnant and agreed to utilize a barrier method contraceptive (e.g., condom or diaphragm with spermicide) tubal ligation, abstinence, or partner with vasectomy) or if post-menopausal for at least 1 year, or surgically sterile.
- Able to provide written informed consent according to Yale HIC guidelines.
- Agree to refrain from elective surgeries (including dental) for a 2-week period following study drug.
- Able to read and write English as a primary language.

Exclusion Criteria:

- Personal history of mood, anxiety, or psychotic axis I DSM-IV disorders confirmed after comprehensive psychiatric evaluation.
- Any history of serious medical or neurological illness.
- Any signs of major medical or neurological illness on examination or as a result of ECG screening or laboratory studies.
- A first-degree family member with history of schizophrenia.
- Lifetime history of psychoactive substance or alcohol dependence or substance or alcohol abuse (other than nicotine or caffeine abuse), or drinking more that 5 drinks/week during the last year.

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- Abnormality on physical examination. A subject with a clinical abnormality may be included only if the study physician considers the abnormality will not introduce additional risk factors and will not interfere with the study procedure (e.g. uncontrolled hypertension, hyperthyroidism, or hypothyroidism will be excluded).
- A positive pre-study (screening) urine drug screen or, at the study physician's discretion on any drug screens given before the scans.
- Pregnant or lactating women or a positive urine pregnancy test for women of child-bearing potential at screening or prior to any imaging day.
- Positive HIV or Hepatitis B/C tests. This test will take place at the screening visit. Subjects will be invited back either for their next study visit or for a HIV/Hep debriefing session. A study clinician will inform them in person of the results. They will be given access to counselling and advised of the appropriate next steps.
- Has received either prescribed or over-the-counter (OTC) centrally active medicine or herbal supplements within the week prior to the MRI scan. Subjects who have taken OTC medication or herbal supplements may still be entered into the study, if, in the opinion of the principal/co-investigator, the medication received will not interfere with the study procedures or compromise safety.
- Any history indicating learning disability, mental retardation, or attention deficit disorder.
- Known sensitivity to ketamine.
- Known sensitivity to lamotrigine.
- Body weight of 250 pounds or greater.
- History of claustrophobia.
- Presence of cardiac pacemaker or other electronic device or ferromagnetic metal foreign bodies in vulnerable positions as assessed by a standard pre-MRI screening questionnaire.
- Donation of blood in excess of 500 mL within 56 days prior to dosing.
- History of sensitivity to heparin or heparin-induced thrombocytopenia.
- Resting blood pressure lower than 90/60 or higher than 150/90, or resting heart rate lower than 45/min or higher than 100/min.

8. How will **eligibility** be determined, and by whom?

Subject eligibility will first be assessed via telephone screening. If the subject seems to be a likely candidate for inclusion in this protocol, he or she will be evaluated for study eligibility in person. Telephone screens will occur by experienced research personnel adept with this process. Suitability for enrollment and randomization will be assessed by experienced study clinicians, via face-to-face evaluations and discussion with research teams.

9. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

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The risks involved with this study include (a) ketamine administration; (b) lamotrigine administration; (c) phlebotomy; (d) MR studies; and (e) clinical assessments.

A. Ketamine administration

Ketamine is a dissociative anesthetic that has been used clinically since the late 1960s [95]. Despite extensive experience, there is no clear and compelling evidence of long-term toxicity associated with ketamine administration in medically supervised settings [97,98]. However, there are acute medical and neuropsychiatric sequelae that deserve special consideration. The ketamine doses employed in the healthy group have been specifically selected to produce mild to moderate behavioral effects without significant sedation. At these doses, ketamine produces a transient alteration of consciousness including altered sensory processing, and thought processes. Initially, subjects frequently feel "drunk" and giddiness is common. As blood levels increase, blood pressure and heart rate increase moderately. This increase is transient and not considered clinically significant. Subjects report differences in complex problem solving evident on frontal lobe and delayed memory tasks, some subjects may report a narrowing of their concentration, feeling distant from surroundings, and enhanced perception of some sensory stimuli. Subjects sometimes report blurred vision and nystagmus. Alterations in the perception of time, body boundaries, and illusions occur. Subjects may experience visual distortions, altered perception of orientation in space, and inability to control thought processes. Subjects may report feeling quite distanced from their surroundings, describe altered awareness of their bodies, and they may close their eyes. During this period, they are still oriented to time and place. They can complete ratings scales testing memory without impairment, their rate of finger tapping is unchanged and the latency of their response on continuous performance tasks of attention is not increased. However, some individuals feel that they cannot control the experience and find it frightening. Vivid dreams and poor sleep quality after infusion of ketamine has also been reported, although dream content was not necessarily unusual and alterations in sleep were not reported on subsequent nights.

The doses used in this protocol produce blood levels that are 1/6 to 1/3 of those produced clinically when ketamine is used as an anesthetic. Short-term safety data showed that adverse events in response to ketamine infusion have been mild and transient, with no evidence of any clinically significant or persistent adverse effects [109]. Adverse events included nausea and vomiting, sedation, anxiety, hypotension, insomnia and nightmares and transient pain in the infusion arm.

B. Lamotrigine

Lamotrigine is an anticonvulsant or anti-epileptic medication. It is also used to delay mood episodes in adults with bipolar disorder. Long-term administration of lamotrigine has been associated with a rash [99]. This rash can be quite serious and require hospitalization and discontinuation of treatment. However, single doses of the drug have not been associated with this side effect and in studies that have used this in conjunction with ketamine, no participants developed rash [12]. There are currently no known factors identified to predict the risk of occurrence or the severity of the rash

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caused by lamotrigine. Nearly all cases of the rash have occurred within 2- to 8-weeks of treatment initiation. Possible allergic reactions to lamotrigine include: hives fever, swollen glands, painful sores in or around the eyes and mouth, difficulty breathing, swelling of the face, lips, tongue, or throat. Possible side effects include worsening mood or behavior changes, depression, anxiety, agitation, irritability, thoughts of suicide and self-harm, restlessness, hyperactivity, dizziness, drowsiness, blurred vision, loss of coordination, fatigue.

C. Phlebotomy

In addition to the IV lines, we may need to draw additional blood for the routine laboratory testing. Inserting a needle into a vein is safe when done by professionals under clean conditions. Sometimes a bruise will occur at the puncture site and on rare occasions a blood clot or an infection may form in the vein. During the intravenous line placement for the infusion, a bruise may occur at the puncture site, and very rarely, an infection may develop. If this occurs, appropriate treatment will be instituted immediately.

Risks associated with blood loss are minimal. Less than 200 cc will be drawn, and this represents 40% of a 500 cc blood donation.

D. MR Studies

Magnetic resonance imaging (MRI) and spectroscopy (MRS) will be performed at the same time at Yale Medical School, Magnetic Resonance Research Center in both a 3T (fMRI) and 7T (MRS) system. The methods used to obtain the MRI are employed for ^{1}H-spectroscopy with the addition of water signal suppression. MRI may be considered ^{1}H-MRS of water.

Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines.

Subjects will be watched closely throughout the MR study. Some people may feel uncomfortable or anxious. If this happens to a subject, they may ask to stop the study at any time and we will take them out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly but subjects are instructed to tell the research staff if they have them. fMRI Task: During the MRI scan, participants will be asked to perform certain tasks. They will be given detailed instructions by the experimenter explaining the task instructions. During some of the cognitive tasks they may see various distracting images some of which will be photographs. As in the behavioral assessment portion, the photographs that they will see may cover a broad range of contents, including slides of flowers, children, families, people's faces, insects, animals, sports, and graphic slides similar to those that might be seen in a newscast or documentary of war footage (including mutilated individuals and dead bodies). Many

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people experience some degree of distress when viewing the most graphic pictures. If they will be shown these graphic images they will have an opportunity to view some sample pictures to make certain that they understand the types of slides they will see during the study and the level of intensity that the pictures may contain. They will be able to choose if they are comfortable with each level of intensity. If they agree to look at the pictures and become upset during the scanning they may indicate their distress and the pictures will be stopped. We will remind them that they may ask to stop viewing the pictures at any time during the testing and still continue in the study.

There are some risks with an MR study for certain people. Applicants may be excluded if they have a pacemaker or some metal objects inside their body because the strong magnets in the MR scanner might harm them. Another risk is a metallic object flying through the air toward the magnet and hitting the subject. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. Subjects will walk through a metal detector prior to entering the magnet room. Nothing metal can be brought into the magnet room at any time. Also, once the subject is in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet.

Subjects are instructed to read and answer very carefully the questions on the MR Safety Questionnaire related to their personal safety. They are instructed during the consent process to take a moment to be sure that they have read the MR Safety Questionnaire and to be sure to tell the staff any information they think might be important. This MR study is for research purposes only and is not in any way a clinical examination. The scans performed in this study are not designed to find abnormalities. The primary investigator, the lab, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a diagnostic evaluation of the images. If a worrisome finding is seen on a subject's scan, a radiologist or another physician will be asked to review the relevant images. Based on his or her recommendation (if any), the primary investigator or consulting physician will contact the subject, inform them of the finding, and recommend that they seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie solely with the subject and their physician. The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that the subject receives based on these findings. The images collected in this study are not a clinical MR exam and for that reason, they will not be made available for diagnostic purposes.

E. <u>Psychiatric Evaluation, Clinical Assessments:</u>

These are all non-invasive, should add no risk, and have been used without difficulty or adverse events in our previous studies with a similar population. The major disadvantage is the time taken to complete them. The psychiatric evaluation and clinical assessments included in the baseline-screening visit are expected to take approximately 60-90 minutes to complete (this includes all clinician-administered interviews and ratings and all self-report measures). The ratings on visit day 2 are expected to require approximately 30 minutes. The measures given on the day of the infusion are expected

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to require approximately 45-60 minutes to complete (again including all clinician-administered and self-report ratings. Our past experience with these measures indicates that they are acceptable to patients. Only subjects' code numbers will be recorded on the forms themselves to protect confidentiality.

10. **Minimizing Risks:** Describe the manner in which the above-mentioned risks will be minimized.

A. Recruitment and Informed Consent

Subjects will be recruited from the Yale-New Haven area by advertisements on the Internet and radio, in local newspapers, posters in the area, and referrals from community clinicians. Prior to enrollment, subjects will have face-to-face interviews with one of the investigators where the nature of the project, the risks, the benefits, and the alternatives to participation in the project will be discussed with the subject and, if necessary, the subject's family. A focused history will be taken and a checklist of hazards will be reviewed with the subject. If, following these discussions, the subject continues to be interested in the project, informed written consent will be obtained on the consent form approved by the Yale University Human Investigation Committee (HIC). Thereafter, the project investigators assume clinical responsibility for the care of the subject.

B. Protection Against Risk

General risk-minimizing strategies: **1.** Effective screening to exclude subjects who would be placed at a greater risk. This includes a comprehensive psychiatric and medical evaluation, physical examination, and the screening studies performed before starting studies (see above). Trained staff, under the supervision of the PI, will conduct all screening procedures. **2.** The investigator or a designated person will explain the benefits and risks of participation in the study to each subject. Subjects will be asked to verbalize their understanding of all aspects of the consent, including risks, benefit and alternatives. The voluntary nature of research studies is always emphasized. **3.** All subject information will be kept confidential and only members of the investigative team with appropriate IRB/HIC and HIPAA training will have access to the study data. Data will be maintained and secured in locked file cabinets or password protected electronic media. A numbering code will be used to assign a unique identifier to each subject. **4.** A Data and Safety Monitoring Plan is described below.

A primary concern is impact by magnetic objects accidentally brought into the magnet room, anxiousness, irritation due to the IV blood sampling, and perceptual disturbances during ketamine infusion. The precautions taken to minimize the risks in these studies are described below:

C. Metal Object

To minimize the chance of a magnetic object entering the magnet room the following precautions are taken:

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1. All personnel involved in the study must go through training provided by the MRRC.
2. The subject is screened in the MRRC for metal objects prior to being allowed into the magnet room
3. Once the subject is in the room only the research team members who have successfully completed the MRRC safety requirements will be allowed into the room.

If an accident occurs, 911/EMS will be activated. All personnel are trained in an established procedure for removing the subject from the magnet. Due to the close proximity to the hospital the time required for the ambulance team to arrive will be under 5 minutes. All personnel involved in the study are trained in this procedure. There will be an M.D. present at all times who will be able to perform immediate resuscitation in the unlikely event it is needed.

D. Subject Monitoring and Emergency Procedures

An experienced clinical research team will closely monitor the subjects. All of the information obtained from subjects participating in this study will be coded by numbers and kept in locked files in the research unit to ensure confidentiality. One member of the two to three person team will watch the subjects while in the magnet room. Visual and verbal contact will be maintained throughout the imaging and spectroscopy. Subjects who become claustrophobic in the magnet will be removed immediately from the magnet room. Subjects who experience distress in the magnetic room for any reason including seizures, loss of consciousness, pain will be removed from the magnet room in accordance with well-established procedures developed for the MRRC. All team members will be thoroughly familiar with the established emergency plan. A nonmagnetic stretcher will be on standby next to the entrance to the magnet. Nonmagnetic oral airways, Ambubag, and stethoscope are stored with the emergency stretcher in the magnet room. Should an emergency arise, one member of the spectroscopy team will telephone the 911/EMS operator and have an ambulance with personnel trained in resuscitation sent to the MRRC. The other members will remove the patient via stretcher from the magnet room taking the patient to the part of the MRRC designated for cardiopulmonary resuscitation. If immediate resuscitation is needed the physician present during the study will perform this procedure.

C. Anxiousness

If a subject is anxious and expresses a desire to leave the magnet then the study will be immediately terminated.

E. IV Irritation

If there is irritation due to IV blood sampling, the physician present during the study will examine the subject, and the study will be terminated if the subject is in pain.

G. Effects of ketamine

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Our group here at Yale pioneered ketamine studies in healthy subjects and in depression, and has been performing these studies since early 1990s. Before participating, healthy subjects undergo careful psychiatric and medical evaluation. A research nurse and study physician are available at all times during test sessions to provide support and consistent "reality testing" for individuals experiencing confusion or transient psychosis. If a subject reports that symptoms cannot be tolerated, the ketamine infusion will be stopped.

Subjects will be observed for at least an hour after the termination of testing, and if intolerable physical or behavioral symptoms persist, subjects will be admitted to the Clinical Neuroscience Research Unit at the Connecticut Mental Health Center for further observation and overnight if necessary. Participants will be informed that they may not drive or operate machinery for 24 hours after end of test day procedures, and study staff will ensure that they are picked up by a responsible adult or safely reach their home on alternate transportation. Subjects will be provided a number to call to reach an on-call research psychiatrist (24-hours/day) should unpleasant effects occur after subjects have left the testing facility. Medication effects will be reviewed and "debriefed" with subjects following each test day by a study clinician. If necessary, subjects will be administered oral diazepam to reduce residual symptoms and their participation in the study will be terminated if necessary. Such subjects will be monitored as found appropriate by the study physicians.

We are also taking a number of precautions to help reduce the chance of having an unpleasant response to ketamine or to reduce the severity of any lingering medication effects. These precautions include:

1) A physician who is trained and has significant experience with ketamine infusion studies will remain in attendance at all sessions ketamine infusion and will be responsible for the infusions. The subjects will also be attended by a research nurse who will accompany them through the study sessions, from the insertion of bilateral cannulae for drug infusion and blood sampling, through to recovery following ketamine infusion.
2) Medications are available (Valium) to relieve distress related to the behavioral effects of ketamine.
3) We will ask the subject to remain at the Yale MRRC recovery room for several hours after the behavioral effects of ketamine should have worn off.
4) We will review the test day with the subjects to deal with their feelings and reactions to each test day before they leave.
5) We will ask the subject to contact us at any time if any unpleasant effects occur.
6) We will ask the subject not to engage in demanding work in the day following the test sessions and we will work with the subject to schedule the test days accordingly.
7) If the subject has any lingering medication effects, such as sedation, we will terminate the remaining test days and work with him/her until these side effects have resolved.

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8) If the subject develops psychiatric symptoms, we may admit him/her to the hospital. This may be involuntary if he/she is in danger of harming yourself or others.
9) We will also contact the subject at 1-week, 4-weeks, and 8-weeks after the ketamine infusion and possible lamotrigine administration to assess for any concerns, side effects, or other AEs.

H. Effects of lamotrigine: Although a single dose of lamotrigine is expected to produce minimal to no side effects[12,13], participants will be informed of potential side effects and will be asked to report any emerging symptoms. Adverse events will be recorded on the test day and during follow-ups.

11. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.) For more information, see the Instructions, page 24.
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study?

The investigator's assessment of the overall risk for subjects participating in this study is moderate.

    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study?

    Not applicable.

    c. Copy, paste, and then tailor an appropriate Data and Safety Monitoring Plan from http://www.yale.edu/hrpp/forms-templates/biomedical.html for
        i. Minimal risk
        ii. Greater than minimal/moderate risk
        iii. High risk

    d. For multi-site studies for which the Yale PI serves as the lead investigator:
        i. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed?
        ii. What provisions are in place for management of interim results?
        iii. What will the multi-site process be for protocol modifications?

Moderate Risk DSMP

1. Personnel responsible for the safety review and its frequency:

The principal investigator, Chadi Abdallah, M.D., will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified

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frequency which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. Either the principal investigator or the HIC have the authority to stop or suspend the study or require modifications.

2. The risks associated with the current study are deemed moderate for the following reasons:

1. We do not view the risks associated with ketamine as minimal.

2. We do not view the risks associated with the combined use of ketamine and ^{1}H-MRS imaging as minimal

3. Given the now established safety and validity of the current research of ketamine in our prior work, we do not view the proposed study as high risk.

Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods.

Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator, Chadi Abdallah, M.D., according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).

b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).

c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).

d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).

e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

4. Plan for Grading Adverse Events:

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The following scale will be used in grading the severity of adverse events noted during the study:

1. Mild adverse event

2. Moderate adverse event

3. Severe

5. Plan for Determining Seriousness of Adverse Events:

Serious Adverse Events:

In addition to grading the adverse event, Dr. Abdallah will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it:

1. is life-threatening

2. results in in-patient hospitalization or prolongation of existing hospitalization

3. results in persistent or significant disability or incapacity

4. results in a congenital anomaly or birth defect OR

5. results in death

6. based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition, or

7. adversely affects the risk/benefit ratio of the study

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the HIC or HSC is necessary.

6. Plan for reporting serious AND unanticipated AND related adverse events anticipated adverse events occurring at a greater frequency than expected, and other unanticipated problems involving risks to subjects or others to the HIC

The investigator will report the following types of adverse events to the HIC:

a) serious AND unanticipated AND possibly, probably or definitely related events;

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b) anticipated adverse events occurring with a greater frequency than expected; and c) other unanticipated problems involving risks to subjects or others.

These adverse events or unanticipated problems involving risks to subjects or others will be reported to the HIC or HSC within 48 hours of it becoming known to the investigator, using the appropriate forms found on the website.

7. Plan for reporting adverse events to co-investigators on the study, funding and regulatory agencies

For the current study, the following individuals, funding, and/or regulatory agencies will be notified:

□ All Co-Investigators listed on the protocol.

The principal investigator, Chadi Abdallah, M.D., will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

12. **Statistical Considerations:** Describe the statistical analyses that support the study design.

The sample size (n=32) was selected based on previous reports[13]. fMRI processing and GBC analyses will be conducted as previously reported[100]. A paired t-test will be conducted to examine the effects of ketamine on GBC (Aim 1). A linear mixed model will examine the effects of ketamine and ketamine-by-lamotrigine interaction on cortical glutamate levels. Delta glutamate (during infusion minus baseline) will be correlated with delta GBC (post infusion minus baseline) (Aim 2). A t-test will compare delta GBC between the lamotrigine and placebo groups (Aim 3). Similar analyses will be conducted to examine the effects of ketamine and lamotrigine on the secondary outcomes, i.e. gln level, gaba level, cortical thickness, subcortical ROIs volumes, fractional anisotropy and mean diffusion, and amygdala activation.

SECTION VI: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS AND DEVICES

If this section (or one of its parts, A or B) is not applicable, state N/A and delete the rest of the section.

A. DRUGS, BIOLOGICS and RADIOTRACERS

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1. **Identification of Drug, Biologic or Radiotracer:** What is (are) the **name(s)** of the drug(s)
biologic(s) or radiotracer(s) being used? Identify whether FDA approval has been granted and for what indication(s).

I. Ketamine has U.S. Food and Drug Administration (USFDA) approval as the only anesthetic for diagnostic and surgical procedures that do not require skeletal muscle relaxation. Ketamine is also indicated for anesthesia induction prior to the administration of other general anesthetic agents or to augment low potency anesthetics such as nitrous oxide.

II. Lamotrigine has U.S. Food and Drug Administration (USFDA) approval as an anticonvulsant. It is also indicated for use in bipolar disorder.

All protocols which utilize a drug, biologic or radiotracer **not** approved by, but regulated by, the FDA, or a radiotracer regulated by the RDRC, must provide the following information:

a. What is the Investigational New Drug (IND) **number** assigned by the FDA?

Not applicable.

b. Who holds the IND?

Not applicable.

c. All protocols which utilize a radiotracer not approved by, but regulated by the FDA must provide the IND number: _____
Alternatively, use of the investigational radiotracer may be under RDRC/RSC oversight: (check if appropriate)_____

For all investigational radiotracers, attach a copy of the RDRC/RSC application (for radioisotopes used in the PET Center, PET Center personnel may complete this step) Go to http://rsc.med.yale.edu/login.asp?url=myApps.asp. When you have logged in, complete the application and attach a copy to this submission.

Alternatively, an **exemption from IND filing requirements** may be sought for a clinical
investigation of a drug product that is lawfully marketed in the United States. If there is no IND and
an exemption is being sought, review the following categories and complete the category that applies
(*and delete the inapplicable categories)*:

Exempt Category 1
The clinical investigation of a drug product that is lawfully marketed in the United States can be

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exempt from IND regulations if all of the following are yes:

i. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug. ☒ Yes ☐ No

ii. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the product. ☒ Yes ☐ No

iii. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. ☒ Yes ☐ No

iv. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56). ☒ Yes ☐ No

v. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs. ☒ Yes ☐ No

Exempt Category 2 (all items i, ii, and iii must be checked to grant a category 2 exemption)

☐ i. The clinical investigation is for an *in vitro* diagnostic biological product that involves one or more of the following (check all that apply):

- ☐ Blood grouping serum
- ☐ Reagent red blood cells
- ☐ Anti-human globulin

☐ ii. The diagnostic test is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure; and

☐ iii. The diagnostic test is shipped in compliance with 21 CFR §312.160.

Exempt Category 3

☐ The drug is intended solely for tests in vitro or in laboratory research animals if shipped in accordance with 21 CFR 312.60

Exempt Category 4

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☐ A clinical investigation involving use of a placebo if the investigation does not otherwise require submission of an IND.

2. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

I. Ketamine has been administered to over 10,000 patients and reported in greater than 100 individual research investigations, including many (as previously cited) from our department. Ketamine has a wide margin of safety and is typically provided in doses of 1-4.5 mg/kg IV over 1 minute as a sole anesthetic agent. In the majority of psychiatric investigations in depressed subjects to date, ketamine has been administered at subanesthetic doses (typically 0.5mg/kg infused over 40 minutes). In healthy subjects, the typical subanesthetic doses used are about 0.23 mg/kg bolus of over 1 minute followed by 0.58 mg/kg/hour infusion (e.g. Refs [12,101-105]; Also, ongoing investigations include: HIC 1111009365, HIC 0908005593, HIC 1009007439). Extensive experience suggests that ketamine administration is not associated with long-term toxicity. The doses of ketamine administered produce blood levels 1/3 - 1/6 those associated with blood levels achieved when ketamine is used as a primary surgical anesthetic. Since 1989, studies at our department administered ketamine on over 800 occasions to over 300 subjects. Our group has extensive experience using ketamine in psychiatric research.

In healthy subjects: In addition to the recently found rapid-acting antidepressant effect, ketamine has been extensively used in human research as a model of psychosis owing to its acute psychotomimetic and cognitive adverse effects [106]. In healthy volunteers, subanesthetic doses of ketamine induce transient perceptual disturbances, dissociative experiences, cognitive dysfunction, affective changes, thought disorder, and mild physical symptoms [107]. These studies showed dose-dependent effects with increased symptoms at higher doses [10,108,109]. However, the symptoms were transient, starting within minutes of ketamine infusion and lasting up to 2-hours [10]. Recently, Perry and colleagues at Yale reviewed a dataset of 450 healthy subjects who collectively received a total of 833 ketamine infusions in a medically supervised setting as part of laboratory studies [97]. This report noticed no evidence of serious adverse events, residual sequelae, or sensitization. Only 2% of subjects experienced non-serious adverse reactions. These emerging side effects resolved by the end of the test day in all subjects, except for one participant who experienced symptoms for 4-days [97].

II. Lamotrigine:

As noted above, lamotrigine is an anticonvulsant drug. It is also used in the treatment of bipolar disorder, specifically in the treatment and prevention of bipolar depressive episodes [78]. Lamotrigine is also believed to act on serotonin reuptake, which may contribute to its antidepressant effects [79,81]. There is evidence of

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peripheral glutamate dysregulation in bipolar disorder [82], and the glutamatergic activity of lamotrigine may also be implicated in its therapeutic and neuroprotective effects. Lamotrigine levels have been shown to peak at 1 to 4 hours after oral administration, with a mean of 2 hours.

Several studies have found that lamotrigine reduced the neuropsychiatric effects of ketamine in healthy subjects [12] as well as preventing some BOLD signal changes in a neuroimaging study [13]. In a pioneering Yale study exploring the impact of lamotrigine on ketamine's influence on glutamate, healthy subjects were given 300 mg of lamotrigine approximately two hours prior to a ketamine infusion (0.25 mg/kg by intravenous bolus and 0.65 mg/kg per hour by intravenous infusion). Those subjects who received lamotrigine demonstrated significantly decreased ketamine-induced perceptual disturbances as assessed by the Clinician Administered Dissociative States Scale, as well as reduced positive and negative psychotomimetic symptoms as assessed by the Brief Psychotic Symptoms Scale, and reductions in learning and memory impairment. Of note, those participants who received lamotrigine showed increases in the rapid mood enhancing effects of ketamine [12]. In another study hoping to identify the mechanisms of action of ketamine in inducing symptoms and to determine the role of increased glutamate release in these alterations, participants were pretreated with lamotrigine, prior to a ketamine infusion to examine which effects of ketamine appear to be mediated by increased glutamate release [13]. They found that ketamine administration induced a rapid and focal decrease in the ventromedial frontal cortex, including the orbitofrontal cortex and subgenual cingulate, which strongly predicted its dissociative effects. They also noted increased activity in the mid-posterior cingulate, thalamus, and temporal cortical regions. They found that lamotrigine pre-treatment prevented many of the BOLD signal changes [13]. In the current proposal, we want to explore further the impact of lamotrigine on ketamine-induced changes in rs-fMRI and the relationship between alterations in glutamate levels and rs-fMRI changes.

3. **Source:** a) Identify the source of the drug or biologic to be used.

I. Ketamine will be obtained from the Yale New Haven Hospital Investigational Drug Service.

II. Lamotrigine will be obtained from the Yale New Haven Hospital Investigational Drug Service.

b) Is the drug provided free of charge to subjects? ☒ Yes ☐ No
If yes, by whom?

No drug will be administered except during the MRS scans, where a physician and a nurse from the research team will administer the drug as described in the methods.

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4. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.

Check applicable Investigational Drug Service utilized:

☒ **YNHH IDS** ☐ **Yale Cancer Center**
☐ **CMHC Pharmacy** ☐ **West Haven VA**
☐ **PET Center** ☐ **None**
☐ **Other:**

Note: If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation.

5. **Use of Placebo: ☒ Not applicable to this research project**

If use of a placebo is planned, provide a justification which addresses the following:

In this study neither ketamine, lamotrigine, or the matched-placebo are used for therapeutic purposes. They will be used in healthy volunteers to determine underlying neurobiological mechanisms and not for treatment purposes.

a. Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this.
b. State the maximum total length of time a participant may receive placebo while on the study.
c. Address the greatest potential harm that may come to a participant as a result of receiving placebo.
d. Describe the procedures that are in place to safeguard participants receiving placebo.

6. **Use of Controlled Substances:**

Will this research project involve the use of controlled substances in human subjects?
☒ Yes ☐ No *See HIC Application Instructions to view controlled substance listings.*

If yes, is the use of the controlled substance considered:
☐ Therapeutic: The use of the controlled substance, within the context of the research, has the potential to benefit the research participant.
☒ Non-Therapeutic: *Note, the use of a controlled substance in a non-therapeutic research study involving human subjects may require that the investigator obtain a Laboratory Research License. Examples include controlled substances used for basic imaging, observation or biochemical studies or other non-therapeutic purposes. See Instructions for further information.*

7. **Continuation of Drug Therapy After Study Closure ☒ Not applicable to this project**

Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended?

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☐ Yes If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access.

☐ No If no, explain why this is acceptable.

B. DEVICES

1. Are there any investigational devices used or investigational procedures performed at YNHH, e.g., YNHH Operating Room or YNHH Heart and Vascular Center? Yes ☐ No ☒
If Yes, please be aware of the following requirements:

a. A YNHH New Product/Trial Request Form must be completed;

b. Your request must be reviewed and approved by a Hospital Committee before patients may be scheduled; and

c. The notice of approval from YNHH must be submitted to the HIC for the protocol file.

Please contact Gina D'Agostino, gina.d'agostino@ynhh.org or 203-688-5052, to initiate the process.

2. What is the name of the device to be studied in this protocol?

Has this device been FDA approved? ☐ Yes ☐ No
If yes, state for what indication.

3. Background Information: Provide a description of previous human use, known risks, and any other factors that might influence risks. If this is the first time this device is being used in humans, include relevant data on animal models.

4. Source:

a) Identify the source of the device to be used.

b) Is the device provided free of charge to subjects? ☐ Yes ☐ No

5. What is the PI's assessment of risk level (significant or non-significant) associated with the use of the device?

☐ **Significant Risk (SR) Device Study:** A study of a device that presents a potential for serious risk to the health, safety, or welfare of a participant and 1) is intended as an implant; 2) is used in supporting or sustaining human life; or otherwise prevents impairment of human health; 3) is of substantial importance in diagnosing, curing, mitigating or treating disease, or otherwise prevents impairment of human health; or 4)

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otherwise presents a potential for serious risk to the health, safety, or welfare of a participant.

Significant Risk Devices require an Investigational Device Exemption (IDE) issued by the FDA.

What is the **IDE number** assigned by the FDA?

Did the FDA approve this IDE as **Category A** (experimental/investigational) or as **Category B** (non-experimental/investigational)?

Who holds the IDE?

☐ **Non-Significant Risk (NSR) Device Study:** A study of a device that does not meet the definition for a significant risk device and does not present a potential for serious risk to the health, safety, or welfare of participants. Note that if the HIC concurs with this determination, an IDE is not required.

6. **Abbreviated IDE or Exempt IDE:** There are abbreviated requirements for an IDE and there also are exemptions to the requirement for an IDE. *See the criteria in the HIC Application Instructions,* Section VI.B.4 at http://www.yale.edu/hrpp/resources/docs/10OFR1aHICProtocol_Application_Instructions5-25-11.pdf *to determine if these pertain to this study.*

☐ **Abbreviated IDE or Exempt IDE** – *If criteria set forth in the HIC Application Instructions are met, copy and paste the completed relevant section from the Instructions into this application.*

7. **Investigational device accountability**:

a. State how the PI, or named designee, ensures that an investigational device is used only in accordance with the research protocol approved by the HIC, and maintains control of the investigational device as follows:

Maintains appropriate records, including receipt of shipment, inventory at the site, dispensation or use by each participant, and final disposition and/or the return of the investigational device (or other disposal if applicable):

Documents pertinent information assigned to the investigational device (e.g., date, quantity, batch or serial number, expiration date if applicable, and unique code number):

Stores the investigational device according to the manufacturer's recommendations with respect to temperature, humidity, lighting, and other environmental considerations:

Ensures that the device is stored in a secure area with limited access in accordance with applicable regulatory requirements:

Distributes the investigational device to subjects enrolled in the IRB-approved protocol:

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SECTION VII: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. Targeted Enrollment: Give the number of subjects:

a. targeted for enrollment at Yale for this protocol: 32

b. If this is a multi-site study, give the total number of subjects targeted across all sites____

2. Indicate recruitment methods below. Attach copies of any recruitment materials that will be used.

☒ Flyers
☒ Posters
☐ Letter
☐ Medical Record Review
☒ Newspaper
☒ Departmental/Center Newsletters
☒ YCCI Recruitment database
☒ Internet/Web Postings
☐ Mass E-mail Solicitation
☒ Departmental/Center Website
☒ Departmental/Center Research Boards
☒ Web-Based Clinical Trial Registries
☒ Clinicaltrials.gov Registry (do not send materials to HIC)
☐ Radio
☐ Telephone
☐ Television
☒ Other (describe): Wallet-sized recruitment cards.

3. Recruitment Procedures:

a. Describe how potential subjects will be identified.

Subjects will be identified via their response to advertisements and/or internal recruiting through the research clinics.

b. Describe how potential subjects are contacted.

Subjects will be contacted via the most convenient means and personal preference, e.g. telephone and e-mail.

c.Who is recruiting potential subjects?

All available research staff is responsible for recruiting potential subjects.

4. Screening Procedures

a. be used to screen potential subjects for eligibility prior to the potential subject coming to the research office? ☒ Yes ☐ No

b. If yes, identify below all health information to be collected as part of screening and check off any of the following HIPAA identifiers to be collected and retained by the research team during this screening process.

HEALTH INFORMATION TO BE COLLECTED:

HIPAA identifiers:

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☒ Names
☒ All geographic subdivisions smaller than a State, including: street address, city, county, precinct, zip codes and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly-available data from the Bureau of the Census: (1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people, and (2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.
☒ Telephone numbers
☒ Fax numbers
☒ E-mail addresses
☐ Social Security numbers
☐ Medical record numbers
☐ Health plan beneficiary numbers
☐ Account numbers
☒ All elements of dates (except year) for dates related to an individual, including: birth date, admission date, discharge date, date of death, all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older
☐ Certificate/license numbers
☐ Vehicle identifiers and serial numbers, including license plate numbers
☐ Device identifiers and serial numbers
☐ Web Universal Resource Locators (URLs)
☐ Internet Protocol (IP) address numbers
☐ Biometric identifiers, including finger and voice prints
☐ Full face photographic images and any comparable images
☐ Any other unique identifying numbers, characteristics, or codes

5. Assessment of Current Health Provider Relationship for HIPAA Consideration:

Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?
☐ Yes, all subjects
☒ Yes, some of the subjects
☐ No

If yes, describe the nature of this relationship.

Some of the subjects recruited into this study will have had prior contact with research clinics and/or may have participated in a prior approved experimental protocol.

6. (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)

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Choose one: For entire study: _____ For recruitment purposes only:
✔☐☐☐☐

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data;
ii. If requesting a waiver of **signed** authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data;

In order to determine if a subject is eligible to come in to the clinic for a screening visit, we conduct phone screens. We ask their permission to record data, and tell them that everything we write down is confidential and will be kept in a locked cabinet. Therefore, due to prescreening on the phone, we cannot get their signed authorization. We can only get their verbal authorization.

By signing this protocol application, the investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

7. **Required HIPAA Authorization:** If the research involves the creation, use or disclosure of protected health information (PHI), separate subject authorization is required under the HIPAA Privacy Rule. Indicate which of the following forms are being provided:
   ☒ Compound Consent and Authorization form
   ☐ HIPAA Research Authorization Form

Consent Personnel:

8. **Process of Consent/Assent:** Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.

If an individual appears to meet enrollment criteria and is interested in participating, a face-to-face interview is conducted, by one of the project investigators. A release of information is obtained for review of any available historical and clinical data. A written authorization form (See Attached) is also obtained from each subject, permitting the research team to use, create, or disclose the subject's PHI for research purposes. The

nature of the project, procedures, relative risks and benefits, and alternatives to participation in the project are discussed with the individual. Following this discussion, the individual is given a copy of the consent form to review at their leisure, and any questions are answered.

If the individual remains interested in the project, written informed consent (see Attached) is obtained, and medical and psychiatric screening procedures are undertaken to confirm eligibility. A copy of the consent form is provided to all participants.

If the individual decides not to participate in this study, the decision not to participate does not affect eligibility to participate in future studies, to receive treatment at the Connecticut Mental Health Center, or to receive treatment on a private basis from a referring clinician.

9. Evaluation of Subject(s) Capacity to Provide Informed Consent/Assent: Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.

As mentioned above, after trained and knowledgeable research personnel explain all aspects of the research protocol with a consent form at hand, the potential subject will be asked to communicate a reciprocal understanding of the protocol. This "say back" method has demonstrated reliability among educators in ascertaining degree of comprehension.

10. Documentation of Consent/Assent: Specify the documents that will be used during the consent/assent process. Copies of all documents should be appended to the protocol, in the same format that they will be given to subjects.

Research personnel capable of determining consent will conduct this process with the most up-to-date copies of the Yale informed consent documentations at hand. Yale consent forms has been appended to this document.

11. Non-English Speaking Subjects: Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. Translated copies of all consent materials must be submitted for approval prior to use.

Not applicable.

12. Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study. If you will

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request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☒ **Not requesting a consent waiver**
☐ **Requesting a waiver of signed consent**
☐ **Requesting a full waiver of consent**

A. Waiver of **signed** consent: (Verbal consent from subjects will be obtained. **If PHI is collected, information in this section must match Section VII, Question 6**)

☐ **Requesting a waiver of signed consent for Recruitment/Screening only**

If requesting a waiver of signed consent, please address the following:
a. Would the signed consent form be the only record linking the subject and the research? ☐ Yes ☐ No
b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☐ No

OR

c. Does the research activity pose greater than minimal risk?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity
☐ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☐ No

☐ **Requesting a waiver of signed consent for the Entire Study (**Note that an information sheet may be required.)

If requesting a waiver of signed consent, please address the following:
a. Would the signed consent form be the only record linking the subject and the research? ☐ Yes ☐ No
b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☐ No

OR

c. Does the research pose greater than minimal risk? ☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** ☐ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☐ No

B. **Full waiver** of consent: (No consent from subjects will be obtained for the activity.)

☐ **Requesting a waiver of consent for Recruitment/Screening only**

a. Does the research activity pose greater than minimal risk to subjects?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity

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☐ No
b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No
c. Why would the research be impracticable to conduct without the waiver?
d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

☐ **Requesting a full waiver of consent for the Entire Study (Note: If PHI is collected, information here must match Section VII, question 6.)**

If requesting a full waiver of consent, please address the following:

a. Does the research pose greater than minimal risk to subjects?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.***
☐ No
b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No
c. Why would the research be impracticable to conduct without the waiver?
d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

SECTION VIII: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

a. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?

b. How will the research data be collected, recorded and stored?
c. How will the digital data be stored? ☒ CD ☒ DVD ☐ Flash Drive ☒ Portable Hard Drive ☒ Secured Server ☒ Laptop Computer ☐ Desktop Computer ☐ Other
d. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?
Do all portable devices contain encryption software? ☒ Yes ☐ No
If no, see http://hipaa.yale.edu/guidance/policy.html

e. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.

After subject participation in the study has ceased, written confidential PHI will be transported from the YDRP to be stored in patient records (CMHC basement). All electronic PHI (ePHI) will continue to be encrypted and password-protected after

subject participation. Both written and ePHI will be destroyed after the protocol is completed at a time designated by the primary investigator.

f. Who will have access to the protected health information (such as the research sponsor, the investigator, the research staff, all research monitors, FDA, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), SSC, etc.)? (please distinguish between PHI and de-identified data).

All pre-identified research personnel, Yale HIC and NIMH will have access to written and ePHI throughout and after the completion of the study until the data is destroyed at the discretion of the primary investigators.

g. If appropriate, has a Certificate of Confidentiality been obtained?

Yes: Because this study contains sensitive information and drug testing and is funded by the NIH, per the Notice of Changes to NIH Policy for Issuing Certificates of Confidentiality (NOT-OD-17-109), effective October 1, 2017, this study is assumed to now have a Certificate of Confidentiality, subjects are anticipated to be protected.

h. Are any of the study procedures likely to yield information subject to mandatory reporting requirements? (e.g. HIV testing – reporting of communicable diseases; parent interview -incidents of child abuse, elderly abuse, etc.). Please verify to whom such instances will need to be reported.

HIV, Hepatitis B and C testing, as part of the screening assessment, may yield information that we are legally required to disclose to the State of Connecticut and/or CDC as reportable infections. Any identifiable information obtained in connection with this protocol remains confidential. Information is disclosed only with the subject's prior authorization or as required by U.S. and State law. Information that we are legally required to disclose includes abuse of a child or elderly person, or certain reportable infectious disease.

SECTION IX: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)

There is no direct clinical benefit for participating in this study. The present studies may benefit others by providing insight into depression and schizophrenia. It may provide

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specific information about the effect of subanesthetic dose of ketamine on the human brain and may provide guidance in further exploring potential mechanisms for the rapid antidepressant effect of ketamine as well as provide potentially important information related to the pathophysiology of psychosis. This information may in turn provide novel diagnostic tests, and may help target new drug development.

The relative risks and inconveniences associated with participation in this study, including ketamine administration, MRS and venipuncture procedures are balanced by the potential benefits to society.

SECTION X: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?

   The current study is not for treatment purposes and the subjects do not need to participate.

2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.

   All participants will be compensated for their participation. Subjects will be paid $40 for screening, $60 for each MRI study (2 studies total), $40 for each cognitive testing session (2 sessions total), and $300 for ketamine infusion test day. Failed imaging studies will be repeated and subjects will be paid additionally for the repeated scan. If participation in the scans has already begun and must be cancelled before completion, then compensation will be based on involvement in the study at a rate of $20 per hour. Reasonable transportation costs up to $100 on the infusion day will be reimbursed. Receipts must be submitted.

   Therefore, we anticipate the subject's total earning – if he/she participates in all sessions – is $540. At the principal investigator's discretion, further subject payment may be made for travel, parking, lodging, food or transportation.

   The debit cards will be offered through the YCCI OnCore ePayments system and will be our preferred payment option. Subjects will be informed that they will receive payment via a Bank of America pre-paid debit card. We will provide their name, address, and telephone number to the Bank of America for processing and their card will be related to their name and number. Their SSN or other information will not be provided. All compensation information will be discussed in the informed consent process.

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3. **Costs for Participation (Economic Considerations):** Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.

The only costs associated with the study are transportation to and from appointments. All services and evaluations are provided free of charge.

4. **In Case of Injury:** This section is required for any research involving more than minimal risk.
   a. Will medical treatment be available if research-related injury occurs?
   b. Where and from whom may treatment be obtained?
   c. Are there any limits to the treatment being provided?
   d. Who will pay for this treatment?
   e. How will the medical treatment be accessed by subjects?

Medical treatment will be offered to the subjects for any physical injuries that they receive as a result of participating in this research. However, the subject or his/her insurance company is responsible for the cost. As required by Federal regulations, all subjects will be told that if they are physically injured, no additional financial compensation is available.

References:

1 Collins, P. Y. *et al.* Grand challenges in global mental health. *Nature* **475**, 27-30, doi:10.1038/475027a (2011).

2 Holma, K. M. *et al.* Incidence and predictors of suicide attempts in DSM-IV major depressive disorder: a five-year prospective study. *The American journal of psychiatry* **167**, 801-808, doi:10.1176/appi.ajp.2010.09050627 (2010).

3 Kessler, R. C. *et al.* Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of general psychiatry* **62**, 593-602, doi:10.1001/archpsyc.62.6.593 (2005).

4 Rush, A. J. *et al.* Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry* **163**, 1905-1917, doi:163/11/1905 [pii]
10.1176/appi.ajp.163.11.1905 (2006).

5 Rush, A. J. *et al.* Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. *N Engl J Med* **354**, 1231-1242, doi:354/12/1231 [pii]
10.1056/NEJMoa052963 (2006).

6 Trivedi, M. H. *et al.* Medication augmentation after the failure of SSRIs for depression. *N Engl J Med* **354**, 1243-1252, doi:354/12/1243 [pii]
10.1056/NEJMoa052964 (2006).

7 Trivedi, M. H. *et al.* Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry* **163**, 28-40, doi:163/1/28 [pii]
10.1176/appi.ajp.163.1.28 (2006).

8 Katz, M. M. *et al.* Onset and early behavioral effects of pharmacologically different antidepressants and placebo in depression. *Neuropsychopharmacology : official*

publication of the American College of Neuropsychopharmacology **29**, 566-579, doi:10.1038/sj.npp.1300341
1300341 [pii] (2004).

9 Mathew, S. J. *et al.* Ketamine for treatment-resistant unipolar depression: current evidence. *CNS drugs* **26**, 189-204, doi:10.2165/11599770-000000000-00000 (2012).

10 Krystal, J. H. *et al.* Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Archives of general psychiatry* **51**, 199-214 (1994).

11 Krystal, J. H. *et al.* Altered NMDA glutamate receptor antagonist response in recovering ethanol-dependent patients. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **28**, 2020-2028, doi:10.1038/sj.npp.1300252 (2003).

12 Anand, A. *et al.* Attenuation of the neuropsychiatric effects of ketamine with lamotrigine: support for hyperglutamatergic effects of N-methyl-D-aspartate receptor antagonists. *Archives of general psychiatry* **57**, 270-276 (2000).

13 Deakin, J. F. *et al.* Glutamate and the neural basis of the subjective effects of ketamine: a pharmaco-magnetic resonance imaging study. *Archives of general psychiatry* **65**, 154-164, doi:10.1001/archgenpsychiatry.2007.37 (2008).

14 Krystal, J. H. *et al.* Potential psychiatric applications of metabotropic glutamate receptor agonists and antagonists. *CNS drugs* **24**, 669-693, doi:10.2165/11533230-000000000-00000 (2010).

15 Krystal, J. H. *et al.* Preliminary evidence of attenuation of the disruptive effects of the NMDA glutamate receptor antagonist, ketamine, on working memory by pretreatment with the group II metabotropic glutamate receptor agonist, LY354740, in healthy human subjects. *Psychopharmacology* **179**, 303-309, doi:10.1007/s00213-004-1982-8 (2005).

16 Berman, R. M. *et al.* Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* **47**, 351-354, doi:10.1016/s0006-3223(99)00230-9 (2000).

17 Diazgranados, N. *et al.* A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. *Archives of general psychiatry* **67**, 793-802, doi:10.1001/archgenpsychiatry.2010.90 (2010).

18 Valentine, G. W. *et al.* The antidepressant effect of ketamine is not associated with changes in occipital amino acid neurotransmitter content as measured by [(1)H]-MRS. *Psychiatry Res* **191**, 122-127, doi:10.1016/j.pscychresns.2010.10.009 (2011).

19 Zarate, C. A., Jr. *et al.* A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Archives of general psychiatry* **63**, 856-864, doi:10.1001/archpsyc.63.8.856 (2006).

20 aan het Rot, M. *et al.* Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biol Psychiatry* **67**, 139-145, doi:10.1016/j.biopsych.2009.08.038 (2010).

21 DiazGranados, N. *et al.* Rapid resolution of suicidal ideation after a single infusion of an N-methyl-D-aspartate antagonist in patients with treatment-resistant major depressive disorder. *The Journal of clinical psychiatry* **71**, 1605-1611, doi:10.4088/JCP.09m05327blu (2010).

22 Ibrahim, L. *et al.* Rapid decrease in depressive symptoms with an N-methyl-d-aspartate antagonist in ECT-resistant major depression. *Prog Neuropsychopharmacol Biol Psychiatry* **35**, 1155-1159, doi:10.1016/j.pnpbp.2011.03.019 (2011).

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23 Machado-Vieira, R. *et al.* Brain-derived neurotrophic factor and initial antidepressant response to an N-methyl-D-aspartate antagonist. *The Journal of clinical psychiatry* **70**, 1662-1666, doi:10.4088/JCP.08m04659 (2009).
24 Mathew, S. J. *et al.* Riluzole for relapse prevention following intravenous ketamine in treatment-resistant depression: a pilot randomized, placebo-controlled continuation trial. *Int J Neuropsychopharmacol* **13**, 71-82, doi:10.1017/s1461145709000169 (2010).
25 Phelps, L. E. *et al.* Family history of alcohol dependence and initial antidepressant response to an N-methyl-D-aspartate antagonist. *Biol Psychiatry* **65**, 181-184, doi:10.1016/j.biopsych.2008.09.029 (2009).
26 Price, R. B., Nock, M. K., Charney, D. S. & Mathew, S. J. Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biol Psychiatry* **66**, 522-526, doi:10.1016/j.biopsych.2009.04.029 (2009).
27 Salvadore, G. *et al.* Increased anterior cingulate cortical activity in response to fearful faces: a neurophysiological biomarker that predicts rapid antidepressant response to ketamine. *Biol Psychiatry* **65**, 289-295, doi:10.1016/j.biopsych.2008.08.014 (2009).
28 Salvadore, G. *et al.* Anterior cingulate desynchronization and functional connectivity with the amygdala during a working memory task predict rapid antidepressant response to ketamine. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **35**, 1415-1422, doi:10.1038/npp.2010.24 (2010).
29 Ibrahim, L. *et al.* Course of Improvement in Depressive Symptoms to a Single Intravenous Infusion of Ketamine vs Add-on Riluzole: Results from a 4-Week, Double-Blind, Placebo-Controlled Study. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, doi:10.1038/npp.2011.338 (2012).
30 Salvadore, G. *et al.* An investigation of amino-acid neurotransmitters as potential predictors of clinical improvement to ketamine in depression. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*, 1-10, doi:10.1017/S1461145711001593 (2011).
31 Zarate, C. A., Jr. *et al.* Replication of Ketamine's Antidepressant Efficacy in Bipolar Depression: A Randomized Controlled Add-On Trial. *Biological Psychiatry*, doi:10.1016/j.biopsych.2011.12.010 (2012).
32 aan het Rot, M., Charney, D. S. & Mathew, S. J. Intravenous ketamine for treatment-resistant major depressive disorder. *Primary Psychiatry* **15**, 39-47 (2008).
33 Duman, R. S. & Voleti, B. Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. *Trends in neurosciences*, doi:10.1016/j.tins.2011.11.004 (2011).
34 Li, N. *et al.* Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biol Psychiatry* **69**, 754-761, doi:10.1016/j.biopsych.2010.12.015 (2011).
35 Abekawa, T., Honda, M., Ito, K. & Koyama, T. Effects of NRA0045, a novel potent antagonist at dopamine D4, 5-HT2A, and alpha1 adrenaline receptors, and NRA0160, a selective D4 receptor antagonist, on phencyclidine-induced behavior and glutamate release in rats. *Psychopharmacology* **169**, 247-256, doi:10.1007/s00213-003-1517-8 (2003).
36 Homayoun, H. & Moghaddam, B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *The Journal of*

APPROVED BY THE YALE UNIVERSITY IRB 2/8/2018 VALID THROUGH 2/11/2019

neuroscience : the official journal of the Society for Neuroscience **27**, 11496-11500, doi:10.1523/JNEUROSCI.2213-07.2007 (2007).

37 Moghaddam, B., Adams, B., Verma, A. & Daly, D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* **17**, 2921-2927 (1997).

38 Moghaddam, B. & Adams, B. W. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science* **281**, 1349-1352 (1998).

39 Razoux, F., Garcia, R. & Lena, I. Ketamine, at a dose that disrupts motor behavior and latent inhibition, enhances prefrontal cortex synaptic efficacy and glutamate release in the nucleus accumbens. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **32**, 719-727, doi:10.1038/sj.npp.1301057 (2007).

40 Liu, J. & Moghaddam, B. Regulation of glutamate efflux by excitatory amino acid receptors: evidence for tonic inhibitory and phasic excitatory regulation. *J Pharmacol Exp Ther* **274**, 1209-1215 (1995).

41 Lorrain, D. S., Baccei, C. S., Bristow, L. J., Anderson, J. J. & Varney, M. A. Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. *Neuroscience* **117**, 697-706 (2003).

42 Bonaventure, P. *et al.* Pharmacological Blockade of Serotonin 5-HT(7) Receptor Reverses Working Memory Deficits in Rats by Normalizing Cortical Glutamate Neurotransmission. *PLoS One* **6**, e20210, doi:10.1371/journal.pone.0020210 (2011).

43 Lopez-Gil, X. *et al.* Clozapine and haloperidol differently suppress the MK-801-increased glutamatergic and serotonergic transmission in the medial prefrontal cortex of the rat. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **32**, 2087-2097, doi:10.1038/sj.npp.1301356 (2007).

44 Chowdhury, G. M. *et al.* (1)H-[(1)(3)C]-nuclear magnetic resonance spectroscopy measures of ketamine's effect on amino acid neurotransmitter metabolism. *Biol Psychiatry* **71**, 1022-1025, doi:10.1016/j.biopsych.2011.11.006 (2012).

45 Li, N. *et al.* mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* **329**, 959-964, doi:10.1126/science.1190287 (2010).

46 Autry, A. E. *et al.* NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature* **475**, 91-95 LID - 10.1038/nature10130 [doi], doi:10.1038/nature10130 (2011).

47 Koike, H., Iijima, M. & Chaki, S. Involvement of AMPA receptor in both the rapid and sustained antidepressant-like effects of ketamine in animal models of depression. *Behav Brain Res* **224**, 107-111, doi:10.1016/j.bbr.2011.05.035 (2011).

48 Maeng, S. *et al.* Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiatry* **63**, 349-352, doi:10.1016/j.biopsych.2007.05.028 (2008).

49 Dwyer, J. M., Lepack, A. E. & Duman, R. S. mTOR activation is required for the antidepressant effects of mGluR2/3 blockade. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*, 1-6, doi:10.1017/S1461145711001702 (2011).

APPROVED BY THE YALE UNIVERSITY IRB 2/8/2018 VALID THROUGH 2/11/2019

50 Cartmell, J., Monn, J. A. & Schoepp, D. D. The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively attenuate phencyclidine versus d-amphetamine motor behaviors in rats. *J Pharmacol Exp Ther* **291**, 161-170 (1999).
51 Cartmell, J., Monn, J. A. & Schoepp, D. D. The mGlu(2/3) receptor agonist LY379268 selectively blocks amphetamine ambulations and rearing. *European journal of pharmacology* **400**, 221-224 (2000).
52 Schlumberger, C. *et al.* Effects of a metabotropic glutamate receptor group II agonist LY354740 in animal models of positive schizophrenia symptoms and cognition. *Behav Pharmacol* **20**, 56-66, doi:10.1097/FBP.0b013e3283242f57 (2009).
53 Hackler, E. A. *et al.* Selective potentiation of the metabotropic glutamate receptor subtype 2 blocks phencyclidine-induced hyperlocomotion and brain activation. *Neuroscience* **168**, 209-218, doi:10.1016/j.neuroscience.2010.02.057 (2010).
54 Homayoun, H., Jackson, M. E. & Moghaddam, B. Activation of metabotropic glutamate 2/3 receptors reverses the effects of NMDA receptor hypofunction on prefrontal cortex unit activity in awake rats. *J Neurophysiol* **93**, 1989-2001, doi:10.1152/jn.00875.2004 (2005).
55 Chin, C. L. *et al.* Awake rat pharmacological magnetic resonance imaging as a translational pharmacodynamic biomarker: metabotropic glutamate 2/3 agonist modulation of ketamine-induced blood oxygenation level dependence signals. *J Pharmacol Exp Ther* **336**, 709-715, doi:10.1124/jpet.110.173880 (2011).
56 Yang, S., Hu, J., Kou, Z. & Yang, Y. Spectral simplification for resolved glutamate and glutamine measurement using a standard STEAM sequence with optimized timing parameters at 3, 4, 4.7, 7, and 9.4T. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* **59**, 236-244, doi:10.1002/mrm.21463 (2008).
57 Rowland, L. M. *et al.* Effects of ketamine on anterior cingulate glutamate metabolism in healthy humans: a 4-T proton MRS study. *The American journal of psychiatry* **162**, 394-396, doi:10.1176/appi.ajp.162.2.394 (2005).
58 Stone, J. M. *et al.* Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology. *Molecular psychiatry*, doi:10.1038/mp.2011.171 (2012).
59 Taylor, M. J., Tiangga, E. R., Ni Mhuircheartaigh, R. & Cowen, P. Lack of effect of ketamine on cortical glutamate and glutamine in healthy volunteers: a proton magnetic resonance spectroscopy study. *J Psychopharmacol*, doi:10.1177/0269881111405359 (2011).
60 Duman, R. S. & Monteggia, L. M. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* **59**, 1116-1127, doi:10.1016/j.biopsych.2006.02.013 (2006).
61 Hardingham, G. E. & Bading, H. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nature reviews. Neuroscience* **11**, 682-696, doi:10.1038/nrn2911 (2010).
62 Turrigiano, G. Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement. *Annual review of neuroscience* **34**, 89-103, doi:10.1146/annurev-neuro-060909-153238 (2011).
63 Beattie, E. C. *et al.* Control of synaptic strength by glial TNFalpha. *Science* **295**, 2282-2285, doi:10.1126/science.1067859 (2002).

APPROVED BY THE YALE UNIVERSITY IRB 2/8/2018 VALID THROUGH 2/11/2019

64 Rutherford, L. C., Nelson, S. B. & Turrigiano, G. G. BDNF has opposite effects on the quantal amplitude of pyramidal neuron and interneuron excitatory synapses. *Neuron* **21**, 521-530 (1998).
65 Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W. & Kelley, K. W. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature reviews. Neuroscience* **9**, 46-56, doi:10.1038/nrn2297 (2008).
66 Duman, R. S. & Aghajanian, G. K. Synaptic dysfunction in depression: potential therapeutic targets. *Science* **338**, 68-72, doi:10.1126/science.1222939 (2012).
67 Popoli, M., Yan, Z., McEwen, B. S. & Sanacora, G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nature reviews. Neuroscience* **13**, 22-37, doi:10.1038/nrn3138 (2012).
68 Kang, H. J. *et al.* Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. *Nature medicine* **18**, 1413-1417, doi:10.1038/nm.2886 (2012).
69 Bessa, J. M. *et al.* The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Molecular psychiatry* **14**, 764-773, 739, doi:10.1038/mp.2008.119 (2009).
70 Yuen, E. Y. *et al.* Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. *Neuron* **73**, 962-977, doi:10.1016/j.neuron.2011.12.033 (2012).
71 Bessa, J. M. *et al.* Stress-induced anhedonia is associated with hypertrophy of medium spiny neurons of the nucleus accumbens. *Translational psychiatry* **3**, e266, doi:10.1038/tp.2013.39 (2013).
72 Vyas, A., Pillai, A. G. & Chattarji, S. Recovery after chronic stress fails to reverse amygdaloid neuronal hypertrophy and enhanced anxiety-like behavior. *Neuroscience* **128**, 667-673, doi:10.1016/j.neuroscience.2004.07.013 (2004).
73 Krystal, J. H., Sanacora, G. & Duman, R. S. Rapid-acting glutamatergic antidepressants: the path to ketamine and beyond. *Biol Psychiatry* **73**, 1133-1141, doi:10.1016/j.biopsych.2013.03.026 (2013).
74 Savitz, J. & Drevets, W. C. Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide. *Neurosci Biobehav Rev* **33**, 699-771, doi:10.1016/j.neubiorev.2009.01.004 (2009).
75 Ota, K. T. *et al.* REDD1 is essential for stress-induced synaptic loss and depressive behavior. *Nature medicine*, doi:10.1038/nm.3513 (2014).
76 Sen, S., Duman, R. & Sanacora, G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biol Psychiatry* **64**, 527-532, doi:10.1016/j.biopsych.2008.05.005 (2008).
77 Verhagen, M. *et al.* Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. *Molecular psychiatry* **15**, 260-271, doi:10.1038/mp.2008.109 (2010).
78 Ng, F., Hallam, K., Lucas, N. & Berk, M. The role of lamotrigine in the management of bipolar disorder. *Neuropsychiatric disease and treatment* **3**, 463-474 (2007).
79 Hahn, C. G., Gyulai, L., Baldassano, C. F. & Lenox, R. H. The current understanding of lamotrigine as a mood stabilizer. *J Clin Psychiatry* **65**, 791-804 (2004).
80 Ketter, T. A., Manji, H. K. & Post, R. M. Potential mechanisms of action of lamotrigine in the treatment of bipolar disorders. *Journal of clinical psychopharmacology* **23**, 484-495, doi:10.1097/01.jcp.0000088915.02635.e8 (2003).

APPROVED BY THE YALE UNIVERSITY IRB 2/8/2018 VALID THROUGH 2/11/2019

81 Bourin, M., Masse, F. & Hascoet, M. Evidence for the activity of lamotrigine at 5-HT(1A) receptors in the mouse forced swimming test. *Journal of psychiatry & neuroscience : JPN* **30**, 275-282 (2005).
82 Berk, M., Plein, H. & Belsham, B. The specificity of platelet glutamate receptor supersensitivity in psychotic disorders. *Life sciences* **66**, 2427-2432 (2000).
83 Avery, J. A. *et al.* Major Depressive Disorder Is Associated with Abnormal Interoceptive Activity and Functional Connectivity in the Insula. *Biol Psychiatry*, doi:10.1016/j.biopsych.2013.11.027 (2013).
84 Cao, L. *et al.* Aberrant functional connectivity for diagnosis of major depressive disorder: a discriminant analysis. *Psychiatry and clinical neurosciences* **68**, 110-119, doi:10.1111/pcn.12106 (2014).
85 Ebmeier, K., Rose, E. & Steele, D. Cognitive impairment and fMRI in major depression. *neurotox res* **10**, 87-92, doi:10.1007/BF03033237 (2006).
86 Kerestes, R., Davey, C. G., Stephanou, K., Whittle, S. & Harrison, B. J. Functional brain imaging studies of youth depression: A systematic review. *NeuroImage. Clinical* **4**, 209-231, doi:10.1016/j.nicl.2013.11.009 (2013).
87 Northoff, G., Wiebking, C., Feinberg, T. & Panksepp, J. The 'resting-state hypothesis' of major depressive disorder-a translational subcortical-cortical framework for a system disorder. *Neurosci Biobehav Rev* **35**, 1929-1945, doi:10.1016/j.neubiorev.2010.12.007 (2011).
88 Scheidegger, M. *et al.* Ketamine Decreases Resting State Functional Network Connectivity in Healthy Subjects: Implications forAntidepressant Drug Action. *PLOS One* **7**, e44799 (2012).
89 Sheline, Y., Price, J., Yan, Z. & Mintun, M. Resting-state functional MRI in depression unmasks increased connectivity between networks via the dorsal nexus. *PNAS* **107**, 11020–11025 (2010).
90 Biswal, B. *et al.* Toward discovery science of human brain function. *Proc Natl Acad Sci* **107**, 4734-4739 (2010).
91 McCabe, C. *et al.* SSRI administration reduces resting state functional connectivity in dorso-medial prefrontal cortex. *Mol Psychiatry* **16**, 592–594 (2011).
92 McCabe, C. & Mishor, Z. Antidepressant medications reduce subcortical-cortical resting-state functional connectivity in healthy volunteers. *NeuroImage* **57**, 1317–1323 (2011).
93 Cole, M. W., Pathak, S. & Schneider, W. Identifying the brain's most globally connected regions. *Neuroimage* **49**, 3132-3148, doi:10.1016/j.neuroimage.2009.11.001 (2010).
94 Machado-Vieira, R., Salvadore, G., Diazgranados, N. & Zarate, C. A., Jr. Ketamine and the next generation of antidepressants with a rapid onset of action. *Pharmacol Ther* **123**, 143-150, doi:10.1016/j.pharmthera.2009.02.010 (2009).
95 Domino, E. F., Chodoff, P. & Corssen, G. Pharmacologic Effects of Ci-581, a New Dissociative Anesthetic, in Man. *Clin Pharmacol Ther* **6**, 279-291 (1965).
96 Rothman, D. L., Petroff, O. A., Behar, K. L. & Mattson, R. H. Localized 1H NMR measurements of gamma-aminobutyric acid in human brain in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 5662-5666 (1993).
97 Perry, E. B., Jr. *et al.* Psychiatric safety of ketamine in psychopharmacology research. *Psychopharmacology* **192**, 253-260, doi:10.1007/s00213-007-0706-2 (2007).
98 Schorn, T. O. & Witwam, J. G. Are there long-term effects of ketamine on the central nervous system? *Br J Anaesth* **52**, 967-968 (1980).

APPROVED BY THE YALE UNIVERSITY IRB 2/8/2018 VALID THROUGH 2/11/2019

99 Hamilton, M. J. *et al.* Carbamazepine and lamotrigine in healthy volunteers: relevance to early tolerance and clinical trial dosage. *Epilepsia* **34**, 166-173 (1993).
100 Anticevic, A. *et al.* Global resting-state functional magnetic resonance imaging analysis identifies frontal cortex, striatal, and cerebellar dysconnectivity in obsessive-compulsive disorder. *Biol Psychiatry* **75**, 595-605, doi:10.1016/j.biopsych.2013.10.021 (2014).
101 D'Souza, D. C. *et al.* Glycine transporter inhibitor attenuates the psychotomimetic effects of ketamine in healthy males: preliminary evidence. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **37**, 1036-1046, doi:10.1038/npp.2011.295 (2012).
102 Gunduz-Bruce, H. *et al.* Glutamatergic Modulation of Auditory Information Processing in the Human Brain. *Biol Psychiatry*, doi:10.1016/j.biopsych.2011.09.031 (2011).
103 Krystal, J. H. *et al.* Interactive effects of subanesthetic ketamine and subhypnotic lorazepam in humans. *Psychopharmacology* **135**, 213-229 (1998).
104 Krystal, J. H. *et al.* Interactive effects of subanesthetic ketamine and haloperidol in healthy humans. *Psychopharmacology* **145**, 193-204 (1999).
105 Krystal, J. H. *et al.* Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function. *Archives of general psychiatry* **62**, 985-994, doi:10.1001/archpsyc.62.9.985 (2005).
106 Kantrowitz, J. T. & Javitt, D. C. Thinking glutamatergically: changing concepts of schizophrenia based upon changing neurochemical models. *Clin Schizophr Relat Psychoses* **4**, 189-200, doi:10.3371/CSRP.4.3.6 (2010).
107 Bubenikova-Valesova, V., Horacek, J., Vrajova, M. & Hoschl, C. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neurosci Biobehav Rev* **32**, 1014-1023, doi:10.1016/j.neubiorev.2008.03.012 (2008).
108 Newcomer, J. W. *et al.* Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **20**, 106-118, doi:10.1016/S0893-133X(98)00067-0 (1999).
109 Oye, I., Paulsen, O. & Maurset, A. Effects of ketamine on sensory perception: evidence for a role of N-methyl-D-aspartate receptors. *J Pharmacol Exp Ther* **260**, 1209-1213 (1992).

APPROVED BY THE YALE UNIVERSITY IRB 2/8/2018 VALID THROUGH 2/11/2019