STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official title: Elucidating the Neurocircuitry of Irritability with High-Field Neuroimaging to Identify Novel Therapeutic Targets

NCT number: NCT05046184

IRB Approved date: 02-16-26

Elucidating the Neurocircuitry of Irritability with High-Field Neuroimaging to Identify Novel Therapeutic Targets

Short Title: Neurocircuitry of Irritability

Principal Investigator: Manish K. Jha, MBBS

Funded by: National Institute of Mental Health

Version Number: v.1.7

04 DECEMBER 2025

Table of Contents

STATEMENT OF COMPLIANCE 1
1 PROTOCOL SUMMARY 2
1.1 Synopsis 2
1.2 Schema 4
1.3 Schedule of Activities (SoA) 5
2 INTRODUCTION 6
2.1 Study Rationale 6
2.2 Background 6
2.3 Risk/Benefit Assessment 8
2.3.1 Known Potential Risks 8
2.3.2 Known Potential Benefits 10
2.3.3 Assessment of Potential Risks and Benefits 10
3 OBJECTIVES AND ENDPOINTS 11
4 STUDY DESIGN 12
4.1 Overall Design 12
4.2 Scientific Rationale for Study Design 14
4.3 Justification for Dose 14
4.4 End of Study Definition 14
5 STUDY POPULATION 15
5.1 Inclusion Criteria 15
5.2 Exclusion Criteria 15
5.3 Lifestyle Considerations 16
5.4 Screen Failures 16
5.5 Strategies for Recruitment and Retention 16
6 STUDY INTERVENTION 20
6.1 Study Intervention(s) Administration 20
6.1.1 Study Intervention Description 20
6.1.2 Dosing and Administration 20

6.2 Preparation/Handling/Storage/Accountability 21
6.2.1 Acquisition and accountability 21
6.2.2 Formulation, Appearance, Packaging, and Labeling 21
6.2.3 Product Storage and Stability 21
6.2.4 Preparation 21
6.3 Measures to Minimize Bias: Randomization and Blinding 21
6.4 Study Intervention Compliance 22
6.5 Concomitant Therapy 22
6.5.1 Rescue Medicine 27
7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL 27
7.1 Discontinuation of Study Intervention 27
7.2 Participant Discontinuation/Withdrawal from the Study 27
7.3 Lost to Follow-Up 28
8 STUDY ASSESSMENTS AND PROCEDURES 28
8.1 Efficacy Assessments 28
8.2 Safety and Other Assessments 30
8.3 Adverse Events and Serious Adverse Events 31
8.3.1 Definition of Adverse Events (AE) 32
8.3.2 Definition of Serious Adverse Events (SAE) 32
8.3.3 Classification of an Adverse Event 32
8.3.4 Time Period and Frequency for Event Assessment and Follow-Up 34
8.3.5 Adverse Event Reporting 34
8.3.6 Serious Adverse Event Reporting 35
8.3.7 Reporting Events to Participants 37
8.3.8 Events of Special Interest 37
8.3.9 Reporting of Pregnancy 37
8.4 Unanticipated Problems 37
8.4.1 Definition of Unanticipated Problems (UP) 37
8.4.2 Unanticipated Problem Reporting 37
8.4.3 Reporting Unanticipated Problems to Participants 38
9 STATISTICAL CONSIDERATIONS 38
9.1 Statistical Hypotheses 38
9.2 Sample Size Determination 38
9.3 Populations for Analyses 39
9.4 Statistical Analyses 39
9.4.1 General Approach 39
9.4.2 Analysis of the Primary Efficacy Endpoint(s) 40
9.4.3 Analysis of the Secondary Endpoint(s) 40
9.4.4 Safety Analyses 41
9.4.5 Baseline Descriptive Statistics 41
9.4.6 Planned Interim Analyses 41
9.4.7 Sub-Group Analyses 41
9.4.8 Tabulation of Individual participant Data 41
9.4.9 Exploratory Analyses 42
10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS 42
10.1 Regulatory, Ethical, and Study Oversight Considerations 42

10.1.1 Informed Consent Process 42
10.1.2 Study Discontinuation and Closure 44
10.1.3 Confidentiality and Privacy 45
10.1.4 Future Use of Stored Specimens and Data 46
10.1.5 Key Roles and Study Governance 46
10.1.6 Safety Oversight 47
10.1.7 Clinical Monitoring 47
10.1.8 Quality Assurance and Quality Control 48
10.1.9 Data Handling and Record Keeping 48
10.1.10 Protocol Deviations 49
10.1.11 Publication and Data Sharing Policy 49
10.1.12 Conflict of Interest Policy 50
10.2 Additional Considerations 50
10.3 Abbreviations 51
10.4 Protocol Amendment History 53
11 REFERENCES 57
12 APPENDICES 62

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, 21 CFR Part 11, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

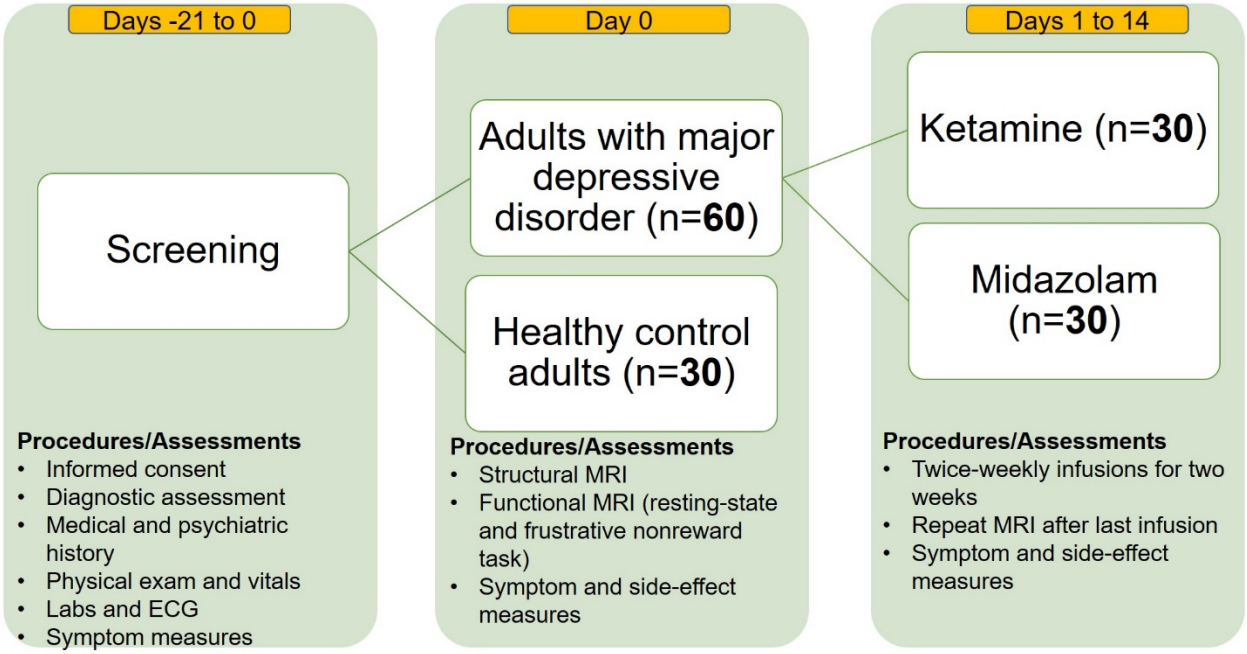
1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Elucidating the Neurocircuitry of Irritability with High-Field Neuroimaging to Identify Novel Therapeutic Targets
Study Description:	The proposed study **aims** to **(Aim 1)** identify dysfunctions in neurocircuitry that engender irritability, and **(Aim 2)** determine how changes in neurocircuit function related to change in irritability. We will accomplish **Aim 1** with resting-state and frustrative nonreward (FNR) task-based fMRI data from n=30 HCs and n=60 subjects with MDD ((Fig 3). To facilitate matching of HCs and MDDs, we will enroll up to 45 HCs and up to 65 subjects with MDD. For **Aim 2**, we will randomize the MDD cohort (n≥60 [up to 65, to facilitate matching with HCs]; same as Aim 1) to 2 weeks of twice-weekly 40-minutes long intravenous infusion of either ketamine (0.5 mg/kg) or midazolam (0.02 mg/kg) in a double-blind parallel-arm fashion, and by repeating clinical assessments and fMRI scans after the last infusion. The central hypothesis of the proposed study is that striatum is a key hub in the neurocircuitry of irritability, and that treatment-related improvement in irritability is associated with normalization of these neurocircuit functioning.
Objectives:	Primary Objectives: Aim 1.1. To identify dysfunctions in resting-state functional neurocircuit that engender irritability. Aim 1.2. To identify task-related dysfunctions neurocircuitry that engender irritability. Aim 2.1. To determine how changes in neurocircuit function relate to changes in irritability. Aim 2.2. To determine changes in neurocircuit function with ketamine versus midazolam.
	Secondary Objectives: To evaluate changes in symptom severity (such as irritability, depression, and anxious arousal) and side effects over two-weeks of treatment with ketamine versus midazolam in adults with major depressive disorder. To explore whether neurocircuit function changes with ketamine versus midazolam are associated with changes in irritability.
Endpoints:	Primary Endpoints: • *Association of irritability with striatum-habenula functional connectivity*. Cross-sectional association between symptoms of irritability and resting-state functional magnetic resonance imaging (fMRI) measures of striatum-habenula functional connectivity in healthy controls and in adults with major depressive disorder (MDD). • *Association of irritability with striatal response to frustrative nonreward (FNR)*. Cross-sectional association between symptoms of irritability and task-based fMRI response of the striatum to FNR in healthy controls and in adults with MDD. • *Treatment-related change in striatum-habenula functional connectivity.* Baseline-to-week-2 changes in resting-state striatum-

	habenula functional connectivity after 2 weeks of twice-weekly infusions of ketamine versus midazolam in adults with MDD. • *Treatment-related change in striatal response to FNR.* Baseline-to-week-2 changes in striatal response to FNR with two weeks of twice-weekly infusions of ketamine versus midazolam in adults with MDD. Secondary Endpoints (Baseline-to-week-2, only subjects with MDD): • Changes in symptoms of irritability after two weeks of twice-weekly infusions of ketamine versus midazolam. • Change in symptoms of depression (including suicidal ideation) and associated symptoms (including anxious arousal) and behaviors (including anger attacks) after two weeks of twice-weekly infusions of ketamine versus midazolam. • Differences in side-effects of ketamine versus midazolam after two weeks of twice-weekly infusions. Exploratory endpoint: To evaluate if changes in neurocircuit function with ketamine mediate treatment-related improvement in irritability. Exploratory analyses will also evaluate neurocircuit mechanisms using measures of cerebral perfusion.
Study Population:	The research plan calls for the enrollment of N=30 healthy control (HC) adults (aged 18-65 years) and N=60 adults with major depressive disorder (MDD, aged 18-65 years) at a single site. To facilitate matching of HCs and MDDs, we will enroll up to 45 HCs and up to 65 subjects with MDD.
Phase:	Phase 2
Description of Sites/Facilities Enrolling Participants:	The human research will be conducted at the University of Texas Southwestern Medical Center (UTSW). Research will be conducted at the Center for Depression Research and Clinical Care (CDRC) and the Clinical Research Unit (CRU). Neuroimaging will be completed at the Advanced Imaging Research Center (AIRC).
Description of Study Intervention:	Subjects with MDD will receive 2 weeks of twice-weekly 40-minutes long IV infusion of either ketamine (0.5 mg/kg) or midazolam (0.02 mg/kg) in a double-blind parallel-arm fashion. Each drug (ketamine or midazolam) will be dissolved in 0.9% saline in a total volume of 100 mL and administered with an infusion pump at a constant rate.
Study Duration:	The proposed study is to be completed in 60 months. The study is anticipated to begin in October 2021 and recruit for the following 5 years until the study is complete (tentatively primary analyses completed by September 2026).
Participant Duration:	For Healthy Control subjects: The duration of an individual HC subject's participation in the study will be a maximum of 3 weeks including the screening and baseline visit. For subjects with MDD: The duration of an individual MDD subject's participation in the study will be a maximum of 5 weeks including the screening, baseline, infusion, and follow-up visits.

1.2 SCHEMA*



** To facilitate matching of healthy controls and major depressive disorder groups, we will enroll up to 45 healthy control adults and up to 65 adults with major depressive disorder.*

1.3 SCHEDULE OF ACTIVITIES (SOA)

Table 1. Schedule of assessments and activities

	Screening	Baseline *	Infusion #1	Infusion #2	Infusion #3	Infusion #4	Study Exit / ET^^
Days	**-21 to 0**	**0**	**2±1**	**5±1**	**8±1**	**11±1**	**14±2^**
Informed consent	x						
Demographics	x						
Medical history	x						
Physical examination	x						
RLHQ, SCQ	x						
Psychiatric history	x						
MINI, ATRQ	x						
LSC-R, CTQ	x						
STOP-Bang	x						
Labs, ECG	x						
Vital signs, weight, BMI	x	x	x	x	x	x	x
Drug screen, β-HCG	x	x	x	x	x	x	x
Concomitant meds	x	x	x	x	x	x	x
Adverse events	x	x	x	x	x	x	x
Neuroimaging#							
Structural MRI		x					x
Resting-state fMRI		x					x
Arterial Spin labeling		x					x
FNR task-based fMRI		x					x
Diffusion tensor imaging		x					x
Symptom and functional impairment measures							
CAST-IRR	x	x	x	x	x	x	x
NIH Toolbox Anger Affect	x	x	x	x	x	x	x
AAQ,$ AQ, BITe, ARI	x	x	x	x	x	x	x
MADRS, QIDS-SR	x	x	x	x	x	x	x
MASQ, GAD-7, P-FIBS	x	x	x	x	x	x	x
C-SSRS, CHRT	x	x	x	x	x	x	x
SHAPS, TEPS	x	x	x	x	x	x	x
Q-LES-Q SF, WPAI	x	x	x	x	x	x	x
Study drug							
Ketamine or midazolam			x	x	x	x	
Monitoring of side effects with study drug							
CADSS			x	x	x	x	x
PRISE			x	x	x	x	x
BPRS-Positive			x	x	x	x	x
Clinician assessment	x	x	x	x	x	x	x

LEGENDS: Grey shaded only in adults with MDD. * HC subjects will be exited from the protocol after the completion of baseline visit. AAQ, Anger Attack Questionnaire; AQ, Aggression Questionnaire; ARI, Affective Reactivity Index; ATRQ, Antidepressant Treatment Response Questionnaire; BITe, Brief Irritability Test; BPRS-Positive, 4-item positive subscale of Brief Psychiatric Rating Scale; CADSS, Clinician-

Administered Dissociative States Scale; CAST-IRR, 10-items extended version of Concise Associated Symptom Tracking irritability domain; CHRT, Concise Health Risk Tracking; C-SSRS, Columbia Suicide Severity Rating Scale; CTQ, Childhood Trauma Questionnaire; GAD-7, 7-item Generalized Anxiety Disorder scale; LSC-R, Life-Stressor Checklist Revised; MADRS, Montgomery-Åsberg Depression Rating Scale; MASQ, Mood and Anxiety Symptom Questionnaire; MINI, Mini-International Neuropsychiatric Interview; MRI, magnetic resonance imaging; P-FIBS, Pain Frequency, Intensity, and Burden Scale; PRISE, Patient rated inventory of side effects; Q-LES-Q SF, Quality of Life Enjoyment and Satisfaction Questionnaire Short Form; QIDS-SR, Quick Inventory of Depressive Symptomatology Self-Report; RLHQ, Reproductive Lifecycle and Hormone Questionnaire; SCQ, Self-administered comorbidity questionnaire; SHAPS, Snaith Hamilton Pleasure Scale; TEPS, Temporal Experience of Pleasure Scale; and WPAI, Work Productivity and Activity Impairment scale.
Participants must complete AIRC MRI procedure screening form on screening visit and on day of neuroimaging to ensure that there are no contraindications to the MRI.
$ AAQ will be completed only at Baseline and Study Exit visits.
^ Study exit visit cannot be completed on the day of last infusion visit.
^^ Early Termination (ET), this visit can be completed at any time point upon decision to early terminate/ withdraw a participant from the study.
List of assessments also provided in Appendix A & B.

2 INTRODUCTION

2.1 STUDY RATIONALE

Irritability is an important yet understudied symptom in adults with major depressive disorder (MDD). It affects 40-50% of adults with MDD and is associated with poorer quality of life and more chronic course of illness. We have also found in three clinical trials of adults with MDD that symptoms of irritability are associated with severity of active suicidal ideation. Despite the large public health burden of irritability, treatment options remain limited as commonly used medications (such as antidepressants) are often ineffective and may even worsen irritability in 5-10% of adults with MDD. As neuroimaging studies to date have focused on research of irritability in pediatric samples, neurocircuit mechanisms of irritability in adults remain ill defined. This knowledge gap has served to limit development of new treatments. The proposed study will fill this knowledge gap by identifying dysfunctions in brain circuits that are associated with irritability and determining how treatment-related reductions in irritability relate to changes in neurocircuit functioning

To accomplish these aims, we will enroll male and female adults aged 18-65 years [n=60 adults with MDD and n=30 age and sex matched healthy controls (HC)]; to facilitate matching of HCs and MDDs, we will enroll up to 45 HCs and up to 65 subjects with MDD. All subjects (HC and MDD) will undergo resting-state and task-based high-field [3 Tesla (3T)] functional MRI (fMRI) and arterial spin labeling to characterize how brain function at a neurocircuit level (both at rest and in response to a behavioral task) are associated with irritability [quantified with the 5-item irritability domain of Concise Associated Symptom Tracking scale (CAST-IRR)]. The MDD cohort (i.e., aforementioned subjects with MDD, n≥60 [up to 65, to facilitate matching with HCs]) will then be randomly allocated to four 40-minute intravenous infusions over two weeks (total of 4 infusion) of either ketamine (at the dose of 0.5 mg/kg per infusion) or midazolam (at the dose of 0.02 mg/kg per infusion) in a double-blind parallel-arm fashion. Clinical assessments and 3T MRI scans will be repeated after the last infusion to evaluate how pre-to-post treatment (ketamine versus midazolam) changes in neurocircuit function using resting-state

and FNR task-based fMRI are related to the association between changes in neurocircuit function and irritability.

2.2 BACKGROUND

2.2.A Irritability is an often-ignored yet debilitating feature of MDD in adults.

Irritability is unique among features of MDD as it is a core criterion symptom for children and adolescents but not for adults.[1] Given that 40-50% of adults with MDD report significant irritability[2-4] and the 12-month prevalence of MDD in adult Americans is 10.4%,[5] irritability may affect 8-10 million adults in the United States (US) annually**.** Irritability in adults with MDD is associated with more chronic course of illness and poorer quality of life.[2,3] We have found that changes in irritability with treatment can independently (i.e., even after controlling for changes in overall depression severity) prognosticate longer-term outcomes.[6,7] These findings suggest that irritability is an important feature of MDD in adults. Recent reports have also linked irritability during childhood/adolescence to suicide-related outcomes later in life.[8,9] Similarly, in three clinical trials of adults with MDD (n=1227), we recently found that irritability was correlated positively (r=0.31-0.73) with suicidal ideation at the same visit. We also found that greater reductions in irritability from baseline-to-week-2 independently predicted lower levels of suicidal ideation from week-2-to-week-8.[10] These associations between irritability and suicidal ideation are of great public health significance given that mortality due to suicide has increased by 33% over the past two decades in the US.[11]
Treatments for irritability remain limited and commonly used antidepressants may even worsen irritability in about 6% of adults with MDD.[12] There is an urgent need to develop novel treatments that specifically target irritability. However, development of novel treatments has been hindered by the gap in scientific knowledge regarding neurocircuit mechanisms of irritability in adults given that research to date has focused on irritability in pediatric samples.[13,14] Therefore, this study aims to identify dysfunctions in neurocircuitry that engender irritability in adults and determine how changes in neurocircuit function relate to changes in irritability.

2.2.B The striatum is a key hub in the functional neurocircuitry of irritability.
Pediatric neuroimaging studies implicate the striatum [dorsal (caudate and putamen) and ventral] as a key hub in the neurocircuitry of irritability.[13] However, irritability-related dysfunctions of the striatum in adults with MDD remain unknown. We recently used 3T resting-state functional magnetic resonance imaging (fMRI) scans from adults with MDD (n=273) who completed the irritability domain of Concise Associated Symptom Tracking scale (CAST-IRR) in the Establishing Moderators and Biosignatures of Antidepressant Response in Clinical Care (EMBARC) study.[15] Using previously reported methods (Chin Fatt, Jha et al.[16]), we computed functional connectivity (FC) among 121 brain regions of interest (n=7260 FC pairs). As n of FC pairs >> n of adults with MDD, we used elastic net for variable selection and found that 78 FC pairs were associated with the CAST-IRR score. We then focused on the 31 pairs that contained a striatal region and used canonical correlation analysis to estimate a linear combination. We found that together these 31 striatum-FC pairs explained over 27% of variance in the CAST-IRR total score. Therefore, our findings support the notion that the striatum is a key hub in the functional neurocircuitry of irritability in adults with MDD.

2.2.C Neuroscience-based behavioral tasks may help characterize irritability-related striatal dysfunctions.
Given the key role of striatum in reward learning and prediction,[17] it's dysfunction may be linked to aberrant **frustrative nonreward (FNR)**, an evolutionarily conserved aversive motivational state that is evoked by the omission of an expected reward.[14,18] Supporting this notion, Perlman et al. found that, in

a cohort of 6-9-year old irritable children (n=26), the severity of irritability was negatively correlated (r = -0.53) with striatal (right dorsal putamen) activation in response to surprising reward omission.[19] Deveney et al. had previously found that a pediatric sample selected for severe irritability (aged 8-17 years old, n=19) exhibited markedly less activation of bilateral striatum than healthy children (n=23) when denied an expected reward.[20] In a task designed to look at both reward feedback and the attentional event that followed feedback, Tseng et al. found that higher irritability was associated with greater activation of bilateral caudate when orienting attention after reward omission.[21] These studies were all limited by their exclusion of adults. Furthermore, these studies did not evaluate the role of habenula (Hb), which signals reward omission (i.e., value of the reward is lower than expected) across species including humans,[22] receives input from striatum,[23] affects midbrain dopaminergic neurons,[22] and modulates response to FNR in preclinical studies.[24] Based on these findings and the paucity of research in adults, we aim to characterize the association of irritability with striatum-Hb neurocircuit function and with striatal response to FNR task.

2.2.D Ketamine is an optimal probe to induce rapid improvement in symptoms of irritability.
Ketamine may reduce irritability as suggested by a recent report in adults with depression.[25] Similarly, we have found, in a transdiagnostic sample of depressed patients with acute suicidality,[26] that a single infusion of ketamine (0.5 mg/kg) rapidly reduced irritability for up to 7 days. We found that the difference between ketamine (n=12) versus midazolam (n=12) was significant (p=0.03) even after controlling for changes in overall depression severity. In additional data from the RAPID Ketamine study,[27] we found that a single infusion of ketamine (0.5 mg/kg, n=22) was associated with greater reduction in irritability (measured with a single item of the Symptoms of Depression Questionnaire) than midazolam (n=19, Cohen's d = 0.77, p<0.01) in patients with depression. Combined with the evidence from preclinical studies[28] and from neuroimaging studies of healthy controls (HCs),[29] and patients with depression[30] that racemic ketamine (or its s-enantiomer) modulates striatal activity, our preliminary data support the use of ketamine as a pharmacological probe in this study of irritability.

2.2.E Gaps in knowledge regarding the neurocircuitry of irritability.
The neurocircuitry of irritability in adults remains ill-defined as research to date has focused on irritability in pediatric samples[13,14] While extant literature and our preliminary findings support the striatum as a key hub, how irritability in adults relates to frustration-induced changes in striatal neurocircuit function remains unknown. Furthermore, studies of striatal response to FNR task have been cross-sectional in nature.[19-21] How striatal responses change after treatment-related symptom improvement remains unclear. While ketamine has been shown to modulate the functioning of striatum and Hb,[29,30,33] its effect on striatum-Hb functional connectivity and on striatal response to FNR has not been characterized previously. Therefore, using 3T multiband fMRI in adults with MDD and HC (the latter to capture a wide range of irritability symptom severity), the proposed project will bridge these gaps in knowledge and elucidate the functional neurocircuitry of irritability with (i) an FNR task to evoke frustration in-scanner and (ii) a pharmacological probe with strong evidence for rapid and robust reduction in irritability. This is of utmost importance because the status quo as it pertains to the study of irritability has resulted in a dearth of research on neurobiology of irritability in adults and therefore has hindered the development of treatments for irritability.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

2.3.1.A Risks of fMRI Procedures.

The fMRI procedure is very safe and exposes subjects to minimal risk or discomfort. The MRI scanning involves the subject placing his/her head in a tube and some subjects may experience a feeling of claustrophobia while in the scanner. The subjects are always in voice contact with a researcher or technician and the scanning session can be terminated quickly if the subject so requests. Subjects with metal objects including surgical clips or metallic prostheses (such as an artificial hip or knee), or shrapnel (metal fragments) will be excluded. The risk to the subject in the event that there is susceptible metal in or on his or her body is significant and may include death. Every subject will be carefully screened using existing standard operating procedures of the Advanced Imaging Research Center (AIRC) for the presence of susceptible metal.

2.3.1.B Ketamine Related Risks (Applicable only to Aim 2).

Ketamine is an analgesic agent and general anesthetic for human and veterinary use. It also has dissociative and psychedelic properties. For that reason, ketamine was categorized as a Schedule III drug in the Controlled Substance Act in August 1999. Over the past several decades, ketamine has been administered as an anesthetic to several million adults and children and has a very favorable safety profile. In addition, it has been used in psychophysiological studies in normal volunteers and patients with severe mental disorders including MDD (with similar doses to that of our proposed study) safely for 3 decades. Furthermore, the s-enantiomer of ketamine was approved for use as an adjunctive treatment for MDD by the Food & Drug Administration, reflecting its favorable risk versus benefit profile. Perceptual disturbances may manifest as vivid dreaming, visualization of psychedelic color, suspension in space, kaleidoscopic floating, and out-of-body experiences. Some patients report the psychic experiences as bizarre or frightening, while others describe them as pleasurable, joyful, or fascinating. When such reactions occur, they are usually mild and short-lived, resolving in several minutes following medication administration. Other side effects commonly associated with ketamine include increases in blood pressure and/or heart rate which will be monitored closely during, and for 1 hour after the end of, the infusions. Use of ketamine may also be associated with nausea/vomiting. Interruption or discontinuation of infusion is usually adequate to resolve these ketamine-associated acute side effects.

2.3.1.C Midazolam Related Risks (Applicable only to Aim 2).

Side effects associated with midazolam at the dose given are expected to be mild. Midazolam has good cardiovascular stability, and induces only a transient and mild respiratory depression compared to other benzodiazepines. Midazolam produces a short-duration anterograde amnesia that is not likely to last beyond 30-60 min following infusion. Fluctuations in vital signs were the most frequently observed findings following parenteral administration of midazolam in adults and included decreased tidal volume and/or respiratory rate decrease (23.3% of patients following IV) and apnea (15.4% of patients following IV administration), as well as variations in blood pressure and pulse rate (Midazolam Package Insert, 2007). The majority of serious adverse effects, particularly those associated with oxygenation and ventilation, have been reported when midazolam is administered with other medications capable of depressing the central nervous system. The incidence of such events is higher in patients undergoing procedures involving the airway without the protective effect of an endotracheal tube, e.g., upper endoscopy and dental procedures.

2.3.1.D Psychological Screening Risks

Answering questions related to mental health and past experiences may be stressful. Research interviews will be interrupted if subjects become distressed or object to answering questions. If in the judgment of the PI or study-affiliated psychiatrist, the patient has worsened to such a degree that further participation would put the patient at risk, then the subject will be discontinued from the study

and provided appropriate clinical care. Also, at any point during the study any subject meeting DSM-5 criteria for a manic episode or psychosis based on clinical interview by the Study PI or study-affiliated psychiatrist will be discontinued and provided appropriate clinical care.

2.3.1.E Medical Screening Risks
Subjects might feel pain during blood draw or when IV is inserted, and can develop bruising, and rarely, infection. The sight of blood or insertion of IV cannula may also cause dizziness or brief loss of consciousness due to vasovagal response. Therefore, all blood draws will be conducted by trained personnel, using aseptic precautions, and in a setting where the subject is resting comfortably and the risk of fall is minimized.

2.3.1.F Privacy and Confidentiality Risks
As the research study involves collection of protected and sensitive health information, there is a potential risk of loss of confidentiality. This risk will be conveyed to subjects as part of informed consent and will be minimized by using SOPs at our Center which comply with Health Insurance Portability and Accountability Act rules.

2.3.1.G Financial risks
Subjects will not be charged for any study procedures. However, in case they experience an adverse effect and seek care, they or their insurance provider will be responsible for this clinical care. This risk will be informed to the subject as part of informed consent process.

2.3.1.H Restrictions to the ongoing psychiatric care
Participation in the study may impose limitations to the ongoing psychiatric care as subjects must either be free of psychotropic medications (including antidepressants, antipsychotics, benzodiazepines, mood stabilizers, sedative/hypnotics, dopamine agonists, stimulants, buspirone, and triptans) and certain anticonvulsants (topiramate and levetiracetam) or be stable on these medications for four weeks prior to the baseline visit, and agree to these restrictions during the course of the study.

2.3.2 KNOWN POTENTIAL BENEFITS
All study subjects will be informed that there may not be any potential benefit to them individually as a result of taking part in the study. All study subjects will receive without cost an extensive psychiatric and medical evaluation which may be of potential benefit for the subjects. Furthermore, subjects with MDD may experience improvement in symptoms, including irritability, with one or both of the study drugs. Participating subjects may gain knowledge about the biological and psychological processes of depression or anxiety disorders, which they may find interesting and helpful in dealing with illness. No other direct benefits result from study participation.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS
While irritability is a prominent feature of illness in 40-50% of adults with MDD, it remains understudied. Commonly used antidepressants are often ineffective and may paradoxically worsen irritability in a sizeable minority of patients. Efforts to identify new treatments for irritability have been hindered by our limited understanding of its underlying biological mechanisms. Therefore, the knowledge gained from the proposed study may elucidate the neurocircuit mechanisms of irritability and potentially facilitate the development of novel circuit-specific treatments. The relevance of this knowledge may extend beyond MDD as irritability is one of the most transdiagnostic symptoms and is associated with

significant burden across a range of illnesses including generalized anxiety disorder, bipolar disorder, post-traumatic stress disorder, and border-line personality disorder.
Based on our previous experience with neuroimaging and ketamine administered intravenously, and on the scientific and medical literature, we believe the subjects' risks to be low. The risks involved in the procedures specific to this study (e.g., neuroimaging and behavioral rating) are very low. There are additional relatively low risks associated with ketamine or midazolam exposure during the clinical trial, which have been briefly described herein. We have taken several precautions (in restricting the eligibility) and in devising mitigating strategies to further reduce the risks of treatment with ketamine or midazolam. We believe the risks are justified as identifying the neurocircuit functions that change with treatment-specific improvement in irritability may inform future mechanistic studies in preclinical models and larger-scale experimental medicine studies in human patients, both of which are needed to ameliorate the public health burden of irritability. Overall, we believe that the totality of risk associated with this research study is reasonable and acceptable in relation to the potential long-term public health benefits. Untreated irritability represents a major public health problem, with considerable cost to the individual, the family, and the community, and potentially increased risk of suicide-related outcomes. By increasing understanding of the neurocircuitry of irritability and identifying potential treatment targets using an experimental medicine approach, this study has the potential to advance urgently needed treatment discovery for irritability.

3 OBJECTIVES AND ENDPOINTS

The overall objective of the proposed study is to characterize the functional neurocircuitry of irritability, and to identify changes in neurocircuit function that are specific to treatment-related symptomatic improvement.

3.A Aims of the study.

Aim 1.1. To identify dysfunctions in resting-state functional neurocircuit that engender irritability.
Hypothesis 1.1: Higher levels of irritability will be associated with greater striatum-habenula connectivity.
Aim 1.2. To identify task-related dysfunctions neurocircuitry that engender irritability.
Hypothesis 1.2: Higher levels of irritability will be associated with greater striatal activation after frustrative nonreward (FNR) task.
Aim 2.1. To determine how changes in neurocircuit function relate to changes in irritability.
Hypothesis 2.1: Treatment with ketamine vs. midazolam will result in greater changes in striatum-habenula FC.
Aim 2.2. To determine changes in neurocircuit function with ketamine versus midazolam.
Hypothesis 2.2: Treatment with ketamine vs. midazolam will result in greater changes in in striatal response to FNR.
Exploratory aim: Evaluate whether neurocircuit function changes with ketamine vs. midazolam are associated with changes in irritability.

3.B Primary endpoints:

- *Association of irritability with striatum-habenula functional connectivity*. Cross-sectional association between symptoms of irritability and resting-state functional magnetic resonance imaging (fMRI) measures of striatum-habenula functional connectivity in healthy controls and in adults with major depressive disorder (MDD).
- *Association of irritability with striatal response to frustrative nonreward (FNR)*. Cross-sectional association between symptoms of irritability and task-based fMRI response of the striatum to FNR in healthy controls and in adults with MDD.

- *Treatment-related change in striatum-habenula functional connectivity.* Baseline-to-week-2 changes in resting-state striatum-habenula functional connectivity after 2 weeks of twice-weekly infusions of ketamine versus midazolam in adults with MDD.
- *Treatment-related change in striatal response to FNR.* Baseline-to-week-2 changes in striatal response to FNR with two weeks of twice-weekly infusions of ketamine versus midazolam in adults with MDD.

3.C. Secondary endpoints (Baseline-to-week-2, only subjects with MDD):

- Changes in symptoms of irritability after two weeks of twice-weekly infusions of ketamine versus midazolam.
- Change in symptoms of depression (including suicidal ideation) and associated symptoms (including anxious arousal) and behaviors (including anger attacks) after two weeks of twice-weekly infusions of ketamine versus midazolam.
- Differences in side-effects of ketamine versus midazolam after two weeks of twice-weekly infusions.

3.D Exploratory endpoint:

To evaluate if changes in neurocircuit function with ketamine mediate treatment-related improvement in irritability. Exploratory analyses will also evaluate neurocircuit mechanisms using measures of cerebral perfusion.

4 STUDY DESIGN

4.1 OVERALL DESIGN

The aims of the proposed research project are to (1) identify dysfunctions in neurocircuitry that engender irritability in adults, and 2) determine how changes in irritability relate to changes in neurocircuit function. To accomplish these aims, we will enroll male and female adults aged 18-65 years [**n=30 healthy controls (HC) and n=60 adults with major depressive disorder (MDD); HC and MDD will be age and sex matched**] [to facilitate matching of HCs and MDDs, we will enroll up to 45 HCs and up to 65 subjects with MDD] over the 5-year grant period (approximately 1.7 subjects per month over the 54 month recruitment period). All subjects are required to be medically healthy as determined by medical history, physical examination, electrocardiogram, screening blood laboratory tests, urine analysis, and urine toxicology drug screen. Adults with MDD must either be free of psychotropic medications or be stable on their concurrent medications at screening visit and for at least four weeks prior to the first magnetic resonance imaging (MRI) scan.

All subjects (HC and MDD) will undergo resting-state and task-based high-field [3 Tesla (3T)] functional MRI (fMRI) and cerebral prefusion to characterize the (i) patterns of functional connectivity (**Aim 1.1**) and (ii) neural responses to a behavioral task eliciting frustrative nonreward (FNR) (**Aim 1.2**) that are associated with irritability [quantified with the 5-item irritability domain of Concise Associated Symptom Tracking scale (CAST-IRR)]. The MDD cohort (n≥60 [up to 65, to facilitate matching with HCs]) will then be randomized to four 40-minute intravenous infusions over two weeks of either ketamine (0.5 mg/kg) or midazolam (0.02 mg/kg) in a double-blind, parallel-arm fashion. Clinical assessments and 3T MRI scans will be repeated after the last infusion to evaluate (i) pre-to-post treatment changes in neurocircuit function using resting-state and FNR task-based fMRI with ketamine versus midazolam (**Aim 2.1**) and (ii) the association between changes in neurocircuit function and irritability (**Aim 2.2**).

All procedures related to this study will be conducted at a **single site**, University of Texas Southwestern Medical Center (UTSW), Dallas, TX.

We have significant experience in the enrollment of adult subjects with MDD and have robust standard operating procedures (SOP) in place to ensure the safety of subjects. Any subject who requires initiation of medication emergently will receive it without delay. In no case will a subject be denied necessary medical or psychiatric treatment for reasons related to study participation. A close collaboration with the clinical services at our center allows for rapid referral and data collection for research prior to the initiation of treatment.

Special populations, including fetuses, neonates, pregnant women, children (<18 years of age), prisoners, and institutionalized individuals will not be included in the proposed research.

STUDY PROCEDURES

Neuroimaging procedures

To identify dysfunctions in neurocircuitry that engender irritability, all subjects (MDD and HC) will undergo resting-state and task-based functional magnetic resonance imaging (fMRI) and ASL scans. To determine how changes in irritability relate to changes in neurocircuit function, adults with MDD will repeat the fMRI scans after 2 weeks of twice-weekly infusions of ketamine or midazolam. We will scan all subjects using the 3T MRI scanner (Magnetom Prisma, Siemens) at AIRC, and will ensure that all subjects are physically comfortable throughout MRI. Each scan session is expected to last 1.5 hours, with less than 75 minutes of subject time in the magnet. Each scan session will include: T1-weighted (T1w) magnetization prepared gradient echo (MPRAGE) and T2w anatomical images; resting-state and FNR task-based multiband-fMRI, arterial spin labeling (ASL) for cerebral perfusion, and diffusion tensor imaging (DTI) for structural connectivity (DTI will provide information about structural connectivity and may be omitted to ensure that subject time in the magnet is less than 75 minutes). The resting-state scan will occur with eyes open before and after task-based scans. Resting-state and task-based scans will have the same acquisition parameters. Physiological data (heart rate, oxygen saturation, electrocardiogram, and respiration rate) that are collected with MRI procedures will also be recorded. To prepare for neuroimaing, study staff will offer participants an opportunity to spend time in a mock scanner that simulates an MRI scanning session to ease any participant apprehension.

Pre-fMRI Training in the Psychological Paradigms: On the day-of-scan, subjects will be familiarized with the frustrative nonreward task where they will complete the task with accurate feedback but no monetary incentive (10 trials). These practice sessions are expected to last less than 5 minutes. Subjects who are unable to demonstrate a clear understanding of the tasks will not be scanned.

Behavioral task for frustrative nonreward: In this task, also referred to as Affective Posner Task, a reward expectation is first established with a monetary incentive for the correct response, which is then unexpectedly omitted to evoke frustration. Subjects are asked to identify a target with a button press (left or right) following a cue presentation. The target appears in same location as the cue for 75% of trials and in the opposite location for 25% of trials. The task comprises of (1) a pre-task training (10 trials outside the scanner trials during which they receive accurate feedback on their performance but do not win or lose money), (2) two non-frustration runs (outside the scanner), (3) a pilot frustration run inside the scanner, and (4) two frustration runs inside the scanner. Subjects are instructed that they need to respond both correctly and quickly to win money. During the two non-frustration runs, which consist of 50 trials each, subjects receive accurate feedback on their performance, earning $0.50 for every correct response and losing $0.50 for every incorrect response. The pilot frustration run of the task consists of 32 trials and is used to acclimate subjects to the frustration runs; subjects receive frustrating feedback on 12.5% of correct trials. On trials with frustrating feedback, subjects are informed that they were “too slow,” and lost money regardless of their actual reaction time. In the two frustration runs, which comprise a total of 100 trials, subjects receive frustrating feedback on 60% of correct trials. After each

run of the task, subjects rate their feelings of unhappiness and frustration using 9-point Likert scales (i.e., 1 = “happy” or “not at all frustrated”; 9 = “sad” or “extremely frustrated”).

Subjects will use a desktop or laptop computer to complete the pre-scanner trials. For the in-scanner portion of the FNR task, stimuli will be displayed on a translucent screen attached to the rear of the scanner bore using 'BOLDscreen32', a television screen in the scanner room on which 1080p high resolution stimuli can be directly displayed. Subjects will view the screen using an angled double mirror system mounted to the head coil. All trials will be presented using E-prime software. Responses will be recorded using an integrated MR-compatible system developed for the collection of behavioral response data during the fMRI scanning.

Procedures for discussing alternative treatments and medication taper (if applicable).

By consenting to this study, subjects with MDD will temporarily forgo the opportunity to receive regular psychiatric care in the community that may involve changes in their antidepressant treatment regimen. For those with MDD, these options would include treatment with antidepressants, antidepressant augmentation strategies (atypical antipsychotics, intranasal esketamine), psychotherapy augmentation, or other neurostimulation (repetitive transcranial magnetic stimulation, electroconvulsive therapy, or vagus nerve stimulation). These alternatives will be clearly explained to all subjects with MDD. Related to **Aim 2**, subjects will also be told that although ketamine is FDA approved for anesthetic purposes, it is not FDA approved for the treatment of depression and therefore would not be available for use after the study. However, the s-enantiomer of ketamine called esketamine is now FDA approved for treatment-resistant depression in combination with an antidepressant medication.

Subjects with MDD who are taking psychotropic medication at the time of screening will not be taken off their medications for the purpose of this study. To be eligible for the study, they will be asked to have taken a stable dose of permitted psychotropic medication for at least four weeks (see Table 2 in section 6.5 about concomitant medications). **No medication will be discontinued if it is believed that the patient has significantly benefited from it.** Should hospitalization be required, patients may be admitted to the inpatient unit at the UT Southwestern Medical Center. The unit staff has extensive experience in treating adults with severe psychiatric illnesses and is adept at maintaining patient safety and comfort.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

We will include HC and MDD (in a current major depressive episode with comorbid anxiety and stress-related disorders allowed) to capture a wide range of irritability symptom severity. We decided on two-week treatment duration as it allows for (1) repeated infusions in order to attain longer-term symptomatic improvement[112] and (2) adequate interval between pre-and-post administration of FNR task (a prior test-retest study[101] used 2-4-week interval). As a previous report found that twice-weekly infusion produced similar improvements (in depression) as thrice-weekly infusion,[114] we decided to use twice-weekly to minimize participant burden. We decided to use midazolam (0.02 mg/kg to minimize sedation per Grunebaum et al.[108]) as control arm to account for non-specific treatment effects.

4.3 JUSTIFICATION FOR DOSE

Subjects will receive an infusion of either 0.5 mg/kg racemic ketamine hydrochloride or 0.02 mg/kg midazolam. The dose of ketamine is (0.5 mg/kg) has been widely used in studies of patients with MDD.[26,27,34-38] As ketamine at this subanesthetic dose causes minimal sedation, to maintain blinding with

active control, we will use the midazolam at 0.02 mg/kg as it minimizes excessive sedation and is consistent with a recent report in adults with MDD.[39] The total dose of ketamine or midazolam will be decided based on the participant's body weight on the baseline visit and will stay the same for all four infusions. The total ketamine dose will not exceed more than 60 mg/day.

4.4 END OF STUDY DEFINITION

HC participant is considered to have completed the study if he or she has completed the screening and baseline visit. Participant with MDD is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3. The overall study is considered completed when the last participant's last study visit has occurred.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

1. Male or female subjects, 18-65 years of age and body weight less than or equal to 120 kg on baseline visit.
2. Participants must have a level of understanding of the English language sufficient to agree to all tests and examinations required by the study and must be able to participate fully in the informed consent process.
3. For Healthy Controls: Subjects must be free of any lifetime psychiatric condition based on the Mini-International Neuropsychiatric Interview (MINI). For MDD: Subjects must meet Diagnostic and Statistical Manual of Mental Disorders (DSM–5) criteria for current unipolar depression [major depressive disorder (MDD) or persistent depressive disorder (PDD) in a current major depressive episode (MDE)] based on MINI.
4. A female of childbearing potential who is sexually active with a male must agree to use an acceptable method of contraception to avoid pregnancy throughout the study. Throughout the study and for 90 days (one spermatogenesis cycle) after receiving the last dose of study drug (ketamine/midazolam), a male who is sexually active with a female of childbearing potential must use an acceptable method of contraception with his female partner and must agree not to donate sperm. Acceptable form of birth control is defined as either one highly effective (permanent sterilization, intrauterine device or hormonal implant) or two other forms of contraception (such as oral contraceptive pill and condom).
5. Subjects must either be free of psychotropic medications (including antidepressants, antipsychotics, benzodiazepines, mood stabilizers, sedative/hypnotics, dopamine agonists, stimulants, buspirone, and triptans) and certain anticonvulsants (topiramate and levetiracetam) or be stable on these medications for four weeks prior to the baseline visit [first magnetic resonance imaging (MRI) scan].
6. Subjects with MDD should be willing to participate in neuroimaging scans before and after infusions, and be willing to undergo infusions with study drug.

5.2 EXCLUSION CRITERIA

1. Lifetime diagnosis of schizophrenia or any psychotic disorder, bipolar disorder, pervasive developmental disorder or intellectual development disorder.

2. Current diagnosis of obsessive-compulsive disorder, anorexia nervosa or bulimia. Comorbid anxiety, stress and trauma-related disorders are permitted as long as unipolar depression is the primary diagnosis.

3. Diagnosis of a moderate or severe substance use disorder within the past 6 months per MINI; all subjects must have a negative urine toxicology test on the day of the MRI, prior to the scan. *

4. Female subjects who are pregnant, nursing, for may become pregnant. Women of childbearing potential must have a negative urine pregnancy test on the day of the fMRI, prior to scan, and on days of study drug infusion, prior to infusion.

5. Any unstable medical illnesses including hepatic, renal, gastroenterologic, respiratory, cardiovascular (including ischemic heart disease), endocrinologic, immunologic, or hematologic disease.

6. Inadequately treated obstructive sleep apnea (STOP-Bang score of 5-8 if untreated, if using positive airway pressure device then past-month apnea hypopnea index ≥ 15 per hour representing moderate or higher severity).

7. Presence of a significant neurological disease such as Parkinson's disease, primary or secondary seizure disorders, intracranial tumors, or severe head trauma.

8. Presence of neurocognitive or dementing disorders.

9. Clinically significant abnormalities of laboratories, physical examination (including unstable hypertension – systolic blood pressure >170, diastolic blood pressure >100), or electrocardiogram at screening visit.

10. Subjects judged to be at serious and imminent suicidal or homicidal risk by the PI or another study-affiliated psychiatrist.

11. Any contraindications to MRI, including pacemakers or metallic objects in the body.

12. Any claustrophobia or other conditions which may result in inability to lie still in the MRI scanner for 1 hour or more.

13. Allergy to ketamine or midazolam in subjects with MDD.

14. Must not be on any prohibited concomitant medication (as specified in section 6.5 of the protocol).

* Positive urine drug screen related to permitted concomitant medication will be allowed.

5.3 LIFESTYLE CONSIDERATIONS

Not applicable.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a *meeting an exclusion criterion or not meeting an inclusion criterion* may be rescreened *based on PI's decision*. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

5.5.A Planned Recruitment Activities

The research strategy for the present study calls for the enrollment of N=30 healthy control (HC) adults (aged 18-65 years) and N=60 adults with major depressive disorder (MDD, aged 18-65 years) over the 5-year project period. Our total enrollment target of N=30 HC and N=60 MDD subjects reflects individuals who are screened, eligible, scanned and/or treated in the protocol. However, to facilitate matching of HCs and MDDs, we will enroll up to 45 HCs and up to 65 subjects with MDD. Anticipating screen fails, drop outs after enrollment, and lost to follow-ups post randomization, we plan on screening a total of N=180 individuals (N=60 HC and N=120) over the proposed 54-month recruitment period (4.5 years allocated to recruitment from the full 5-year study duration). This enrollment period provides for a highly feasible **~3.3 subjects screened and ~2 subjects who complete baseline visits per month**. This 54-month enrollment period allows for up to 3 months at the beginning of the study for finalization of the study protocol, manual of procedures, development of the study database, and other clinical trial start up activities. Therefore, we will plan to initiate enrollment in Q2 of Year. Following the 3-month start up period and 54-month enrollment period, there will be an additional 3 months remaining in the 60-month project period to allow for data cleaning, analyses and manuscript preparation. With the proposed timeline, the PI and the team have ensured that *there is sufficient time to account for regulatory activities, study staff training, and activities related to data management and statistical analyses*.

The projected enrollment rate of **~3.3 subjects enrolled (i.e., signed consent form) per month** is a highly feasible goal based on current flow of subjects in neuroimaging studies and clinical trials at the Center for Depression Research and Clinical Care (CDRC), and the demonstrated track record of enrollment at similar rates in prior studies recruiting from similar populations. As one of the major research and clinical programs at the University of Texas Southwestern Medical Center, CDRC currently has fifteen full-time faculty members including three psychiatrists, two research psychologists, three PhD biostatisticians, one full-time postdoctoral fellow, three full-time Masters-level clinical raters, five full-time research coordinators, and a program administrator. Investigators at CDRC have a long track record in successfully recruiting subjects and completing studies involving the assessment and treatment of healthy subjects as well as subjects with MDD.

5.5.B Mood Disorders Network

The Center for Depression Research and Clinical Care (CDRC), in the Department of Psychiatry at UT Southwestern Medical Center is one of the foremost depression research facilities in the nation. The Center has a special emphasis on Treatment Resistant Depression (depression that does not remit readily with current antidepressant treatment regimens) and brings together broad expertise, resources and needed infrastructure in research and clinical care. In addition, the CDRC has extensive experience conducting research studies requiring high rates of subject recruitment and retention, as exemplified by these previous multi-site studies: Sequenced Treatment Alternatives to Relieve Depression and Establishing Moderators and Biosignatures of Antidepressant Response in Clinical Care.

The CDRC has developed The Mood Disorders Network (MDN), comprised of affiliates: clinical (e.g., primary care practices, charity clinics) and community (e.g., schools), who are interested in collaborating with CDRC on activities and projects geared toward improving care for those suffering from depression. Many MDN clinical sites use the Vital Sign 6 (VS6) software, an application that allows screening and management of depression, using the principles of Measurement-Based Care (MBC). Using VS6 application, participants are systematically assessed for depression and treatment outcomes are

monitored utilizing multi-step evidence-based treatment algorithms. Providers are then given treatment feedback by CDRC clinicians to assist with depression treatment decisions.

CDRC is creating the UT Southwestern Depression Cohort: a research registry of volunteers consisting of MDN patients who have been consented to learn more about participation in research into depressive disorders (Protocol Title: UTSW Depression Cohort: A Longitudinal Study of Depression; STU: 092015-049; NCT02697487). The CDRC also has an ongoing natural observational study of individuals with major depression and healthy controls(Protocol Title: Dallas 2K: A Natural History Study of Depression; NCT02919280; STU: 112015-021) and those who are healthy controls or may be at risk for depression (Protocol Title: Resilience in Adolescent Development; STU 062016-042; and Protocol Title: Characterizing Inflammatory Profiles and Suicidal Behavior in Adolescents; STU-2020-1297). Study participants for the proposed study will be recruited from these ongoing studies such as UTSW Depression Cohort, RAD, and Dallas 2K. Other registries such as the UT Southwestern Research Volunteer Registry, local providers and IRB-approved study advertisements and various recruitment strategies, will also be used for study recruitment.

Additionally, the CDRC has implemented a robust workflow for identifying and screening candidates for clinical research and based on project-specific needs, employs direct-to-patient advertising through radio, newspaper, internet-based and other media outlets. A centralized programmatic intake process is employed that includes a central phone line, a program-specific email, and a website featuring a web-based survey that patients can take to see if they qualify for studies. Individuals who contact CDRC by completing the pre-screener online survey are automatically entered into a secure web-based database in order to be contacted by a member of the research staff for additional screening. The aforementioned observational study, the Dallas 2K study has an enrolled over 800 adults with depression. Based on the eligibility criteria, we anticipate that ~40% will likely be eligible for the proposed study. While not every individual will be appropriate for the current project, our census speaks to a robust workflow for screening and enrolling patients with depression into clinical research studies.

In addition to recruiting individuals through the CDRC centralized programmatic intake process, recruitment is also anticipated from the following additional sources: (1) patients with MDD who are currently receiving clinical care from providers (psychiatrists and psychologists) in CDRC; (2) the Adult Outpatient Psychiatry Clinic at UT Southwestern; (3) self-referrals from patients who have completed other research protocols through CDRC; (4) clinicians within and outside of the UT Southwestern Medical Center; and (5) self-referrals from individuals attending community outreach programs in the Dallas Fort Worth (DFW) Metroplex and from media advertisements. To foster close collaborations with providers within the UT Southwestern Medical Center and across DFW Metroplex more broadly, PI and study team will send out “Dear Doctor” letters and emails.

Participants will also be recruited from registries such as the UT Southwestern Research Volunteer Registry and Southwestern Health Resources Volunteer Research Participant Registry.

The PI will review subject enrollment at weekly project meetings with the study coordinator and PI’s primary mentor, Dr. Madhukar Trivedi, so that any deviation from the anticipated enrollment schedule will be detected quickly. All outreach and advertising activities will also be reviewed on a weekly basis

5.5.C Engagement Strategies for Retention

The PI will hold weekly project meeting with the study coordinator in order to monitor enrollment and ensure that enrolled subjects are completing all study procedures as intended.

In terms of infrastructure, the data coordinating facilities at UTSW provides a secure, validated, centralized electronic data management facility that is HIPAA and FDA-compliant (Research Electronic Data Capture, or REDCap). The REDCap system employed in this study includes several components, including a Screening Database and a Subject Tracking Database, in addition to the Study Database proper. As subjects complete visits the date of the visit will be entered into the database. Missed visits will be coded for reason (such as ill, scheduling problem, inability to contact, etc.). If subjects terminate early, the date and reason will be coded in the Subject Tracking Database. On a weekly basis, the study research coordinator, who will also function as the data manager, will run a report on patient accrual and completion status for review by the PI and the team. On an ongoing basis, the study data manager, the PI, and other team members will review the status of patient accrual and retention, as well as form completion, to identify and resolve any bottlenecks or problem areas that arise during the course of the study.

At CDRC, the study coordinator will be the subject's point of contact during the study. Subjects will be provided with phone and email contact information for their coordinator, in addition to the contact information for the study PI. Upon enrollment, subjects provide detailed contact information for themselves, their clinical providers (if applicable), and a family member or close friend who is aware of the patient's study participation. At each visit, the study coordinator "checks in" with the patient regarding protocol adherence and study satisfaction, in addition to any protocol specific procedures. This system provides the opportunity to detect possible reasons for study non-adherence or early discontinuation by the patient in a timely fashion and to address any issues, if possible.

5.5.D Strategies that will be used to ensure a diverse, representative sample of participants

The UTSW has fostered community partnerships that are mutually beneficial to the School and nearby underserved populations. Faculty members have benefited from collaborations with underserved and often hard-to-reach populations and front-line clinicians, to recruit for research, develop research partnerships and to ensure that community expertise, experiences and priorities inform their work. The CDRC at UT Southwestern is facilitating the formation and transformation of these partnerships so that they become the catalyst for groundbreaking research that uncovers and addresses important social, environmental, and health problems; builds skills among academic and community partners; and improves health of communities.

The UTSW is part of a large urban medical center, and benefits from a large and diverse catchment area. Located in the Dallas city of Dallas County, the racial and ethnic distribution of UTSW's catchment area is 42% Hispanic, 25% African American, and 28.8% white non-Hispanics. For the proposed clinical trial there are no special restrictions with regard to ethnicity or race. It is expected that the study sample will closely approximate the racial and ethnic composition of the greater catchment area of the UT Southwestern Medical Center.

5.5.E Potential recruitment/enrollment challenges and strategies that can be implemented in the event of enrollment shortfalls

Anticipated study related barriers may involve issues including staff workload and scheduling windows. This will be addressed by including coordinators who serve as back-up for the primary study coordinator responsible for the proposed project. These back-up staff members will allow the study team to screen and enroll subjects and complete study visits within subject's availability. All study staff will be trained by the PI and study procedures will be reviewed at weekly project meetings to ensure consistency of study procedures across both primary and back-up study personnel.

Some participant related barriers include appointment scheduling, transportation issues, and maintaining contact between study visits. To ensure that subjects can attend appointments, whenever possible, the visit schedule will include flexibility. To help address transportation issues, the study team will offer patients metro cards and other transportation resources available through CDRC. The research staff will discuss routes of contact with each participant during the screening phase in order to establish multiple routes of contact along with preferred contact methods to maintain contact between appointments. The research staff will check voicemail and email daily to be able to respond to any patient contact and will reach out before each scheduled appointment to ensure that subjects are still available and able to complete the visit as scheduled.

Unique to the times, the current COVID-19 pandemic represents a potential barrier to enrollment to nearly all clinical research activities. CDRC is working closely with Departmental and Health System leadership to ensure optimization of SOPs related to mitigating risk related to COVID-19 infection. Strategies that the study team will use to help mitigate against this barrier include the use of clear and consistent messaging to potential study subjects regarding the safety precautions against the spread of COVID-19 at CDRC, including the implementation of thorough and consistent hospital grade cleaning and disinfecting, vaccinations, the use of masks by study personnel and subjects, and social distancing.

In case of slower than anticipated enrollment, PI and primary mentor, Dr. Trivedi, will scrutinize reasons for pre-screen and screen failures during the weekly CDRC research meetings that are led by Dr. Trivedi and one-on-one weekly supervision meetings between PI and Dr. Trivedi. If the accrual is below 33% of the total targeted enrollment by end of Year 2, we will consider additional advertising method in form of social media campaigns and advertising on the website of national organizations such as Anxiety and Depression Association of America.

There will be no involvement of special vulnerable populations, such as adults unable to consent, fetuses, neonates, pregnant women, children (<18 years of age), prisoners, institutionalized individuals, or any other vulnerable populations.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Subjects will receive an infusion of either 0.5 mg/kg racemic ketamine hydrochloride or 0.02 mg/kg midazolam. We will use commercially available ketamine and midazolam for these infusions. Both drugs have FDA approval for use via this route of administration.

6.1.2 DOSING AND ADMINISTRATION

Procedures for Ketamine/Midazolam Infusion.

All infusion procedures and monitoring will take place at the Clinical Research Unit (CRU) of UTSW using the existing Standard Operating Procedures that are in place for the administration of ketamine in research studies. Participants will be asked to fast prior to their infusion. They should not consume solid foods and non-clear liquids in the eight hours prior to the infusion. They should not consume clear liquids and water in the two hours prior to the infusion. An indwelling catheter will be placed in the antecubital vein of the nondominant arm (preferred). If this vein is deemed unusable for any reason,

another vein can be used on the nondominant arm. If all veins in the nondominant arm are unusable, the dominant arm may be used. Pulse, blood pressure, and digital pulse-oximetry monitoring will be instituted. All physiologic monitoring data will be recorded on a standard anesthesia record beginning five minutes prior to ketamine administration. A study PI or study-affiliated provider with privileges to administer ketamine will be present at the CRU throughout the administration of ketamine or midazolam so that potential adverse events can be evaluated and treated promptly. For the purpose of this study, the term "study-affiliated provider" is defined as a physician, physician-in-training, physician's assistant, or nurse practitioner. The study PI or study-affiliated provider will remain available until at least one hour following the termination of the study drug infusion or until the patient meets Aldrete criteria (good respiration, O2 saturation, consciousness, circulation, and activity) for post-anesthesia care, whichever period is longer.

Subjects will receive an infusion of either 0.5 mg/kg racemic ketamine hydrochloride or 0.02 mg/kg midazolam. The total dose of the study drug at each infusion will be based on the subject's weight at baseline visit. The dose of ketamine is (0.5 mg/kg) has been widely used in studies of patients with MDD.[26,27,34-38] As ketamine at this subanesthetic dose causes minimal sedation, to maintain blinding with active control, we will use the midazolam at 0.02 mg/kg as it minimizes excessive sedation and is consistent with a recent report in adults with MDD.[39] Subjects will be randomly allocated to one of the two arms prior to first infusion and will receive the same drug at each of the four infusions. Subjects and study team members, including the PI, study-affiliated providers, coordinators, nursing staff, and data analysts will be blinded to the drug assignment until the completion of the proposed project, except for those study team members who are involved with monitoring for safety such as the medical monitor (i.e., ublinded study team members). Only the research pharmacist and unblinded study team members will know the identity of the drug. Each drug (ketamine or midazolam) will be dissolved in 0.9% saline in a total volume of 100 mL and administered with an infusion pump at a constant rate. A study team rater will collect self-report symptom and side-effect ratings during and following the infusion. Inter-rater reliability is established for all outcome measures prior to study. The schedule of assessments is presented in Table 1. A study-affiliated provider will evaluate the subject at least 1 hour after the end of infusion to assess discharge readiness, documenting that vitals are within normal range (systolic blood pressure < 140 mm of Hg, and diastolic blood pressure <90 mm of Hg on two separate occasions that are at least 15 minutes apart).

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The study drug will be dispensed to the study team by the investigational drug service.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The research pharmacy will dispense the study drug mixed in normal saline (total volume of 100 ml) and will label the package with patient identifiers. The study team will be blinded to the identity of study drug, except for those study team members who are involved with monitoring for safety such as the medical monitor.

6.2.3 PRODUCT STORAGE AND STABILITY

The research pharmacy will store the study drug per their standard operating procedures. Study team will receive the study drug mixed with normal saline for infusion on the morning of the infusion. Any study drug mixture that remains unused will be discarded per standard operating procedures.

6.2.4 PREPARATION

The preparation of study drug mixture (I.e., mixing of study drug with normal saline) will be done by the investigational drug services.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Subjects with MDD who have completed all baseline assessments including pre-treatment fMRI scan will be randomly allocated to one of the two study arms (ketamine or placebo) randomly by a permuted block procedure consisting of blocks of 6 patients (10 total blocks) and stratified by whether they are taking psychotropic medications or not at baseline visit (per inclusion criterion #5). The study statistician will generate the randomization sequence prior to the enrollment of first participant and will provide it to the UTSW research pharmacist, who is not otherwise involved in patient assessment. The research pharmacist will prepare the study medication (ketamine or midazolam) for intravenous infusion in a 100 ml normal saline bag or syringe, and send it to the Clinical Research Unit the morning of infusion, such that a fixed dose of ml per kg is administered. Only the UTSW pharmacist and unblinded study team members will have access to the randomization code to link it to the subject ID (and be responsible for providing this information per Data Safety Monitoring Plan for their unblinded review); thus, all subjects, clinicians (including administering provider), clinical raters, and data analysts will remain masked to treatment assignment until the completion of the proposed project.

6.4 STUDY INTERVENTION COMPLIANCE

The infusion will be completed in the presence of study PI and/or other study-affiliated provider. If the study drug is not fully utilized, the remainder will be discarded per standard operating procedures.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements. Subjects must be free of psychotropic medications (including antidepressants, antipsychotics, benzodiazepines, mood stabilizers, sedative/hypnotics, dopamine agonists, stimulants, buspirone, and triptans) and certain anticonvulsants (topiramate and levetiracetam) or be stable on these meds at screening visit and for at least four weeksprior to the baseline visit [first magnetic resonance imaging (MRI) scan]. Subjects with MDD must also agree to not use aforementioned medications during the course of their study participation if they were free of psychotropic medications. For those who were on psychotropic medications at screening visit, they must be willing to continue on these during the course of study. This will ensure any psychotropic medication-change related effects do not confound the effects of study drug.

Table 2. Concomitant Medications. Medications Allowed (Y) and Not Allowed (N) as Concomitant Medications.

Drug Name or Class	Episodic Use	Chronic Use	Restrictions/Comments
Analgesics	Y	Y	Nonnarcotic analgesics are allowed. Medically appropriate episodic use of

Drug Name or Class	Episodic Use	Chronic Use	Restrictions/Comments
			narcotic analgesics is allowed for acute medical indications but is limited to no more than 3 days for each episode, and may not be taken the day of the infusion starting at midnight. Tramadol is not allowed.
Anesthetics, general	Y (except for ketamine, which is excluded)	N	If procedures requiring general anesthesia are or occur/have occurred, study team will evaluate whether continuation in the study is safe and medically appropriate.
Anesthetics, local	Y	N	
Anorexics/antiobesity/ Appetite suppressants	N	N	OTC Alli (Xenical), sibutramine (Meridia), and phentermine (Adipex-P and others) are not allowed. Use of GLP-1 agonists will be allowed.
Antacids	Y	Y	
Antiacne	Y	Y	Topical agents only, including topical antibiotics. Isotretinoin is not allowed
Antanginal agents	N	N	
Antiarrhythmics	N	N	Amiodarone is excluded
Antiasthma agents	Y	Y	
Antibiotics	Y	Y	Chronic use of topical antibiotics for acne is allowed, with the exception of the MAOI linezolid (Zyvox) and isoniazid, which are not allowed. Erythromycin, clarithromycin, rifampin are excluded.
Anticoagulants	N	N	Warfarin (Coumadin) is not allowed. Antiplatelet agents are allowed (see “Antiplatelets”).
Anticholinergics	Y	Y	Scopolamine not allowed
Anticonvulsants	N	Y	Gabapentin, and pregabalin are allowed. Other anticonvulsants are not allowed, including lamotrigine and carbamazepine. Stable in dosing at least four weeks prior to randomization is required.
Antidepressants	N	Y	Stable (for at least 4 weeks prior to baseline) and during the course of the study is required. No dose changes are allowed during the study. Monoamine oxidase inhibitors (which may have unknown drug-drug interactions) are excluded. Concomitant use of trazodone (up to 200mg daily) is allowed. Nefazodone is excluded.
Antidiarrheal preparations	Y	N	Only loperamide, bismuth subsalicylate, and kaolin preparations are allowed
Antifungals, systemic	N	Y	

Drug Name or Class	Episodic Use	Chronic Use	Restrictions/Comments
Antifungals, topics	Y	Y	Ketoconazole and itraconazole are excluded
Antihistamines	Y	Y	The use of combinations containing pseudoephedrine or phenylephrine is not allowed. Combination products containing the word nighttime or are specifically marketed for before sleep routinely that only include an antihistamine are allowed. Combination products ending in "-D" routinely contain a stimulant such as pseudophedrine, and the appropriate limits above apply to them. (See "Cough and Cold Preparations" for combination products.)
Antihypertensives	N	Y	Diltiazem, verapamil are excluded
Anti-impotence meds	Y	Y	
Antiinflammatory drugs	Y	Y	Indomethacin and systemic corticosteroids are not allowed
Antimigraines	N	N	Triptans are not allowed
Antinausea/antiemetics	Y	N	Phosphoric acid preparations (Emetrol, Emecheck), bismuth subsalicylate (Pepto-Bismol), cola syrup, 5-HT3 receptor antagonists (e.g., ondansetron), and prokinetic agents (metoclopramide) are allowed. Scopolamine is not allowed (see section on anticholinergics).
Antineoplastic/ immunosuppresants	N	Y	Interferon, methotrexate, and other immunosuppressant not allowed. For patients on cancer maintaining therapy, their oncological provider will be consulted to ensure that use of ketamine/midazolam is safe.
Antiplatelet agents	N	Y	Aspirin (maximum 325 mg/day) and clopidogrel (Plavix) are allowed. Note that use of an SSRI or of a triple uptake inhibitor may increase bleeding times and possibly prothrombin times.
Antipsoriatic treatments	Y	Y	Acitretin is not allowed. Topical agents and monoclonal antibodies are allowed
Antipsychotics	N	Y	Stable in dosing at least four weeks prior to randomization is allowed
Antismoking	N	Y	Varenicline is not allowed. Other treatment should be stable for four weeks prior to randomization
Antiviral agents	Y	Y	Only oral or topical agents are allowed. Only acyclovir, famciclovir, valacyclovir,

Drug Name or Class	Episodic Use	Chronic Use	Restrictions/Comments
			penciclovir, docosanal, trifluridine, and vidarabine are allowed. Amantadine,rimantadine, indinavir, nelfinavir, ritonavir, saquinavir are not allowed. Tamiflu (oseltamivir phosphate), and Relenza (zanamivir) inhalants are permitted for influenza prophylaxis but use is limited to a 7- to 14-day course in accordance with prescribing information. Interferons are not allowed. Use of drugs for COVID-19 will be reviewed to ensure continuation in research study is appropriate for the subject.
Anxiolytics	N	Y	Chronic, stable treatment with benzodiazepines is allowed. Stable in dosing at least four weeks prior to randomization is required. Benzodiazepines should not be taken within 2 hours of infusion. Total daily dose not to exceed 6 mg equivalent of lorazepam.
Barbiturates	N	N	
Benign prostratic hyperplasia treatments	N	Y	Male patients who have symptoms of obstructed voiding should not be included in the study. Surgically or medically treated patients must be asymptomatic and receiving a stable dosage of allowed medications (α-1 blockers, finasteride, or dutasteride) for 1 month before screening.
Buspirone	N	Y	Stable in dose at least four weeks prior to randomization
Cough/cold preparations	Y	N	Use of cough and cold preparations containing pseudoephedrine or dextromethorphan is not allowed, as are those containing phenylephrine. Combination products ending in “-D” routinely contain a stimulant such as pseudophedrine, and the appropriate limits apply to them. (See “Antihistamines”.)
Diuretics	Y	Y	Episodic use of diuretics is restricted to treatment of premenstrual symptoms. For chronic use, medication and dosage should be stable for 1 month before screening.
Dopaminergics	N	Y	Dopamine agonists for restless leg syndrome are allowed.

Drug Name or Class	Episodic Use	Chronic Use	Restrictions/Comments
Gastrointestinal H2-blockers Proton Pump inhibitors Prokinetics	Y	Y	Cimetidine and metoclopramide are not allowed
Hormone suppressants	N	Y	Only finasteride and dutasteride are allowed
Hormones, reproductive	N	Y	Systemic hormonal contraceptives (oral contraceptives of estrogen and progestin combinations, depot injections such as Depo-Provera, the contraceptive implant Implanon, or transdermally delivered contraceptives such as Ortho Evra) are allowed
Hormones, thyroid	N	Y	Thyroid hormone replacement is allowed (dosage of thyroid medication should be stable for 3 months before screening). Therapeutic use for psychiatric disorders (e.g., T3 augmentation) is not allowed
Hypoglycemic agents	N	Y	Oral agents, GLP-1 agonists are allowed. Insulin is not allowed
Hypolipidemics	N	Y	Niacin and niacinamide are not allowed, bilde acid sequesterants are not allowed. FInrates, exetimibe, and statins are allowed
Laxatives	Y	Y	Only fiber-based products and docussate are allowed
Lithium	N	Y	Stable in dosing at least four weeks prior to randomization is allowed
Medications that are primarily metabolized by CYP2C8 (e.g., cerivastatin, paclitaxel, repaglinide, sorafenib, and torsemide)	N	N	
Muscle relaxants	N	N	
NMDA receptor antagonists	N	N	
Opioid agonists/analgesics (e.g., codeine, hydrocodone, methadone, morphine, maperidine, propoxyphene) and antagonists (e.g.,	N	N	See section on analgesics for exceptions

Drug Name or Class	Episodic Use	Chronic Use	Restrictions/Comments
naltrexone, naloxone, nalmefene)			
Sedative/hypnotics	N	Y	Ongoing, stable hypnotic therapy (e.g., zolpidem, zaleplon, benzodiazepine hypnotics, and low-dose trazodone 50-200mg) will be allowed during the course of the study. Eszopiclone is not allowed. Patients should not take benzodiazepines, within 2 hours of infusion
Steroids/systemic	Y	N	Allowed for medical urgency/emergencis, such as severe allergic reactions.
Steroids: topical/inhaled	Y	Y	
Steroid: intraarticular	Y	--	
Stimulants	N	N	Oral or transdermal methylphenidate, amphetamine products or prodrugs, pseudoephedrine, modafinil (Provigil), and other medications of same category are not allowed
Vaccine	Y	NA	

6.5.1 RESCUE MEDICINE

The study site will supply antiemetic rescue medication that will be obtained from the investigational drug service as needed. The following rescue medications may be used: ondansetron 8 mg sublingual as needed. The date and time of rescue medication administration as well as the name and dosage regimen of the rescue medication will be recorded.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from study drug (ketamine or midazolam) does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include those listed under exit visit in Table 1.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive study treatment for 7 days after baseline visit.

The reason for participant discontinuation or withdrawal from the study will be recorded on the subject disposition Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for two scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within specified time frame in Table 1 and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

See Appendix A & B for tables (clinician/assessor administered as well as self-report) of assessments.

Diagnostic and Behavioral Rating Procedures.

The diagnostic interviews and behavioral ratings will be conducted by well-trained raters under the supervision of the PI and/or Dr. Trivedi [Director of Center for Depression Research and Clinical Care (CDRC)]. When applicable, bi-annual inter-rater reliability procedures are conducted at CDRC to ensure adequate calibration of inter-rater reliability. In the event of staff turnover, new raters must achieve this threshold of reliability prior to participation.

The **Mini International Neuropsychiatric Interview (MINI)** is a diagnostic interview used to determine psychiatric diagnoses in research settings based on DSM-5 criteria. The brief structured interview assesses the 17 psychiatric disorders that are thought to be most prevalent.[40]

The **Concise Associated Symptom Tracking scale irritability domain (CAST-IRR)** is a measure of irritability that participants complete at each visit. Individual items are "I wish people would just leave me alone", "I feel very uptight", "I find myself saying or doing things without thinking", "Lately everything seems to be annoying me" and "I find people get on my nerves easily". These items are rated on a five-point Likert scale with responses of "strongly disagree", "disagree", "neither agree nor disagree", "agree", and "strongly agree" corresponding to scores of 1, 2, 3, 4, and 5 (total score range: 5-25). Cronbach's alpha of CAST-IRR in previous studies were 0.83, 0.77, and 0.78 respectively. Previous reports have found strong associations of CAST-IRR scores with other measures of anger and irritability (convergent validity) and minimal associations with measures of mania, insomnia, and anhedonia (divergent validity), underscoring its strong psychometric properties.[7,12,41] To capture additional aspects of irritability, we will use an extended version of this scale which includes the following additional items: "I often feel irritable", "I often overreact with anger or rage over minor things", "I am grouchy or grumpy most of the time", "I lose my temper if I feel threatened", and "I get terribly angry if I don't get something that I was expecting". As additional measures of irritability, we will also ask participants to complete the **Brief Irritability Test (BITe)** and the **Affective Reactivity Index (ARI)**. The five-item BITe scale was devleoped from a larger pool of 63 candidate irritability-related items and was found to be unidimensional with Cronbach's alpha of 0.88. The BITe was found to be strongly correlated with other measures of irritability such as the Born Steiner Irritability Scale.[42] The ARI is a 7-item scale of irritability that is widely used in pediatric literature.[43] A study of ARI in adults supported its strong psychometric properties and provided evidence for good test-retest validity.[44]

To assess constructs that are frequently associated with irritability, we will use the **Buss-Perry Aggression Questionnaire (AQ)** which is a 29-item questionnaire that measures anger (8 items), physical aggression (9 items), verbal aggression (5 items) and hostility (8 items),[45] the **Anger Affect domain** of the **National Institute of Health (NIH) Toolbox Emotion Battery**,[46] and the **Massachusetts General Hospital Anger Attack Questionnaire (AAQ)** at baseline in both subjects with MDD and HC, and at the study exit visit in subjects with MDD. Using this AAQ, participants can be categorized as having anger attacks (per Fava et al., "*if they exhibited the following four criteria over the previous 6 months: 1) irritability, 2) overreaction to minor annoyances, 3) occurrence of anger attacks, at least one of which occurred within the past 1 month, and 4) experience during at least one of the attacks four or more of the following: tachycardia, hot flashes, chest tightness, paresthesia, dizziness, shortness of breath, sweating, trembling, panic, feeling out of control, feeling like attacking others, attacking physically or verbally, and throwing or destroying objects.*"[47] Additionally, participants who meet criteria for anger attacks are asked to report the number of anger attacks in the past month as follows: 0, 1-2, 3-4, 5-8, and 9 or more.[47]

To assess depression and associated symptoms, we will use the **Montgomery-Åsberg Depression Rating Scale (MADRS)** which is a 10-item clinician-administered instrument used for the evaluation of depressive symptoms in adults and for the assessment of any changes in those symptoms.[48] Each of the 10 items is rated on a scale of 0 to 6. These individual item scores are added together to form a total score, which can range between 0 and 60 points. To assess overall depression symptoms with a self-report scale, we will use the **Quick Inventory of Depressive Symptomatology self-report (QIDS-SR)** version. [49] The total score of QIDS-SR (range of 0-27) is based on the nine criterion symptom domains each of which is scored from 0-3. The Cronbach's α of QIDS-SR has ranged from 0.86 to 0.87 in previous reports.[49,50] We will use the **Mood and Anxiety Symptoms Questionnaire (MASQ),** a 30-item self-report, designed to measure dimensions of depression and anxiety that has been validated in both clinical and non-clinical samples.[51] The MASQ provides three factors with good construct validity:

negative affect, positive affect and anxious arousal (AA). To assess symptoms of generalized anxiety, we will use the 7-item **Generalized Anxiety Disorder scale (GAD-7)**.[52] To assess symptoms of anhedonia, we will use the **Temporal Experience of Pleasure Scale (TEPS) (self-report)** scale.[53] The TEPS is an 18-item self-report measurement of anticipatory (10 items) and consummatory (8 items) components of anhedonia which consists of a series of statements that must be rated according to how accurate they are for the individual. The scale differentiates the role of anticipatory pleasure ('wanting') from consummatory pleasure ('liking'). Anticipatory pleasure involves the prediction of pleasure from future reward and the experience of pleasure associated with a positive prediction; in contrast, consummatory pleasure involves the in-the-moment experience of pleasure in the presence of reward. We will also assess anhedonia with the **Snaith Hamilton Pleasure Scale (SHAPS)**.[54] Symptoms of pain will be assessed with the self-reported **Pain Frequency Intensity and Burden Scale (P-FIBS)**.[55] To assess quality of life and functional impairments, we will use the 14-item short form version (the general activities summary scale) of the **Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q)**[56] and the **Work Productivity and Activity Impairment (WPAI)**[57] scales. These measures of depression, associated symptoms, and functional impairments will be used in secondary analysis to identify changes in neurocircuit function that are specific to irritability even after controlling for these features. Furthermore, these measures will be used to assess changes in these symptoms and functional impairments with treatment (ketamine versus midazolam).

The **Life Stressor Checklist-Revised (LSC-R)** systematically records the occurrence and severity of past traumatic or stressful life events.[58] The questionnaire includes 30 life events, including experiences with natural disasters, physical or sexual assault, death of a relative, and other events and follows a yes/no format. For endorsed events, respondents are asked to provide the following: age when event began, age when event ended, belief that they were in danger ("yes" or "no"), feelings of helplessness ("yes" or "no"). In addition, subjects rate the events' effect on life and how upsetting the event was at the time on a five-point intensity scale (1 = "not at all or never to 5 = "extremely"). We will use this instrument and the **Childhood Trauma Questionnaire (CTQ)[59]** to quantify prior trauma exposure and evaluate its effect on irritability and frustrative nonreward in an exploratory fashion. The **Reproductive Lifecycle and Hormone Questionnaire (RLHQ)** is a standard brief questionnaire used in clinical trials to assess reproductive lifecycle status and the use of exogenous hormones.[60] The proposed study will use Modules 1 and 2 in all women at screening visit. The instrument will be used in the current study to systematically record menstrual and hormonal status of female subjects in order to explore the impact of these factors on outcomes of interest. If relationships are detected, then we will include a covariate in the model adjusting for hormonal and reproductive-related factors in secondary analyses.

8.2 SAFETY AND OTHER ASSESSMENTS

The **Columbia Suicide Severity Rating Scale (C-SSRS)** is a comprehensive, semi-structured interview measure that uniquely measures the full spectrum of suicidality including passive and active suicidal ideation, suicidal intent as well as suicidal behaviors.[61] All study subjects will be assessed for suicidal ideation and behavior by trained members of the study team; both "past month" and lifetime versions of the C-SSRS will be completed at screening. Individuals who score a 4 or greater on the past month suicidal section, or who have engaged in any suicidal behavior in the past month, will be excluded and referred promptly for clinical care. Our clinicians and staff at the Depression Center all undergo formal web-based training on administering the C-SSRS and have extensive experience in its utilization. The Center has established SOPs for handling suicidality (detailed elsewhere in this document). For patients who do not show suicidality at the described threshold and proceed in the research protocol, variability in C-SSRS scores will explored in relation to outcomes of interest (e.g., irritability, frustrative nonreward and neurocircuit measures). To assess active suicidal ideation with a self-report scale, we will use the **3-item suicidal thoughts factor of the Concise Health Risk Tracking (CHRT)** scale.[62] These items include, "I

have been having thoughts of killing myself", "I have thoughts about how I might kill myself", and "I have a plan to kill myself". These items are rated on a five-point Likert scale with responses of "strongly disagree", "disagree", "neither agree nor disagree", "agree", and "strongly agree" corresponding to scores of 0, 1, 2, 3, and 4 respectively (total score range: 0-12).

The **Patient Rated Inventory of Side Effects (PRISE) Adverse Event Visit Checklist** is a clinician-administered questionnaire used to qualify side effects by identifying and evaluating the tolerability of each symptom.[63] This scale will be completed before and after each infusion and at the study edit visit, only in subjects with MDD. The **Brief Psychiatric Rating Scale (BPRS)** is a clinician-administered assessment used to capture acute behavioral changes throughout treatment.[64] We will use only the 4 items assessing positive (+) symptoms of psychosis such as conceptual disorganization, hallucinatory behavior, suspiciousness, and unusual thought content for this proposed study. The scale will be conducted before and after each infusion. The **Clinician-Administered Dissociative States Scale (CADSS)** will be used before and after each infusion to assess the severity of dissociative symptoms associated with study drug administration.[27]

Screening procedures.

After receiving complete disclosure about the research and opportunity to fully review the consent form, potential subjects will be given the opportunity to ask questions. If they choose to take part in the study, then they will be asked to sign the informed consent form. All subjects will undergo a structured diagnostic interview with MINI and will complete forms related to demographics, medical history, concomitant medications along with symptom measures (detailed in section above), Reproductive Lifecycle and Hormone Questionnaire, Life Stressor Checklist-Revised, Self-administered Comorbidity Questionnaire (SCQ), Childhood Trauma Questionnaire, and STOP-Bang questionnaire. Study PI, a study-affiliated psychiatrist, or trained study personnel will complete clinician-rated symptom measures (Montogomery-Åsberg Depression Rating Scale and C-SSRS), collect medical and psychiatric history, document subjects' commitment to use appropriate contraceptive(s), and verify that subject meets all the inclusion criteria and none of the exclusion criteria. As part of screening visit, subjects will also undergo a physical examination by the PI or a study-affiliated psychiatrist (including documentation of height, weight, blood pressure, heart rate, and respiratory rate), electrocardiogram (ECG), blood tests (comprehensive metabolic panel, thyroid function tests, hemoglobin A1c, c-reactive protein, and complete blood count), urine drug screen, and urine pregnancy test (only in women of childbearing potential). These screening procedures will take place either the Center for Depression Research and Clinical Care or at the Clinical Research Unit, both of which are within the University of Texas Southwestern Medical Center.

At the time of initial screening, patients with MDD will be asked to identify if they have a current mental health provider. Patients with a current provider will be asked to provide the name and contact information of this provider.

Subject Follow-up procedures.

As patients complete the study treatment phase (early or planned) a study clinician (PI or psychiatrist who is covering for PI) will meet with them to discuss the transition of care plan. As part of the study exit visit, the study clinician will complete an end of study disposition form which includes a description of the treatment plan and the next scheduled follow up appointment with their provider, if applicable. This will be documented in the participant's chart. For patients that have an established psychiatrist or other treatment provider, the study team will work closely to ensure that there is a smooth transition of care from the end of the study to their clinical treatment. A study clinician may contact the participants

primary provider for coordination of clinical care. For patients who do not have pre-established care, the study team will provide referrals within the UT Southwestern and Texas Health Hospital system or larger community. At this exit visit, the study team will be check in on the status of the disposition plan and any follow up appointments will be discussed.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The Principal Investigator is responsible for study oversight, including ensuring human research subject protection by designating appropriately qualified and trained study personnel to assess, report, and monitor adverse events. The Research Team will review each adverse (AE)/ serious adverse event (SAE) and unanticipated problem as needed. These reviews will include an assessment of the possible relatedness of the event to the study intervention or other study procedures. The Investigators will also provide advice for decisions to exclude, refer, or withdraw participants as required. The study staff will be trained to monitor for and report AEs, SAEs, and UPIRSOs per the guidelines described below.

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

An AE is any untoward medical occurrence in a study subject administered an investigational product and that does not necessarily have a causal relationship with this treatment.
An unexpected AE is any adverse investigational product related-event, the specificity or severity of which is not consistent with the current IB or prescribing information for a marketed compound. Also, reports which add significant information on specificity or severity of a known, already documented AE constitute unexpected AEs. For example, an event more specific or more severe than described in the IB would be considered “unexpected”.
An AE therefore can be any unfavorable and unintended sign (including laboratory finding), symptom or disease temporally associated with participation in an investigational study, whether or not considered investigational product related. In addition to new events, any increase in the severity or frequency of a pre-existing condition that occurs after the subject signs a consent form for participation is considered an AE. This includes any side effect, injury, toxicity, or sensitivity reaction.
Any condition, laboratory abnormality, or physical finding with an onset date prior to the subject signing consent for study participation is considered to be pre-existing in nature and part of the subject’s medical history.

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

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

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

- Any death occurring within 30 days of the subject receiving the Investigational Product, regardless of the subject having discontinued from the protocol, must be reported as an SAE.

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

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

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

List criteria for AEs according to the Medical Dictionary for Regulatory Activities (MedDRA) Version 19.0 (March 2016) should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. If the experience is not covered in the abovementioned criteria, the guidelines shown in Table 3 below should be used to grade severity. Notably, the term “severe” is a measure of intensity and that a severe AE is not necessarily serious.

Table 3. AE Severity Grading

Severity (Toxicity Grade)	**Description**
Mild (1)	Transient or mild discomfort (< 48 hours); no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention or therapy required.
Severe (3)	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible.

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

The relationship of an AE to the Investigational Product should be assessed using the following the guidelines in Table 4.

Table 4. AE Relationship to the Study Drug

Relationship to Study Drug	**Comment**
Definitely related	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the Investigational Product; that follows a known or expected response

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

As this study involves procedures, such as placement of intravenous lines, any AE relationship to study procedures will be determined in similar fashion to the AE relationship to the Study Drug (Table 4).

8.3.3.3 EXPECTEDNESS

The PI or study psychiatrist will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI or study-affiliated provider will record all reportable events with start dates occurring any time after informed consent is obtained until the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

To UT Southwestern IRB
AEs are reportable to the IRB within 5 business days *when they meet the following definition*:

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

All other AEs with an onset date after the subject signs consent for study participation must be reported to the IRB *at the time of annual renewal*. Details of the event must include severity, relationship to the study Investigational Product, duration, action taken, and outcome.
All AEs that are considered related to the study Investigational Product must be followed to resolution or stabilization if improvement is not expected. AEs that completely resolve and then recur should be recorded as a new AEs. AEs continuing at exit visit should have a comment in the source documents by the PI that the event has stabilized or is not expected to improve.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

To UT Southwestern IRB
SAEs are reportable to the IRB within 24 hours of knowledge of the event *when they meet the following definition*:

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

All other SAEs with an onset date after the subject signs consent for study participation must be reported to the IRB *at the time of annual renewal*. Details of the event must include severity, relationship to the study Investigational Product, duration, action taken, and outcome.
All SAEs that are considered related to the study drug must be followed to resolution or stabilization if improvement is not expected. SAEs that completely resolve and then recur should be recorded as a new SAE. SAEs continuing at 30 days post-last dose should have a comment in the source documents by the PI that the event has stabilized or is not expected to improve.

To Independent Safety Monitor
Any unexpected, fatal or life-threatening adverse events will be reported to the ISM within 3 days of becoming aware of the event. Other serious and unexpected adverse events are to be reported within 7 days, and any other unanticipated events will be reported within 30 days to the ISM. Anticipated or unrelated SAEs and all other AEs will be handled in a less urgent manner but will be reported to the ISM on an annual basis.

To FDA
A report on the MedWatch 3500A form must be sent to the FDA when the event is:

(1) serious, unexpected suspected reaction (the investigator judges there is evidence to suggest a causal relationship);

(2) findings from other clinical, animal, or *in-vitro* studies that suggest significant human risk, and
(3) a clinically important increase in the rate of a serious suspected adverse reaction no later than 15 days after determining that the information qualifies for reporting.

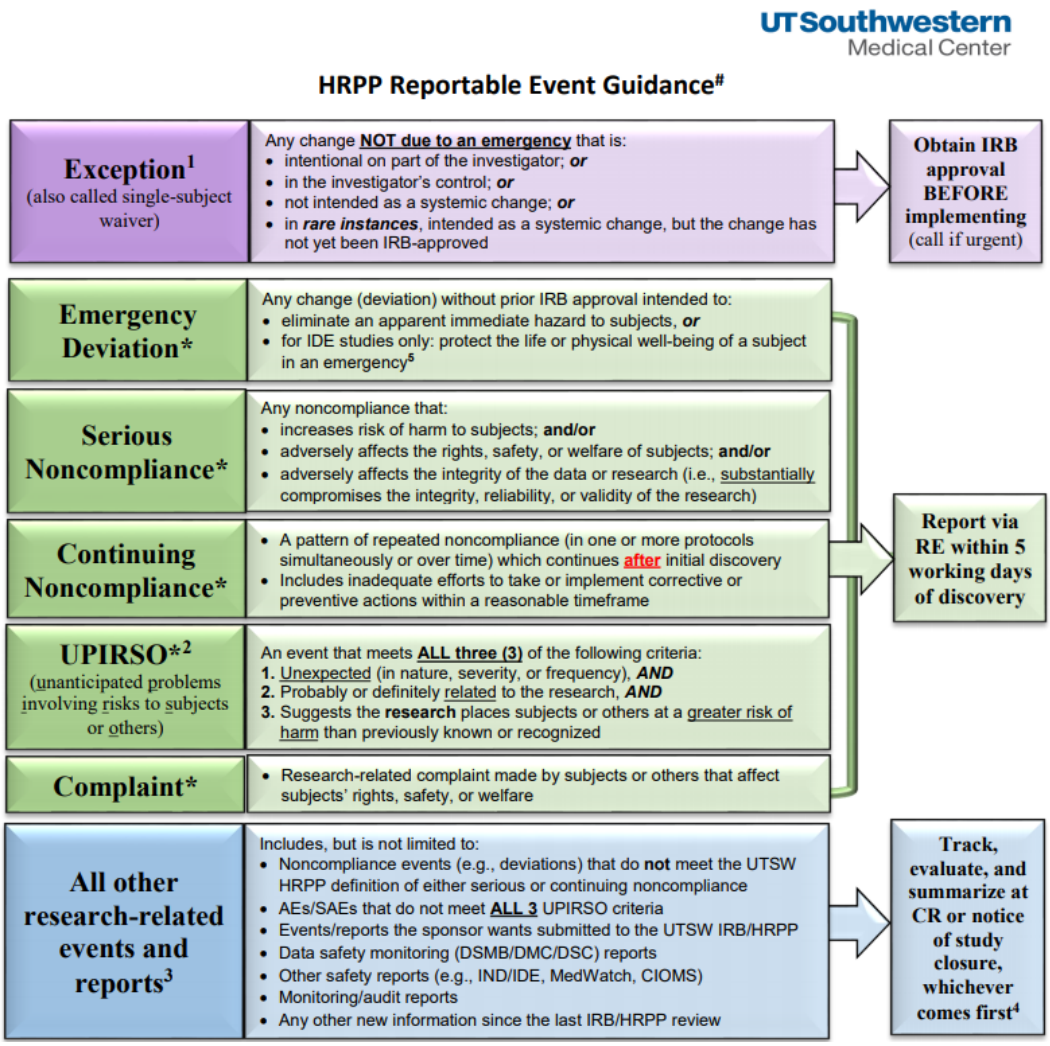
Unexpected fatal or life-threatening suspected SAEs will be reported no later than 7 calendar days after initial receipt of the information.

REPORTING TO THE SPONSOR [NATIONAL INSTITUTE OF MENTAL HEALTH (NIMH)]

The PI will report the following events in writing to the NIMH Program Official (PO) and will indicate that the monitoring entities (i.e., the PI and IRB, and ISM) and appropriate regulatory entities (e.g., OHRP, FDA) have been notified in accordance with the approved monitoring plan and federal regulations. For each reportable event, the documentation will include identifying information for the protocol, the date on which event occured and the date at which the PI became aware of the event, a detailed description of the event and impact on participant(s), a detailed description of the measures taken (including clinical) in response to the event (if any), confirmation that the appropriate monitoring entities and regulatory bodies have been notified, and a description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the event.

1. **IRB/ISM/OHRP/FDA Suspensions or Terminations.** Within 3 business days of receipt of any suspension or termination of approval and include a statement of the reason(s).
2. **Deaths related to study participation.** Must be reported immediately (no later than 5 business days) of the PI first learning of the event.
3. **Unexpected Serious Adverse Events that are related to study participation.** Within 5 business days of the study team becoming aware of the SAE.
4. **Unanticipated Problems Involving Risks to Subjects or Others**. Within 5 business days of the PI becoming aware of the event.
5. **Adverse Events.** For all AEs and SAEs that are deemed expected and/or unrelated to the study, a summary will be submitted to the NIMH PO with the annual progress report.
6. **Protocol violations.** PI will report any protocol violations to NIMH with the annual progress report.

HRPP Reportable Event Guidance at UT Southwestern Medical Center



8.3.7 REPORTING EVENTS TO PARTICIPANTS

Per standard protocols, a study-affiliated provider will review screening and laboratory test whereas the MRI scans are reviewed by the department of radiology. There is the possibility that while reviewing the test results an abnormality may be identified. In this eventuality, patients will be contacted and informed. The study PI or study-affiliated provider of the research team will provide information to the patient regarding the incidental finding. Study participants will be offered information about this incidental finding and referred to their primary care doctor or will be provided with an appropriate doctor referral. In case of an emergency study participants will be referred to the UT Southwestern Emergency Department or to their closest Emergency Department.

8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable.

8.3.9 REPORTING OF PREGNANCY

The subject must immediately inform her doctor if any of the following occur:

- She becomes pregnant while taking the study drug
- She misses her menstrual period, or experiences unusual menstrual bleeding

Modification / Update, MOD013-STU-2021-0667, Manish Jha, 2/16/2026

- She stops using birth control
- She thinks, FOR ANY REASON, that she may be pregnant

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The PI will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the ISM. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB in 24 hours and to the ISM within 3 days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB at annual review and to the ISM within 30 days of the investigator becoming aware of the problem.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Study participants will be offered information about the unanticipated problem that they may be suffering and referred to their primary care doctor or will be provided with an appropriate doctor referral. In case of an emergency study participants will be referred to the UT Southwestern University Hospital Emergency Department or to their closest Emergency Department.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Hypothesis 1.1: Higher levels of irritability will be associated with greater striatum-habenula connectivity.

Hypothesis 1.2: Higher levels of irritability will be associated with greater striatal activation after frustrating vs. positive feedback.

Hypothesis 2.1: Treatment with ketamine vs. midazolam will result in greater changes in striatum-Hb FC.

Hypothesis 2.2: Treatment with ketamine vs. midazolam will result in greater changes in (2.1) striatum-Hb FC.

9.2 SAMPLE SIZE DETERMINATION

For Aim 1, we plan to enroll n=60 adults with MDD and n=30 HC adults during the five years of the study period in a cross-sectional fMRI design. However, to facilitate matching of HCs and MDDs, we will enroll up to 45 HCs and up to 65 subjects with MDD. We expect ~10% data loss from motion artifact and other technical problems, yielding total of n=81 for analysis. As we have a priori identified 13 striatal ROIs, we will use **two-tailed alpha of 0.0038** (Bonferroni adjustment; alpha of 0.05 divided by 13 and rounded to four places after decimal) **for ALL power estimation**.

Power analysis to test hypothesis 1.1: In our preliminary data, we found that significant correlations between CAST-IRR and striatum-Hb FC pairs ranged between 0.38-0.52. The lower value of this range (0.38) corresponds to R^2 (variance) of 0.1444 for the tested condition. We expect some degree of correlation is between the dependent variable (CAST-IRR) and the covariates (age, sex, race, ethnicity, and diagnosis given that we found a Cohen's d = 2.82 for difference in CAST-IRR between MDD and Healthy Controls participants of Establishing Moderators and Biosignatures of Antidepressant Response in Clinical Care; preliminary data). Staying conservative, we assumed that these covariates had $R^2 = 0.09$ (a conservative estimate as we expect the diagnosis of MDD vs. HC to be significantly correlated with the total scores of CAST-IRR based on our findings in EMBARC study where mean CAST-IRR). Therefore, our analytic sample size of n=81 (after accounting for data loss from motion artifact) will provide >80% power to detect an R^2 =0.144 for the tested condition at two-tailed alpha of 0.0038, assuming that the R^2 for covariate is 0.16. If we assume higher correlation of 0.50 (R^2 = 0.25) between covariates and CAST-IRR, we will need n=64 to test our hypothesis with >80% power.

Power analysis to test hypothesis 1.2: Tseng et al.[79] found proportion of irritability that was explained by the activation within striatal region during the attentional even following frustrating vs. positive feedback was **0.13**. We will have >80% power to detect R^2 =0.13 for the tested condition is ≥0.13 at two-tailed alpha of 0.0038, assuming that the R^2 for covariates is 0.16. If we vary our assumption of R^2 for covariates to 0.09 or 0.25, we will need an estimated sample size of 89 and 72 respectively. In summary, we have adequately powered our study based on our preliminary findings and those of Tseng et al.[79] to test hypotheses 1.1 and 1.2.

For Aim 2, we will include the n≥60 [up to 65, to facilitate matching with HCs] adults with MDD who were also included in Aim 1. An estimated loss of fMRI data from 6 subjects (10%) due to motion artifact and a 1:1 randomization will yield 27 subjects in the ketamine condition and 27 subjects in the control midazolam condition.

Power analysis to test hypotheses 2.1 and 2.2: We did not have preliminary estimates for the effect size of ketamine vs. midazolam differences in striatum-Hb FC or in the striatal activation during the

attentional even following frustrating vs. positive feedback. Therefore, we based our power calculation by assuming an effect size of 0.50 which by convention is considered a moderate effect size. Our statistical analysis plan calls for the use of an ANCOVA with following covariates: age, sex, race, ethnicity, and baseline values (of striatum-Hb FC or contrast for N+1 trial following frustrating vs. positive feedback). As we are including the baseline value as covariate, we anticipate the R^2 for covariates to be 0.25. Therefore, our planned sample size of n=27 (after accounting for loss of fMRI data due to motion artifact, will provide us with >80% power to test detect an effect size of 0.50 for differences in striatum-Hb FC (2.1) and in striatal activation during the attentional even following frustrating vs. positive feedback (2.2) between ketamine and midazolam arm at a two-tailed alpha of 0.0038, assuming R^2 for covariates to be 0.25. If we vary our assumption of R^2 for covariates to 0.16 or 0.36, we will need an estimated sample size of 26 and 21 respectively in each treatment arm. In summary, we have adequately powered our study to detect a moderate effect size (0.50) for ketamine vs. midazolam comparison.

We did not conduct a sample size estimation for mediation analyses and other additional analyses given that they are exploratory in nature.

9.3 POPULATIONS FOR ANALYSES

To test hypotheses 1.1 and 1.2, we will use all subjects (both HC and MDD) who complete MRI scans and clinical assessments at baseline. To test hypotheses 2.1 and 2.2,we will use all subjects with MDD who complete MRI scans at both baseline and post-treatment visits, and undergo two weeks of twice-weekly infusion of study drug (ketamine or midazolam). For secondary analyses of clinical outcome (symptom severity and side effects), we will use a modified intent to treat analysis where subjects with MDD who receive at least 1 study drug infusion will be included.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

We will use descriptive statistics to characterize the sample (HCs and adults with MDD). For Aim 2, while randomization should protect against baseline group differences, we will compare the treatment groups (ketamine and midazolam) with univariate tests, as appropriate, on key baseline clinical and sociodemographic features. If any features differ significantly, we will account for those differences in our analyses that test for hypotheses 2.1 and 2.2. Analyses of primary, secondary and exploratory objectives are described below.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Hypothesis 1.1: Higher levels of irritability will be associated with greater striatum-habenula connectivity.

Plan to test hypothesis 1.1: In full sample (n=30 HC and n=60 MDD, updated to the final enrollment number given that in order to facilitate matching of HCs and MDDs, we will enroll up to 45 HCs and up to 65 subjects with MDD) using baseline data only, we will use linear regression analyses [separate for each striatal region of interest; ROIs parcellated per Choi et al.;[104] 13 regions in total] with the total score of irritability domain of the Concise Associated Symptom Track scale (CAST-IRR) as the dependent variable and functional connectivity between striatal ROI and habenula [therefore a total of 13 striatum-Hb functional connectivity (FC) pairs] from resting-state functional magnetic resonance imaging (fMRI) as the independent variable. We will include age, sex, race, ethnicity, and diagnosis as covariates.

Hypothesis 1.2: Higher levels of irritability will be associated with greater striatal activation after frustrating vs. positive feedback.

Plan to test hypothesis 1.2: In full sample (n=30 HC and n=60 MDD; updated to the final enrollment number given that in order to facilitate matching of HCs and MDDs, we will enroll up to 45 HCs and up to 65 subjects with MDD) using baseline data only, we will use linear regression analyses (separate for each striatal ROI) with the total score of CAST-IRR as the dependent variable and the **contrast (Δ) for Trial N+1** (see Figure 4B of RESEARCH STRATEGY FOR DETAILS) **following frustrating vs. positive feedback** (from FNR task-based fMRI**)** as the independent variable. We will include age, sex, race, ethnicity, and diagnosis as covariates.

Hypothesis 2.1: Treatment with ketamine vs. midazolam will result in greater changes in striatum-Hb FC.

Plan to test hypothesis 2.1: In MDD cohort only (n≥60 [up to 65, to facilitate matching with HCs]), we will use separate analysis of covariance (ANCOVA) for each striatal ROI with **post-infusion striatum-Hb FC** (resting-state fMRI repeated after the last infusion) as the **dependent variable**, **treatment arm** (ketamine versus midazolam) **as the independent variable**, and age, sex, race, ethnicity, and baseline striatum-Hb FC (for the same individual) as covariates.

Hypothesis 2.2: Treatment with ketamine vs. midazolam will result in greater changes in (2.1) striatum-Hb FC.

Plan to test hypothesis 2.2: In MDD cohort only (n≥60 [up to 65, to facilitate matching with HCs]), we will use separate ANCOVA for each striatal ROI with **post-infusion contrast (Δ) for Trial N+1 following frustrating vs. positive feedback** (FNR task-based fMRI repeated after the last infusion) as the **dependent variable**, **treatment arm** (ketamine versus midazolam) **as the independent variable**, and age, sex, race, ethnicity, and baseline contrast (Δ) for Trial N+1 following frustrating vs. positive feedback as covariates.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Neuroimaging analyses.

Using the previously described methods, we will compute the n=7260 functional connectivity pairs using the 121 regions of interest, as described in Chin Fatt, Jha et al.,[33] that include the 13 striatal regions of interest. Using the methods described for preliminary data, we will use elastic net analyses (10-fold cross-validation repeated 5 times) with CAST-IRR total score at baseline as the outcome of interest and include the 7260 FC pairs in the model. We will repeat the elastic model by including age, sex, race, ethnicity, and diagnosis in order to evaluate whether the retained FC pairs differ. We will then use canonical correlation analyses to compute the proportion of variance in the CAST-IRR total scores that are explained by the connectivity of the striatum with other brain regions.

As exploratory analyses using a whole-brain approach for functional connectivity at baseline, we will use the 13 striatal ROIs as seeds and apply Fisher R-Z transform to the Pearson seed-to-whole brain connectivity maps, factoring in dataset-specific terms for degrees of freedom. We will then enter subject-level Z-maps into an ANCOVA framework, controlling for age, gender, race, ethnicity, and diagnosis, using the AFNI 3D multivariate modeling (3dMVM) approach, with whole-brain family-wise error (FWE) correction using 3dClustSim, accounting for false positives due to multiple comparisons and spatial autocorrelation.

We will also conduct exploratory analyses where we will test for interactions of age and sex for analyses used to test hypotheses 1.1., 1.2., 2.1., and 2.2. Specifically, we will test for there is a neurocircuit functioning-by-age or -by-sex interaction for Aim 1 to evaluate if the association between neurocircuit functioning and CAST-IRR differs on the basis of age or sex. Similarly, we will include a treatment arm-

by-age or -by-sex interaction for Aim 2 to evaluate if the neurocircuit-functioning-differences between ketamine and midazolam vary by age or sex. We will use contrast estimates to evaluate any significant interaction.

Analyses of clinical data.

As we will collect **measures** of **irritability** (CAST-IRR), **overall depression** (Montgomery Asberg Depression Rating Scale and Quick Inventory of Depressive Symptomatology Self-Report version), **anxious arousal** (Anxious Arousal subscale of the Mood and Anxiety Symptom Questionnaire), **suicidality** (Columbia-Suicide Severity Rating Scale and Suicide Thoughts factor of the Concise Health Risk Tracking Scale), and **side effects** (Patient Rated Inventory of Side Effects) at repeated observations (baseline, each infusion visit, and study exit), we will use separate repeated-measures mixed model analyses with each of these **measures** as the dependent variable and treatment arm, visit (entered as class variable), and visit-by-treatment interaction as independent variables along with age, sex, race, ethnicity as covariates. Like the neuroimaging analyses, we will conduct exploratory analyses that evaluate for any age or sex interactions.

9.4.4 SAFETY ANALYSES

Summary statistics during treatment and/or as change scores from baselines for structured measures of safety analyses will be conducted. Furthermore, AEs occurring throughout the study will be tabulated based on treatment arm.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

We will use descriptive statistics to characterize the sample (HCs and adults with MDD). For Aim 2, while randomization should protect against baseline group differences, we will compare the treatment groups (ketamine and midazolam) with univariate tests, as appropriate, on key baseline clinical and sociodemographic features.

9.4.6 PLANNED INTERIM ANALYSES

A planned interim analysis with baseline data only will be conducted after 50% of the sample has been recruited to generate pilot data for subsequent grant submission. No interim analyses with data from the randomized controlled trial (two weeks of twice weekly infusions) are planned.

9.4.7 SUB-GROUP ANALYSES

In exploratory analyses, we will specifically test for sex-specific differences.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual participant data may be tabulated, in a de-identified fashion, to evaluate how changes in symptom severity relate to changes in neurocircuit functioning.

9.4.9 EXPLORATORY ANALYSES

Exploratory mediation analyses for Aim 2.

To determine how changes in neurocircuit function mediate changes in irritability with ketamine, we will use the McArthur approach as outlined by Kraemer.[65] According to this approach, three criteria must be fulfilled to show that a mediating feature (M) mediates the effect of treatment (T) on outcome (O). These are:

1. First criterion, **temporal precedence**, relates to study design and stipulates that T precedes M. By design, the T in our proposed study (4 infusions of ketamine or midazolam) preceded the M

(post-infusion striatal neurocircuit functioning as measured with resting-state and task-based fMRI).

2. The second criterion, **correlation**, stipulates that M and T are not independent, i.e., the distribution of M differs between the two treatment arms. We will **test this criterion with hypotheses 2.1 and 2.2 respectively** where we will identify the M as the striatum-Hb FC pairs (M2.1) and the contrasts (Δ) for Trial N+1 following frustrating vs. positive feedback (M2.2) in striatal ROIs that differ significantly between ketamine versus midazolam.
3. And the third criterion, **analytic**, states that the effect size on T on O depends wholly or in part on M. In our proposed study, Outcome will be the post-treatment severity of irritability, T is ketamine and midazolam, and M identified earlier are the neurocircuit functioning that differ significantly between ketamine versus midazolam (M2.1 and M2.2). Following the analytic approach in previous publications using this approach, mediators that differ significantly between ketamine and midazolam will be subjected to the third criterion, analytic, using the following steps:
    A. Compute overall effect size of difference in post-infusion total CAST-IRR between ketamine and midazolam;
    B. Rescale mediator variable (above-mentioned M2.1 and M2.2) by dividing it with squared root of the sum of estimated variance in the two treatment arms;
    C. Compute the difference between the means of rescaled M between the two treatment arms and their average;
    D. Perform linear regression analysis with post-infusion score of irritability as the outcome variable, treatment arm (ketamine coded as +1/2 and midazolam coded as -1/2), rescaled mediator, and the interaction between treatment arm and rescaled mediator;
    E. Compute effect size assuming that T and M are independent (null condition);
    F. Compute the moderator effect size by calculating the difference between overall effect size and the effect size under null condition; and
    G. Use bootstrap methods to compute 95% confidence interval for the mediator effect size and ascribe statistical significance if zero is not contained within this confidence interval.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol: 1. Informed consent form for healthy control subjects. 2. Informed consent document for adults with major depressive disorder (MDD).

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Voluntary informed consent will be obtained from all subjects prior to completing any study-related procedures or assessments for the proposed project. The initial consent form describes the nature of the procedures and time requirements, potential risks, the confidentiality of information, and the rights of re-search subjects. Individuals who are enrolled in ongoing observational studies or other studies that permit future contact for research will be contacted by the study team members, with the IRB's approval.

Interested individuals contacting CDRC by phone will be told that the information they give over the phone is written down and discussed by the research team. They will be advised that if they do not enroll in research with CDRC, the information will be destroyed, and that if they do enroll, the information becomes part of their research chart, which is destroyed after seven years. A phone screening will be completed after they give verbal authorization. If an individual appears to meet enrollment criteria and is interested in participating, a face-to-face interview (either conducted in person or via IRB approved televideo platform) will be conducted at UT Southwestern by the PI or study-affiliated provider. As the proposed study involves study drug infusions, the informed consent will be obtained by the PI or a study-affiliated provider (e.g., physician, physician-in-training, physician's assistant, nurse practitioner). Referring clinicians will be asked to inform potential subjects of the possible alternatives to participating in the study. A release of information will be obtained for review of any available historical and clinical data. A written authorization form will also be 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 will be discussed with the individual. Following this discussion, the individual will be given a copy of the consent form to review at their leisure, and any remaining questions are answered.

If the individual remains interested in the project, written informed consent will be obtained, and medical and psychiatric screening procedures are undertaken to confirm eligibility. A copy of the consent form is provided to all subjects. If the individual decides not to participate in this study, a staff member provides reasonable and timely assistance in obtaining an alternative referral, if so desired. The decision not to participate does not affect eligibility to participate in future studies, to receive treatment at UT Southwestern, or to receive treatment on a private basis from a referring clinician. Subjects will also be given the opportunity to withdraw from the study at any point. The consent process also includes documentation of permission to obtain previous medical records, including contact with previous physicians, pharmacies, and family members.

Protections Against Risk

There are three areas in which safeguards to protect subjects from undue risk require discussion. These include the procedures used to obtain informed consent, the procedures used to ensure confidentiality of the subjects' data, and the procedures used to minimize possible risks associated with the study procedures.

In the consent form, and in discussion with an investigator, subjects are advised fully of the procedures to be used, the amount of time required of them, the possible risks and benefits of the procedures, their right to refuse participation in the study without prejudice, their right to terminate participation at any moment without prejudice, and the name and telephone number of the Principal Investigator.

In the informed consent form, subjects are told that the information they provide, and all findings will be kept strictly confidential, with access limited to the research staff at the research sites and the possible exception of state or federal regulatory personnel. Only the PI and study team will have access to the code which links that participant study ID number to protected health information. This

information will be kept on a password protected computer on the UT Southwestern campus and backed up on a protected UT Southwestern server that is maintained by IT personnel and designed to house confidential patient information. The electronic data capture system used to collect data for this proposed study will collect it in a de-identified fashion, using only the subjects assigned study ID number (after eligibility is verified). As the study requires MRI scans and infusion of study drug, subjects will be informed that their protected health information may be entered in the electronic medical record system that is used by UT Southwestern (Epic). Results from this proposed study will be published as group data without the use of characteristics that would identify individual subjects. We quote information only by number in conference discussions, scientific reports, or publications, in order to maintain anonymity.

We have described above the potential risks of the research procedures and the safeguards that will be used to minimize risks. These include termination of subjects from research participation if it is believed that such participation endangers their welfare. Monitoring procedures are used to evaluate potential side effects of treatment or of research procedures. The protocol stipulates an extensive medical and psychiatric evaluation of all subjects as a condition for research participation.

Subjects are monitored for potential reactions during the clinical trial by the PI or study-affiliated psychiatrist, including treatment-emergent suicidality.

Suicidal Ideation and Imminent Harm: Despite treatment, subjects with MDD may get worse and become withdrawn and uncommunicative. In these cases, it is helpful and potentially lifesaving for doctors to have access to a family member or friend who can inform them of the subject's condition. To ensure safe participation, it is helpful to ask each subject to designate an emergency contact person. All suicide threats are taken seriously. It is important to assess the risk of suicide carefully when working with these individuals. Due to these reasons, as part of informed consent process, subject will be asked to identify an emergency contact person, provide their contact information, and complete a release of information form so that the study team may contact these individuals in case of an emergency.

Many subjects may admit to fleeting thoughts of death or briefly wishing for death; these thoughts need to be considered in context of the subject's overall history, along with a consideration of other risk factors for suicide. Study psychiatrists will assess every patient at each visit for suicide risk and potential. Individuals at such risk will be treated appropriately, including options such as increased contact, more frequent clinical visits, or emergency psychiatric hospitalization. Please note that subjects who score 4 or more on the past month suicidal ideation module of C-SSRS or had any suicidal behavior within the past month on C-SSRS will be excluded. Our Center, and the PI, have extensive experience in working with individuals with elevated risk of suicide and maintaining the safety of individual subjects.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, other investigators, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants

- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

In the informed consent form, subjects are told that the information they provide and all findings will be kept strictly confidential, with access limited to the research staff at the research sites and the possible exception of state or federal regulatory personnel. Only the PI and members of study team will have access to the code which links that participant study ID number to protected health information. This information will be kept on a password protected computer on the UT Southwestern campus and backed up on a protected UT Southwestern server that is maintained by IT personnel and designed to house confidential patient information. The electronic data capture system used to collect data for this proposed study will collect it in a de-identified fashion, using only the subjects assigned study ID number (after eligibility is verified). As the study requires MRI scans and infusion of study drug, subjects will be informed that their protected health information may be entered in the electronic medical record system that is used by UT Southwestern (Epic). Results from this proposed study will be published as group data without the use of characteristics that would identify individual subjects. We quote information only by number in conference discussions, scientific reports, or publications, in order to maintain anonymity.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Modification / Update, MOD013-STU-2021-0667, Manish Jha, 2/16/2026

Data collected for this study will be analyzed and stored at the UT Southwestern. After the study is completed, the de-identified, archived data will be transmitted to and stored at the UT Southwestern, for use by other researchers including those outside of the study. Per NIH policies, data may be uploaded in a de-identified fashion to comply with sponsor instructions.
When the study is completed, access to study data and/or samples will be provided through the study team at UT Southwestern.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Medical Monitor**
Manish Kumar Jha, M.B.B.S., *Assistant Professor of Psychiatry*	*Madhukar H. Trivedi* *Professor of Psychiatry*
UT Southwestern	*UT Southwestern*
5323 Harry Hines Blvd.	*5323 Harry Hines Blvd.*
+1-214-648-0156	*+1-214-648-0188*
manish.jha@utsouthwestern.edu	*Madhukar.trivedi@utsouthwestern.edu*

Overall Structure of the Study Team

This is a single-site study to be conducted at the University of Texas Southwestern Medical Center and will be led by Dr. Manish Jha.

As the PI of the study, **Dr. Jha will be responsible for all aspects of this research study**. A research study coordinator will assist Dr. Jha in the conduct of this study and will also serve as the data manager. As part of the Standard Operating Procedures at the Center for Depression Research and Clinical Care (CDRC), each study provider and coordinator has a back-up person who covers in case of their absence. Dr. Madhukar Trivedi, will serve the unblinded medical monitor. Other psychiatrists affiliated with CDRC may also serve as study psychiatrist for this protocol. Similarly, a research coordinator, TBD, will serve as the back-up for primary research coordinator. All study personnel will be trained by the PI and added to the IRB protocol before they can perform any study-related tasks or procedures.

Role of Co-Investigators

Dr. **Trivedi** will oversee the PI's conduct of the research study and will also serve as the medical monitor (in which role, he will have access to the unblinded data). Dr. Jha will regularly report the progress of the study to Dr. Trivedi who will arrange for regular meetings with other members of the team to ensure study milestones are met with diligent monitoring of subjects' safety. Dr. Trivedi will meet with Dr. Jha in regular 1-on-1 meetings and will be available to address any issues as they arise Based on Dr. Trivedi's several decades-long experience in the conduct of clinical trials and neuroimaging studies as PI, he is well qualified not only to conduct the study but also serve as medical monitor.

A statistical collaborator (Dr. Minhajuddin) will oversee biostatistics-related aspects of the study conduct. This will include supervising the creation of database in Research Electronic Data Capture (REDCap), setting up the reports to alert study team about missing data as well as provide summary statistics for recruitment and retention activities, setting up the block randomization with the research pharmacy to ensure that all members of the study team are blinded, to assist Dr. Jha in preparation of reports for the Independent Safety Monitor, and to supervise the conduct of interim analysis as well as final data analyses after the study enrollment is completed.

Role of other study personnel

A research pharmacist will prepare the study drug for infusion on the day of infusion. Research affiliated investigator with masters-level or higher statistical training will be responsible to providing unblinded data to the Medical Monitor and the Independent Safety Monitor (ISM) so that they can monitor the safety of study participants. Research nurses affiliated with the CRU will assist Dr. Jha or the study-affiliated psychiatrist in conducting the infusion of study drug.

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of an Independent Safety Monitor (ISM). For this study, Dr. Chadi Abdallah at Baylor College of Medicine has agreed to serve as an ISM. He has extensive experience in the conduct of clinical trials of ketamine for the purposes of neuropsychiatric disorders. He has served as PI on randomized controlled trials of ketamine of adults with major depressive disorder as well as post-traumatic stress disorder. The ISM (Dr. Abdallah) is independent from the study conduct and free of conflict of interest with regards to this team. This ISM will meet with study team at least semiannually to assess safety and efficacy data on each arm of the study. The ISM operates separately from the study team, and will be provided with unblinded data by an affiliated unblinded investigator who will not directly participate in the conduct of clinical trial (to maintain blinding of study team members). Each data element that the ISM needs to assess will be clearly defined.

10.1.7 CLINICAL MONITORING

The PI, Dr. Jha, will meet with co-Investigator, Dr. Trivedi, on a regular basis to monitor potential risks and protecting against risks. Study team will also meet as part of the larger weekly group meetings at our Center for Depression Research and Clinical Care (CDRC) which will include the PI, study-affiliated psychiatrist, and research coordinators. The PI will monitor the risks of evaluations and treatments on an ongoing basis. During the weekly meetings, the PI, study-psychiatrists, study coordinator, and other study team members will discuss any AEs or unexpected problems (UPs) along with the review of study progress and safety.

The PI will meet with Dr. Trivedi and other co-investigators at a minimum of twice a year to review all aspects of study progress and safety. This review will include the following: (i) plan for grading adverse events and attribution of adverse events; (ii) plans for unanticipated adverse events reporting; (iii) plans for anticipated adverse events reporting; and (iv) safety reviews and the frequency of this reviewThe PI and study coordinators will also evaluate the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, and other factors that can affect study outcome. They will also consider factors external to the study, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study. There will be no separate clinical monitoring of this single site study that is being led by the PI.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

The PI and study coordinators will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be clarified or resolved.

The PI and study coordinators will ensure that the clinical trial is conducted, and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and copies of all source documentation related to that subject. This will be stored in a locked cabinet in a secure area.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap, a 21 CFR Part 11-compliant data capture system provided by the UT Southwestern. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. MRI data is stored on secure server provided through the AIRC.

Data will be collected for research purposes only. Participants in this proposed study will provide the following data: (A) neuroimaging data which will include structural and functional images of participants' brains; (B) behavioral data (e.g., self-reported symptom measures, childhood trauma history, data from the frustrative nonreward task); (C) psychiatric history data (e.g., prior hospitalization, structured diagnostic interview with MINI International Neuropsychiatric Interview,[40] clinical evaluation); and (D) data related to medical history, physical examination including review of systems (and screening for obstructive sleep apnea with the STOP-Bang questionnaire[66]), vital signs, ECG, routine laboratory tests that are needed to monitor safety of participants; and (E) basic demographics (e.g., age, sex, race) and indices of social functioning (e.g., education attainment, socioeconomic status).

10.1.9.2 STUDY RECORDS RETENTION

Study records acquired during this research project will be saved for at least 7 years following the end of the research study.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the PI and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the PI to use continuous vigilance to identify and report deviations within 14 working days of identification of the protocol deviation, or as specified by the sponsor. All deviations must be addressed in study source documents, reported to National Institute of Mental Health Program Official. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Furthermore, study will comply with any sponsor requirement for sharing of de-identified clinical and neuroimaging data.

In addition, this study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research.

Data use agreements will also be initiated with the following entities: Katharine Dunlop and Venkat Bhat with University of Toronto/Unity Health, Wan-Ling Tseng with Yale University, Sanjay Mathew with Texas A&M, and Charles Nemeroff with The University of Texas at Austin. A limited data set with de-identified data will be shared with these groups, so that they can assist with clinical and fMRI data analyses.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design,

conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the policies of UT Southwestern has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

Not applicable.

10.3 ABBREVIATIONS

3dMVM	3D multivariate modeling
3T	3 Tesla
AAQ	Anger Attack Questionnaire
AE	Adverse events
AIRC	Advanced Imaging Research Center
ANCOVA	Analysis of covariance
AQ	Aggression Questionnaire
ARI	Affective Reactivity Index
ATRQ	Antidepressant Treatment Response Questionnaire
BITe	Brief Irritability Test
BPRS	Brief Psychiatric Rating Scale
CADSS	Clinician-Administered Dissociative States Scale
CAST-IRR	10-items extended version of Concise Associated Symptom Tracking irritability domain
CCARP	Centers for Community and Academic Research Partnerships
CDRC	Center for Depression Research and Clinical Care
CFR	Code of Federal Regulations
CHRT	Concise Health Risk Tracking
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	novel coronavirus disease 2019
CRF	Case Report Form
CRU	Clinical Research Unit
C-SSRS	Columbia Suicide Severity Rating Scale
CTQ	Childhood Trauma Questionnaire
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5th Edition
DTI	Diffusion tensor imaging
EMBARC	Establishing Moderators and Biosignatures of Antidepressant Response in Clinical Care
ECG	Electrocardiogram
FC	Functional connectivity
FDA	Food and Drug Administration
fMRI	functional magnetic resonance imaging
FNR	Frustrative nonreward
FWE	family-wise error
GAD-7	7-item Generalized Anxiety Disorder scale
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
Hb	habenula
HC	Healthy Control
ICH GCP	International Conference on Harmonisation Good Clinical Practice
IDS	investigational drug services

Modification / Update, MOD013-STU-2021-0667, Manish Jha, 2/16/2026

IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IT	Information Technology
IV	Intravenous
kg	kilogram
LSC-R	Life-Stressor Checklist Revised
MADRS	Montgomery-Åsberg Depression Rating Scale
MASQ	Mood and Anxiety Symptom Questionnaire
MDD	Major Depressive Disorder
mg	miligram
MINI	Mini-International Neuropsychiatric Interview
mL	mililiter
MPRAGE	Magnetization prepared gradient echo
MRI	magnetic resonance imaging
NIH	National Institute of Health
P-FIBS	Pain Frequency, Intensity, and Burden Scale
PHI	Protected Health Information
PI	Principal Investigator
PRISE	Patient rated inventory of side effects
QIDS-SR	Quick Inventory of Depressive Symptomatology Self-Report
Q-LES-Q SF	Quality of Life Enjoyment and Satisfaction Questionnaire Short Form
REDCap	Research Electronic Data Capture
RLHQ	Reproductive Lifecycle and Hormone Questionnaire
SAE	serious adverse event
SCQ	Self-administered comorbidity questionnaire
SD	standard deviation
SHAPS	Snaith Hamilton Pleasure Scale
SoA	Schedule of Activities
SOPs	Standard Operating Procedures
T1w	T1-weighted
TEPS	Temporal Experience of Pleasure Scale
UNIKET	Using Neuroimaging to identify therapeutic targets of Irritability using Ketamine
UPs	unanticipated problems
US	United States
UTSW	UT Southwestern
WPAI	Work Productivity and Activity Impairment scale

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1.1	08/07/2021	Reporting to Sponsor, change from Mount Sinai to UT Southwestern University Hospital, risks about restrictions to psychiatric treatment	Added per sponsor comments. Correction of referral hospital from Mt. Sinai to UTSW, limitations to medications.
1.2	02/14/2022	Added 21 CFR Part 11 to the Statement of Compliance	As this study will capture electronic signature in electronic records
		Age limit corrected to 18-65 years throughout the protocol	Errors in the protocol regarding age limit were corrected.
		Participants must complete AIRC MRI procedure screening form on screening visit and on day of neuroimaging to ensure that there are no contraindications to the MRI.	This is to ensure that scanning with MRI is safe on each day of neuroimaging. The screening visit check is required for study eligibility.
		AAQ will be completed only at Baseline and Study Exit visits.	Clarification that this scale will be collected only.
		Study exit window increased to ±2 days.	To provide more flexibility in scheduling while clarifying that last infusion and study exit cannot be on the same day.
		Resting-state fMRI to be completed before and after task-based scan.	Clarified that resting-state fMRI will be conducted twice in each scanning session.
		“in-scanner” deleted prior to pilot frustration run	The pilot frustration run will occur outside the scanner.
		In section 4.1, updated that 'BOLDscreen32' a television screen will be used to display stimuli, and physiological data that are measured with MRI (heart rate, oxygen saturation, ECG, and respiration rate) will be recorded.	Clarified that AIRC is using a television screen instead of a projector system. Also, physiological data that are measured with MRI scans will also be recorded.
		Section 5.5..D, deleted “Furthermore, Dr. Trivedi, who serves as back-up for PI’s current professional responsibilities may also serve as a back-up physician within the research team.”	Dr. Trivedi will serve as medical monitor, so will not be able to serve as back-up physician as he will be unblinded.
		In Section 6.1.2., inserted “The total dose of the study drug at each infusion will be based on the subject’s weight at baseline visit.”	Clarified that weight at baseline visit will be used to determine the dose of study drug at each visit.
		In section 6.1.2., “except for those study team members who are monitoring for safety such as the medical monitor.”	Clarified that study team members who are monitoring for safety, including the medical monitor, will be unblinded.

		In section 6.3, "and stratified by whether they are taking psychotropic medications or not at baseline visit (per inclusion criterion #5)".	Clarified that the randomization will be stratified by whether participants with MDD are taking psychotropics or not.
		Labs of hemoglobin A1c and c-reactive protein added.	Added labs for safety to detect poor glycemic control and active infection and injury.
		In section 6.5, stable dose of 4 weeks.	Clarified that participants with MDD who
		Tables of assessments added to appendix A & B	To provide an easy-to-view list of the different scales used in the study.
		The grading of AE reporting updated.	The table of AE reporting updated as life threateninggrade is an SAE and additional clarifications to AE gradings added.
		Inserted in 8.3.3.2, "As this study involves procedures, such as placement of intravenous lines, any AE relationship to study procedures will be determined in similar fashion to the AE relationship to the Study Drug (Table 4)."	Relationship of an adverse event to study procedure clarified.
		Section 8.3.6, reporting timeline to sponsor updated per NIMH policy. UTSW Rerpotable Event Guidance infographic included.	Our reporting deadlines to NIMH were not in compliance, therefore updated. HRPP event guidance included for clarity.
1.3	03/24/2022	In section 4.1, added "inside the scanner" after pilot frustration run.	To clarify that pilot frustration run will be completed inside the scanner.
		In section 4.1, added "and diffusion tensor imaging (DTI) for structural connectivity (this may be omitted to ensure that subject time in the magnet is less than 70 minutes)."	We will add diffusion tensor imaging as scans that will be obtained as part of this study. Clarified that DTI scans may not be collected to ensure within magnet time for a person stays at less than 70 minutes.
		In section 5.5.B, added information about STU 062016-042 and STU-2020-1297.	Clarified additional observational studies at CDRC for recruitment.
		In section 5.1, inclusion criterion #4 updated to make it clearer.	Language updated to make it clearer. The criterion itself has not changed.
1.4		Section 8.2 – Removed urinalysis that was listed as part of screening procedures.	A urinalysis is not performed—only urine drug screens and urine pregnancy tests, if applicable.

		Section 4.1 – Changed subject time in scanner to allow for up to 75 minutes.	Increasing the time the subject is allowed in the scanner will allow for more time to conduct ASL scans that have been omitted previously due to time constraints.
		Section 6.1.2 – Added that the participant should be fasting prior to infusion.	The informed consent form states that the participant should be fasting prior to infusion. This is typical protocol pre-ketamine infusion. Protocol has been amended for the sake of clarity and consistency.
		Section 10.1.1.2 – Amended consent process to allow consent to be obtained by study staff other than the PI or study-affiliated physician. Consent can be obtained by the PI, study-affiliated physician (including resident physicians), or study-affiliated mid-level provider (PA, NP) after training and approval by the PI.	This will allow for back up providers in case the study PI is unavailable.
		Section 6.1.2 – Amended IV placement to allow for use of veins other than the antecubital vein of the nondominant arm.	While the antecubital vein of the nondominant arm is preferred, it may not be feasible or accessible for each infusion. Therefore, use of another vein should be permitted.
		Section 6.1.2 – Removed requirement of the presence of a crash cart during the infusion process.	There is no crash cart available at the UTSW CRU. As indicated in the protocol, the study PI or study-affiliated provider is available at CRU for emergencies during the infusion.
		Section 8.2 – Added “other trained study personnel” to allow other staff to complete clinician-administered assessments.	Staff can administer clinician-administered assessments such as MADRS and C-SSRS after completion of appropriate training.
		Changed “study-affiliated physician” to “study-affiliated provider” throughout the protocol.	This change was made to incorporate different level providers that are practicing within their scope of practice. “Provider” includes anyone with the titles physician, physician-in-training, resident or fellow, physician’s assistant, or nurse practitioner.

		Section 6.5, Table 2 – Removed "PRN" from episodic use.	This is to add clarity as certain medications can be used on chronic basis as PRN (such as benzodiazepines for anxiety/panic). Therefore, removal of PRN improves the clarity.
1.5	11/17/2023	Section 1.3, Table 1 – Amended so that Study Exit also functions as Early Termination	This is to clarify that if a patient withdraws from treatment, they will still complete the final visit (Study Exit / Early Termination).
1.6	10/28/2024	Throughout the protocol, clarified that we will enroll up 45 HCs and 65 subjects with MDD to facilitate the age and sex match.	We will need to exceed the initially planned n=30 HC to ensure that there are at least 1 HC subjects who are matched to all enrolled subjects with MDD by sex and age (+- 3 years). Additionally, n=60 for MDD may be exceeded to facilitate matching with HCs who are already enrolled in the protocol.
	10/28/2024	Added to section 5.5.B, Participants will also be recruited from registries such as the UT Southwestern Research Volunteer Registry and Southwestern Health Resources Volunteer Research Participant Registry.	Additional recruitment source of UT Southwestern Research Volunteer Registry and Southwestern Health Resources Volunteer Research Participant Registry added to the protocol.
1.7	12/04/2025	Added to section 10.1.11: Added intent to initiate data sharing agreements to share limited dataset with several entities	These groups have expertise in analyzing the types of data collected as part of this study, such as fMRI data.

11 REFERENCES

1. Association AP. *Diagnostic and statistical manual of mental disorders (DSM-5®).* American Psychiatric Pub; 2013.

2. Fava M, Hwang I, Rush AJ, Sampson N, Walters EE, Kessler RC. The importance of irritability as a symptom of major depressive disorder: results from the National Comorbidity Survey Replication. *Molecular psychiatry.* 2010;15(8):856.

3. Judd LL, Schettler PJ, Coryell W, Akiskal HS, Fiedorowicz JG. Overt irritability/anger in unipolar major depressive episodes: past and current characteristics and implications for long-term course. *JAMA Psychiatry.* 2013;70(11):1171-1180.

4. Perlis RH, Fava M, Trivedi MH, et al. Irritability is associated with anxiety and greater severity, but not bipolar spectrum features, in major depressive disorder. *Acta Psychiatrica Scandinavica.* 2009;119(4):282-289.

5. Hasin DS, Sarvet AL, Meyers JL, et al. Epidemiology of Adult DSM-5 Major Depressive Disorder and Its Specifiers in the United States. *JAMA Psychiatry.* 2018;75(4):336-346.

6. Jha MK, Minhajuddin A, South C, Rush AJ, Trivedi MH. Irritability and Its Clinical Utility in Major Depressive Disorder: Prediction of Individual-Level Acute-Phase Outcomes Using Early Changes in Irritability and Depression Severity. *The American journal of psychiatry.* 2019;176(5):358-366.

7. Minhajuddin A, Jha MK, Chin Fatt C, Trivedi MH. Psychometric Properties of Concise Associated Symptom Tracking (CAST) Scale and Validation of its Clinical Utility in Patients with Major Depressive Disorder: Findings from the EMBARC Study. *Psychiatric Research and Clinical Practice.* 2019;In press.

8. Orri M, Galera C, Turecki G, et al. Association of Childhood Irritability and Depressive/Anxious Mood Profiles With Adolescent Suicidal Ideation and Attempts. *JAMA Psychiatry.* 2018;75(5):465-473.

9. Pickles A, Aglan A, Collishaw S, Messer J, Rutter M, Maughan B. Predictors of suicidality across the life span: The Isle of Wight study. *Psychological Medicine.* 2009;40(9):1453-1466.

10. Jha MK, Minhajuddin A, Chin Fatt C, et al. Association between Irritability and Suicidal Ideation in Three Clinical Trials of Adults with Major Depressive Disorder. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.* 2020;45(13):2147-2154.

11. Hedegaard H, Curtin SC, Warner M. Suicide mortality in the United States, 1999–2017. National Center for Health Statistics Data Brief, no 330. Accessesd on 06 JUN 2020 at: *https://www.cdc.gov/nchs/data/databriefs/db330-h.pdf*. 2018.

12. Jha MK, Minhajuddin A, South C, Rush AJ, Trivedi MH. Worsening Anxiety, Irritability, Insomnia, or Panic Predicts Poorer Antidepressant Treatment Outcomes: Clinical Utility and Validation of the Concise Associated Symptom Tracking (CAST) Scale. *The international journal of neuropsychopharmacology.* 2018;21(4):325-332.

13. Brotman MA, Kircanski K, Stringaris A, Pine DS, Leibenluft E. Irritability in Youths: A Translational Model. *The American journal of psychiatry.* 2017;174(6):520-532.

14. Eshel N, Leibenluft E. New Frontiers in Irritability Research-From Cradle to Grave and Bench to Bedside. *JAMA Psychiatry.* 2019.

15. Trivedi MH, McGrath PJ, Fava M, et al. Establishing moderators and biosignatures of antidepressant response in clinical care (EMBARC): Rationale and design. *Journal of psychiatric research.* 2016;78:11-23.

16. Chin Fatt CR, Jha MK, Cooper CM, et al. Effect of Intrinsic Patterns of Functional Brain Connectivity in Moderating Antidepressant Treatment Response in Major Depression. *The American journal of psychiatry.* 2020;177(2):143-154.

17. O'Doherty J, Dayan P, Schultz J, Deichmann R, Friston K, Dolan RJ. Dissociable roles of ventral and dorsal striatum in instrumental conditioning. *Science (New York, NY).* 2004;304(5669):452-454.

18. Amsel A. The role of frustrative nonreward in noncontinuous reward situations. *Psychological bulletin.* 1958;55(2):102-119.

19. Perlman SB, Jones BM, Wakschlag LS, Axelson D, Birmaher B, Phillips ML. Neural substrates of child irritability in typically developing and psychiatric populations. *Developmental cognitive neuroscience.* 2015;14:71-80.

20. Deveney CM, Connolly ME, Haring CT, et al. Neural mechanisms of frustration in chronically irritable children. *The American journal of psychiatry.* 2013;170(10):1186-1194.

21. Tseng WL, Deveney CM, Stoddard J, et al. Brain Mechanisms of Attention Orienting Following Frustration: Associations With Irritability and Age in Youths. *The American journal of psychiatry.* 2019;176(1):67-76.

22. Namboodiri VM, Rodriguez-Romaguera J, Stuber GD. The habenula. *Current biology : CB.* 2016;26(19):R873-r877.

23. Hong S, Amemori S, Chung E, Gibson DJ, Amemori KI, Graybiel AM. Predominant Striatal Input to the Lateral Habenula in Macaques Comes from Striosomes. *Current biology : CB.* 2019;29(1):51-61.e55.

24. Coffey KR, Marx RG, Vo EK, Nair SG, Neumaier JF. Chemogenetic inhibition of lateral habenula projections to the dorsal raphe nucleus reduces passive coping and perseverative reward seeking in rats. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.* 2020.

25. McIntyre RS, Lipsitz O, Rodrigues NB, et al. The Effectiveness of Ketamine on Anxiety, Irritability, and Agitation: Implications for Treating Mixed Features in Adults with Major Depressive or Bipolar Disorder. *Bipolar disorders.* 2020.

26. Murrough JW, Soleimani L, DeWilde KE, et al. Ketamine for rapid reduction of suicidal ideation: a randomized controlled trial. *Psychol Med.* 2015;45(16):3571-3580.

27. Fava M, Freeman MP, Flynn M, et al. Double-blind, placebo-controlled, dose-ranging trial of intravenous ketamine as adjunctive therapy in treatment-resistant depression (TRD). *Mol Psychiatry.* 2018.

28. Kokkinou M, Ashok AH, Howes OD. The effects of ketamine on dopaminergic function: meta-analysis and review of the implications for neuropsychiatric disorders. *Mol Psychiatry.* 2018;23(1):59-69.

29. Vollenweider FX, Vontobel P, Oye I, Hell D, Leenders KL. Effects of (S)-ketamine on striatal dopamine: a [11C]raclopride PET study of a model psychosis in humans. *Journal of psychiatric research.* 2000;34(1):35-43.

30. Sterpenich V, Vidal S, Hofmeister J, et al. Increased Reactivity of the Mesolimbic Reward System after Ketamine Injection in Patients with Treatment-resistant Major Depressive Disorder. *Anesthesiology.* 2019;130(6):923-935.

31. Morris LS, Kundu P, Costi S, et al. Ultra-high field MRI reveals mood-related circuit disturbances in depression: a comparison between 3-Tesla and 7-Tesla. *Translational psychiatry.* 2019;9(1):94.

32. Lawson RP, Nord CL, Seymour B, et al. Disrupted habenula function in major depression. *Mol Psychiatry.* 2017;22(2):202-208.

33. Carlson PJ, Diazgranados N, Nugent AC, et al. Neural correlates of rapid antidepressant response to ketamine in treatment-resistant unipolar depression: a preliminary positron emission tomography study. *Biological psychiatry.* 2013;73(12):1213-1221.

34. Murrough JW, Iosifescu DV, Chang LC, et al. Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *The American journal of psychiatry.* 2013;170(10):1134-1142.

35. Murrough JW, Perez AM, Pillemer S, et al. Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. *Biological psychiatry.* 2013;74(4):250-256.

36. Phillips JL, Norris S, Talbot J, et al. Single, Repeated, and Maintenance Ketamine Infusions for Treatment-Resistant Depression: A Randomized Controlled Trial. *The American journal of psychiatry.* 2019;176(5):401-409.

37. Singh JB, Fedgchin M, Daly EJ, et al. A Double-Blind, Randomized, Placebo-Controlled, Dose-Frequency Study of Intravenous Ketamine in Patients With Treatment-Resistant Depression. *The American journal of psychiatry.* 2016;173(8):816-826.

38. Zheng W, Zhou YL, Liu WJ, et al. Rapid and longer-term antidepressant effects of repeated-dose intravenous ketamine for patients with unipolar and bipolar depression. *Journal of psychiatric research.* 2018;106:61-68.

39. Grunebaum MF, Galfalvy HC, Choo TH, et al. Ketamine for Rapid Reduction of Suicidal Thoughts in Major Depression: A Midazolam-Controlled Randomized Clinical Trial. *The American journal of psychiatry.* 2018;175(4):327-335.

40. Sheehan D. The mini-international neuropsychiatric interview, version 7.0 for DSM-5 (MINI 7.0). *Medical Outcomes Systems.* 2014.

41. Trivedi MH, Wisniewski SR, Morris DW, et al. Concise Associated Symptoms Tracking scale: a brief self-report and clinician rating of symptoms associated with suicidality. *The Journal of clinical psychiatry.* 2011;72(6):765-774.

42. Holtzman S, O'Connor BP, Barata PC, Stewart DE. The Brief Irritability Test (BITe): a measure of irritability for use among men and women. *Assessment.* 2015;22(1):101-115.

43. Stringaris A, Goodman R, Ferdinando S, et al. The Affective Reactivity Index: a concise irritability scale for clinical and research settings. *Journal of child psychology and psychiatry, and allied disciplines.* 2012;53(11):1109-1117.

44. Mulraney MA, Melvin GA, Tonge BJ. Psychometric properties of the affective reactivity index in Australian adults and adolescents. *Psychological assessment.* 2014;26(1):148-155.

45. Buss AH, Perry M. The aggression questionnaire. *Journal of personality and social psychology.* 1992;63(3):452-459.

46. Salsman JM, Butt Z, Pilkonis PA, et al. Emotion assessment using the NIH Toolbox. *Neurology.* 2013;80(11 Suppl 3):S76-86.

47. Fava M, Anderson K, Rosenbaum JF. "Anger attacks": possible variants of panic and major depressive disorders. *The American journal of psychiatry.* 1990;147(7):867-870.

48. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. *The British journal of psychiatry : the journal of mental science.* 1979;134:382-389.

49. Rush AJ, Trivedi MH, Ibrahim HM, et al. The 16-Item Quick Inventory of Depressive Symptomatology (QIDS), clinician rating (QIDS-C), and self-report (QIDS-SR): a psychometric evaluation in patients with chronic major depression. *Biological psychiatry.* 2003;54(5):573-583.

50. Rush AJ, Bernstein IH, Trivedi MH, et al. An evaluation of the quick inventory of depressive symptomatology and the hamilton rating scale for depression: a sequenced treatment alternatives to relieve depression trial report. *Biological psychiatry.* 2006;59(6):493-501.

51. Wardenaar KJ, van Veen T, Giltay EJ, de Beurs E, Penninx BW, Zitman FG. Development and validation of a 30-item short adaptation of the Mood and Anxiety Symptoms Questionnaire (MASQ). *Psychiatry research.* 2010;179(1):101-106.

52. Spitzer RL, Kroenke K, Williams JB, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Archives of internal medicine.* 2006;166(10):1092-1097.

53. Gard DE, Gard MG, Kring AM, John OP. Anticipatory and consummatory components of the experience of pleasure: a scale development study. *Journal of research in personality.* 2006;40(6):1086-1102.

54. Snaith RP, Hamilton M, Morley S, Humayan A, Hargreaves D, Trigwell P. A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale. *The British journal of psychiatry : the journal of mental science.* 1995;167(1):99-103.

55. dela Cruz AM, Bernstein IH, Greer TL, et al. Self-rated measure of pain frequency, intensity, and burden: psychometric properties of a new instrument for the assessment of pain. *Journal of psychiatric research.* 2014;59:155-160.

56. Endicott J, Nee J, Harrison W, Blumenthal R. Quality of Life Enjoyment and Satisfaction Questionnaire: a new measure. *Psychopharmacology bulletin.* 1993;29(2):321-326.

57. Reilly MC, Zbrozek AS, Dukes EM. The validity and reproducibility of a work productivity and activity impairment instrument. *PharmacoEconomics.* 1993;4(5):353-365.

58. Wolfe J, Kimerling R. Gender issues in the assessment of posttraumatic stress disorder. 1997.

59. Bernstein DP, Fink L, Handelsman L, et al. Initial reliability and validity of a new retrospective measure of child abuse and neglect. *The American journal of psychiatry.* 1994;151(8):1132-1136.

60. Freeman MP, Walker R, Laughren TP, Miller KK, Fava M. Female reproductive life cycle and hormones: methodology to improve clinical trials. *The Journal of clinical psychiatry.* 2013;74(10):1018-1021.

61. Posner K, Brown GK, Stanley B, et al. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. *The American journal of psychiatry.* 2011;168(12):1266-1277.

62. Trivedi MH, Wisniewski SR, Morris DW, et al. Concise Health Risk Tracking scale: a brief self-report and clinician rating of suicidal risk. *The Journal of clinical psychiatry.* 2011;72(6):757-764.

63. Rush A, O'Neill B. Patient Rated Inventory of Side Effects (PRISE). *Dallas, TX: University of Texas Southwestern Medical Center.* 1999.

64. Overall JE, Gorham DR. The brief psychiatric rating scale. *Psychological reports.* 1962;10(3):799-812.

65. Kraemer HC. A mediator effect size in randomized clinical trials. *International journal of methods in psychiatric research.* 2014;23(4):401-410.

66. Chung F, Yegneswaran B, Liao P, et al. STOP questionnaire: a tool to screen patients for obstructive sleep apnea. *Anesthesiology.* 2008;108(5):812-821.

12 APPENDICES

Appendix A. Clinician/Assessor Administered Assessments

Assessment	Description	Purpose
Antidepressant Treatment Response Questionnaire (ATRQ)	A questionnaire used to determine treatment resistance in major depressive disorder.	Medical history
Brief Psychiatric Rating Scale (BPRS-Positive)	A 4 item scale to assess for positive symptoms of psychosis.	Study-drug side-effect monitoring/safety
Clinician Administered Dissociative States Scale (CADSS)	A 23 item clinician administered questionnaire and observational scale to assess possible dissociative symptoms associated with ketamine treatment.	Study-drug side-effect monitoring/safety
Columbia Suicide Severity Rating Scale (C-SSRS)	A clinician administered assessment to quantify the severity of suicidal ideation and behavior.	Safety
Montogomery-Åsberg Depression Rating Scale (MADRS)	A 10 item structured interview to assess symptoms of depression.	Disease history and treatment efficacy
MINI Neuropsychiatric Inventory (MINI)	A brief structured diagnostic interview used to assess the most common (17) psychiatric disorders in the DSM-V.	Psychiatric history
Patient Rated Inventory of Side Effects (PRISE)	A clinician administered questionnaire used to qualify treatment side effects by identifying and evaluating the tolerability of each symptoms addressing 9 domains: Gastrointestinal, Heart, Skin, Nervous System, Eyes/Ears, Genital/Urinary, Sleep, Sexual Functioning, and Other.	Study-drug side-effect monitoring/safety
Reproductive Lifecycle and Hormone Questionnaire (RLHQ) – Module I & II	A questionnaire to assess reproductive lifecycle status and the use of exogenous hormones.	Medical History

Appendix B. Self-report assessments

Assessment	Description	Purpose
Affective Reactivity Index (ARI)	A 7-item scale of irritability.	Disease history and treatment efficacy.
Buss-Perry Aggression Questionnaire (AQ)	A 29-item scale used to measure anger, physical aggression, verbal aggression, and hostility.	Disease history and treatment efficacy.
Massachusetts General Hospital Anger Attack Questionnaire (AAQ)	A brief self-report scale to identify participants who exhibit anger attacks and quantify anger attack frequency.	Disease history and treatment efficacy.
Brief Irritability Test (BITe)	A 5-item self-report measure of irritability.	Disease history and treatment efficacy.

Modification / Update, MOD013-STU-2021-0667, Manish Jha, 2/16/2026

Concise Associated Symptom Tracking Scale – Irritability domain (CAST-IRR)	A self-report measure to track symptoms of irritability in major depressive disorder.	Disease history and treatment efficacy.
Concise Health Risk Tracking Scale – Self-Report (CHRT-SR)	A self-report Likert scale to assess symptoms of suicidal ideation.	Disease history and treatment efficacy.
Childhood Trauma Questionnaire (CTQ)	A questionnaire to capture traumatic experiences in childhood.	Psychiatric history
NIH Toolbox Emotion Battery – Anger Affect Domain	A three-part measure of the Anger Affect domain to assess symptoms of anger, physical aggression, and hostility.	Disease history and treatment efficacy.
Generalized Anxiety Disorder scale (GAD-7)	A 7-item scale to assess symptoms of anxiety.	Disease history and treatment efficacy.
Life Stressor Checklist-Revised (LSC-R)	A checklist to capture past traumatic experiences and life stressors.	Psychiatric history
Mood and Anxiety Symptoms Questionnaire (MASQ)	A 30-item self-report scale to assess symptoms of depression and anxiety.	Disease history and treatment efficacy.
Pain Frequency Intensity and Burden Scale (P-FIBS)	A scale to assess symptoms of pain	Disease history and treatment efficacy.
Quality of Life and Enjoyment Satisfaction Scale (Q-LES-Q SF)	A 14-item scale to assess quality of life and functional impairment related to disease.	Disease history and treatment efficacy.
Quick Inventory of Depressive Symptomatology – Self-report (QIDS-SR)	16 item assessment self-report measure to assess the symptom criteria of major depressive disorder.	Disease history and treatment efficacy.
Self-administered Comorbidity Questionnaire (SCQ)	A self-report questionnaire to assess the presence of medical history problems and any corresponding treatments for the condition.	Medical history
Snaith Hamilton Pleasure Scale (SHAPS)	A 14- item scale to assess symptoms of anhedonia.	Disease history and treatment efficacy.
STOP-Bang Questionnaire	A self-report questionnaire to assess for obstructive sleep apnea.	Medical history
Temporal Experience of Pleasure Scale (TEPS)	A 16-item scale to assess symptoms of anhedonia.	Disease history and treatment efficacy.
Work Productivity and Activity Impairment (WPAI)	A scale to assess functional impairment related to disease.	Disease history and treatment efficacy.