A Systematic Investigation of Neurophysiological Correlates of Low Dose Intravenous Ketamine in Treatment Resistant Depression Patients

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Protocol Title: A systematic investigation of neurophysiological correlates of low dose intravenous Ketamine in treatment resistant depression patients

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Population: 20 patients, age: 18-60
Number of Sites: 1
Study Duration: 2 years
Subject Duration: 2-3 weeks

General Information

Major depressive disorder (MDD) is one of the most disabling mental illnesses worldwide with a lifetime prevalence of about 17% in the United States [1, 2] and is associated with increased mortality [3, 4]. Treatment resistant depression (TRD), defined as reduced clinical response to adequate doses and duration of antidepressants, is a severe, chronic and most disabling form of MDD [5, 6]. The introduction of antidepressants revolutionized the field of mood disorders since 1950's. Antidepressants significantly reduced the morbidity, hospitalization and overall mortality of patients suffering from depression [7]. Despite this impressive progress, nearly 50% of depressed patients do not, unfortunately, fully respond to treatment [6, 8] and current antidepressants takes few weeks to work. Furthermore, there are no reliable clinical biomarkers available to predict who will respond to antidepressant treatment and thus patients often go through several trial-and-error attempts of different treatment strategies with only modest benefits. Identifying novel therapeutic targets for TRD is a critical priority as prolonged illness is a strong predictor of poor functional outcome [6, 9]. Consequently, there are two primary challenges in antidepressant research: (a) developing rapidly acting, safe and effective antidepressant treatments, and (b) developing noninvasive clinically useful biomarkers of antidepressant response with a focus on early indicators of treatment response. The serendipitous discovery of rapid and protracted antidepressant effect of Ketamine, a widely used anesthetic drug, has opened up development of novel glutamate based antidepressant treatment strategies. The overarching objective of the current project is to address the challenge of **developing biomarkers of Ketamine and its rapid antidepressant effect** through the use of well-validated, reliable and non-invasive clinical neurophysiological techniques such as electroencephalography (EEG) and transcranial magnetic stimulation (TMS).

Background Information

Ketamine hydrochloride (HCl) is widely used anesthetic agent with intravenous bolus injection at of 1 to 4.5 mg/kg at a rate of 0.5 mg/kg/min can rapidly induce anesthesia starting within 30 seconds and lasting for 5–10 min [10]. The sub-anesthetic dose (0.5 mg/kg over 40 minutes) of Ketamine improves depression symptoms in patients with TRD [11-13], bipolar depression [14] and post-traumatic stress disorder [15] and also in reducing suicidal ideation (SI) in depressed patients [16, 17]. The improvement in depression scores were seen as early as 2 hours and the antidepressant effects persisted in up to 35% of patient at the end of 7 days [11]. Murrough et al 2013 [12] conducted a relatively larger (N=67 TRD), two site, randomized, parallel design clinical trial and confirmed the superior efficacy of Ketamine even compared to active placebo anesthetic drug, midazolam in reducing depression symptoms.]The response rates to Ketamine vs. midazolam were 64% and 28%, respectively with strong effect size (Cohen's d 0.7) and 21 out of 47 patients Ketamine-treated patients continued to exhibit improved scores over the 7-day period and thus highlighting inter-individual variability in duration of response to Ketamine. A subsequent study found that **early antidepressant response to the first infusion**

was highly predictive of a sustained response to subsequent infusions [18]. IThus, even though the efficacy of ketamine has been clearly established by many studies, the exact underlying neural mechanisms involved in Ketamine's rapid antidepressant effect is still unclear [23]. Ketamine is a n-methyl aspartate (NMDA) antagonist and administration at low doses transiently increases glutamate release in the frontal cortex, thus enhancing glutamatergic neurotransmission presumably through non-NMDA mechanisms such as α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), metabotropic glutamate, and kainate receptors [24, 25]. Indeed, AMPA antagonist pretreatment abolishes Ketamine's antidepressant effect [26]. Anesthetic dose of Ketamine is however not associated with glutamate release [25]. NMDA receptor antagonism decreases the activity of cortical parvalbumin-positive interneurons and causes cortical excitation by disinhibition of pyramidal neurons [25]. Recent neurobiological studies suggest that Ketamine induced NMDA blockade triggers a complex intracellular cascade - Ketamine upregulates BDNF expression by inhibiting eukaryotic elongation factor (eEF2) kinase [27], and increases synaptic protein synthesis and spine formation in the prefrontal cortex (PFC) [28]. In summary, a sub-anesthetic dose of Ketamine induces increased glutamate activity and initiates synaptic plasticity processes presumably mediating the antidepressant effects [29, 30]. The challenge is to be able to validate these preclinical findings in patients with depression. In addition, it is unclear if the early increases in glutamatergic neurotransmission are related to the antidepressant action. If there are **non-invasive biomarkers** available, then it is possible to directly test in TRD patients if the **early antidepressant effect of Ketamine is related to enhanced glutamate neurotransmission and synaptic plasticity**.

Clinical markers of glutamate neurotransmission and synaptic plasticity

Substantial evidence has shown abnormalities in glutamatergic neurotransmission causing impairments in neural plasticity in mood disorders [31]. Magnetic resonance spectroscopy (MRS) has been employed to measure brain glutamate and glutamine. MRS Ketamine studies in healthy subjects have been inconsistent, showing no change or increased glutamate or glutamine. One study in TRD patients found Ketamine pretreatment low Glx/glutamate ratio (as a marker of glutamine) in the prefrontal cortex was associated with greater improvement [32]. Proton MRS measurements of glutamate and glutamine, especially at low field MRI, has poor spatial and chemical resolution. A 1H-[13C]-MRS at high MRI field show sub-anesthetic Ketamine dose increase glutamate/glutamine cycle in rats [33] but these findings require confirmation in human clinical subjects.
Peripheral BDNF levels: Antidepressant treatment increases brain BDNF expression. While it is currently not possible to measure brain BDNF in patient, peripheral BDNF levels are decreased in patients with MDD [34] and serum BDNF increases is associated with antidepressant treatment response [35]. Ketamine significantly increased plasma Brain Derived Neurotrophic Factor (BDNF) levels in treatment responders compared to non-responders at 4 hours post-infusion [36, 37] and negatively correlated with the depression scores [38]. These results suggest that plasma BDNF at 4 hours post-infusion could be markers of Ketamine treatment response. However it is not clear if the BDNF increase is related to enhanced glutamate neurotransmission or synaptic potentiation.

Biomarker of prognosis and response to ketamine treatment

There is an urgent need of a biomarker of prognosis and response to treatment in patients with MDD. Pharmacogenetic studies have been trying to use genetic variants to predict the response to specific medications in patients, but most of the studies are still underpowered and the high genetic heterogeneity of the MDD population is hindering replication of most findings (Fabbri and Serretti, 2015). Specifically, associations with genetic variants are especially complicated because they require very large sample sizes and not often look into the functionality of those variants. In this context, switching the focus to functional genetic markers, such as DNA methylation and gene expression levels, to predict a priori the response to treatment, would overcome those limitations and allow for a more accurate and dynamic measure of how the cells of a given patient will actually respond to potential medications. This approach has been proposed by a recent study showing that the ex vivo cellular response to medications can predict the clinical outcome of the treatment in vivo. Specifically, the degree to which paroxetine reduced the phosphorylation of the enzyme DNA methyltransferase (thereby reducing its activity and increasing the expression of neurotrophic genes) in peripheral blood mononuclear cells of depressed patients significantly correlated with the reduction of depressive symptoms after six weeks of treatment (Gassen & Fries et al., 2015). In other words, the failure to improve symptoms was detected after seventy two hours of ex vivo treatment in blood cells. In this sense, the study aims to observe specific expression and/or methylation

markers measured in blood cells isolated treated ex vivo with a single dose of ketamine and it's correlation with the clinical response to treatment in patients with treatment-resistant depression.

Transcranial magnetic stimulation (TMS) measures of Cortical Excitability

TMS is a widely employed non-invasive brain stimulation (NIBS) technique to characterize neurophysiological function underlying neuropsychiatric illnesses [39], including markers of cortical excitability and plasticity that can elucidate the neurobiology of drug action [40]. TMS paradigms include measurements of motor evoked potentials (MEP) with surface electromyography (EMG) applied to hand muscles such as the abductor pollicis brevis (APB) after applying single or paired TMS pulses on the motor cortex [41, 42]. The motor cortex is an extensively studied target area for neurophysiological studies because changes in motor activation and excitability can be readily and reliably assessed by MEP [42]. **MEP amplitude, cortico-motor threshold (MT) and intra-cortical facilitation (ICF)** are commonly reported measures of cortical excitability function [42]. The MEP amplitude is measured as the average response to a series of TMS pulses applied at a consistent TMS intensity [43]. MEP amplitude is thought to be dependent on NMDA and voltage gated channels and thus modulated by NMDA and GABAergic drugs and a reliable measure of the excitatory/inhibitory balance of cortical pyramidal cells [39]. MT is a measure of corticospinal excitability and depends on the excitability of axons and that of synaptic connections at corticospinal level [40, 42]. Voltage-gated sodium (Na) channels regulate axon excitability and NMDA regulates fast excitatory synaptic neurotransmission in the neocortex [39]. Anticonvulsants with Na channel blocking properties increase MT. Ketamine, a NMDA antagonist, paradoxically decreases MT [44]. ICF assessments by TMS involve pairing a subthreshold (80% of RMT) conditioning stimulus followed by a suprathreshold test stimulus, with an interstimulus interval of 10–20 ms [39, 45]; such pre-conditioning facilitates (i.e. increases amplitude) the MEP. ICF is thought to index NMDAR-mediated excitatory neurotransmission in the cortex [39, 46] though GABA and other neuromodulators may also contribute to ICF. A **review of TMS studies of cortical excitability** in patients with MDD indicated no significant differences in cortical excitability measures [47]. However, there were cortical inhibitory deficits as measured by intra-cortical inhibition and silent cortical period, both thought to be linked GABA function. Interestingly, Levinson at al. found increased resting motor threshold (RMT) in TRD compared with unmedicated MDD patients, medicated/ euthymic MDD patients, and healthy subjects [48]. While chronic (30 days) administration of paroxetine in healthy subjects induced a significant increase in ICF, single intravenous administration of serotonergic antidepressants, citalopram and clomipramine reduced motor cortical excitability in TRD patients [49]. These findings raise the intriguing question whether any reductions in cortical excitability in TRD patients could be ameliorated by Ketamine's ability to increase cortical excitability.

Auditory steady state response (ASSR) and Gamma band oscillations

Gamma band oscillations (30-80 Hz) are thought to play a critical role sensory and cognitive processing, and in the pathophysiology of a number of neuropsychiatric disorders. The auditory steady state response (ASSR) is a widely used non-invasive electroencephalography (EEG) technique to study cortical gamma oscillations in auditory cortex. The ASSR evoked power is thought to be regulated by interaction of NMDA receptor and parvalbumin (PV) gamma aminobutyric acid (GABA) inhibitory interneuron activity on sensory cortical pyramidal [50, 51]. Ketamine and NMDA antagonists dose-dependently increases ASSR power and gamma oscillations [52, 53] and causes increased cortical excitability likely by shifting excitatory/inhibitory balance at the circuit level [25, 54, 55]. Further, there has been recent initial validations of ASSR at 40 Hz frequency as a potential marker of NMDA activity [56]. Only one study looking at ASSR using MEG study reported no change in MDD patients compared to healthy subjects but significantly larger ASSR power if compared with BD patients [57]

Objectives

The primary goal of the project is to study the effect of Ketamine on cortical neurophysiological function in TRD patients. There are three key preclinical findings regarding Ketamine antidepressant effects that motivate the current study: **a)** low dose Ketamine causes early increase in glutamate neurotransmission; **b)** Ketamine initiates synaptic plasticity; **c)** ketamine infusion leads to rapid improvement in depression symptoms; **d)** specific expression and/or methylation markers measured in blood cells isolated treated ex vivo with ketamine will significantly correlate with the clinical response to treatment in patients with treatment-resistant depression; The proposal essentially employs robust and non-invasive neurophysiological techniques, ASSR-gamma oscillatory response and TMS cortical excitability to investigate the above findings in patients with treatment resistant

depression. This project is innovative in multiple respects; this proposal is the **first systematic investigation of neurophysiological correlates of rapid antidepressant effect** of low dose intravenous Ketamine in TRD patient and is highly innovative and original. The study design will be an open label intervention and by measuring cortical excitability at baseline and at different post Ketamine infusion time points, it is possible to **study temporal dynamics of neurophysiological changes** and closely relate these to the changes in depression symptoms. This proposal will be significant contribution to our understanding of **Ketamine's early antidepressant effect**. This project will be the first attempt to study the **effect of Ketamine on cortical gamma ASSR and index of NMDA activity** in TRD patients. Another innovative component of this project is the integration of clinical, neurophysiological and blood based BDNF markers in the study of Ketamine antidepressant effect. This proposal will potentially yield substantial data on markers of treatment response. As reported above, BDNF measures were only reported at 4 hours post-infusion time-point, in addition to this time point, this project also aims to obtain BDNF measures at 24 hours to examine correlates of treatment response.

The main objectives of this project are to investigate the antidepressant effect of low dose Ketamine on cortical excitability and plasticity in the Dorsal Lateral Prefrontal Cortex (DLPFC) of patients with TRD using TMS-EEG**.** We had previously collected cortical excitability data from 4 health volunteers. We first determined resting motor thresholds (RMT) as the lowest stimulator output intensity which elicited an MEP greater than 50 μV in at least 5 out of 10 trials. RMTs were found to be comparable across participants (*M*=53.8*, SE*=5.0;, purple) and commensurate with previous investigations in the literature. Bipolar EMG recordings of the APB muscle during TMS at 100% RMT demonstrated a mean peak to peak amplitude of 569 μV. To measure ICF, an initial conditioning pulse applied at 80% of RMT was followed by a test pulse at 100% RMT at an inter-stimulus interval of 10 ms. MEP responses at this latency resulted in an increased mean peak to peak MEP amplitude of 1381 μV- a 233% increase over the single pulse response. Our assessment of motor cortex excitability was determined to be robust and reliable with a total data collection time of less than four minutes and minimal data processing. Thus, our TMS indices are a straightforward and ideal index of Ketamine-induced cortical excitability changes.

Exploratory Aim: To investigate Ketamine's acute excitatory glutamatergic effect on cortex as indexed by the ASSR. The first goal of the study is to deploy auditory steady state response (ASSR) and gamma band oscillations as a marker of Ketamine's early excitatory effect on cortex. We previously studied the feasibility of quantifying the effect of pharmacological manipulations on cortical ASSR power. We studied the effect of amphetamine, which increases synaptic dopamine neurotransmission. Twelve healthy subjects (mean age 30.3±10, M:F 7:5) took part in a double-blind, cross-over, placebo-controlled study of single-dose amphetamine (0.5 mg/kg) administration. Subjects performed the ASSR task, involving auditory click trains consisting of binaural presentations of 1 ms duration tones repetition frequencies of 40 Hz, 30 Hz, or 20 Hz. To ensure attention to stimuli, click trains were presented as an oddball paradigm. Electroencephalographic (EEG) data during task performance were collected on a 128 channel Geodesic Sensor Net. Off-line de-noising and averaging will be performed with EEGLAB (62), and wavelet analyses with Brain Vision Analyzer. The average evoked gamma power was derived for the 40 Hz stimuli over the interval 225–525 ms after stimulus onset. Amphetamine showed decreases in gamma power specifically to the 40 Hz stimuli ($t=2.3$, $p<.05$, $dz=0.66$). Time frequency plots of gamma power after amphetamine for 40 Hz stimuli demonstrated amphetamine induced decreased gamma power. ASSR-EEG paradigm may be well-suited to tracking Ketamine induced modulations in gamma oscillations as implemented in this project

Study Design and Procedure

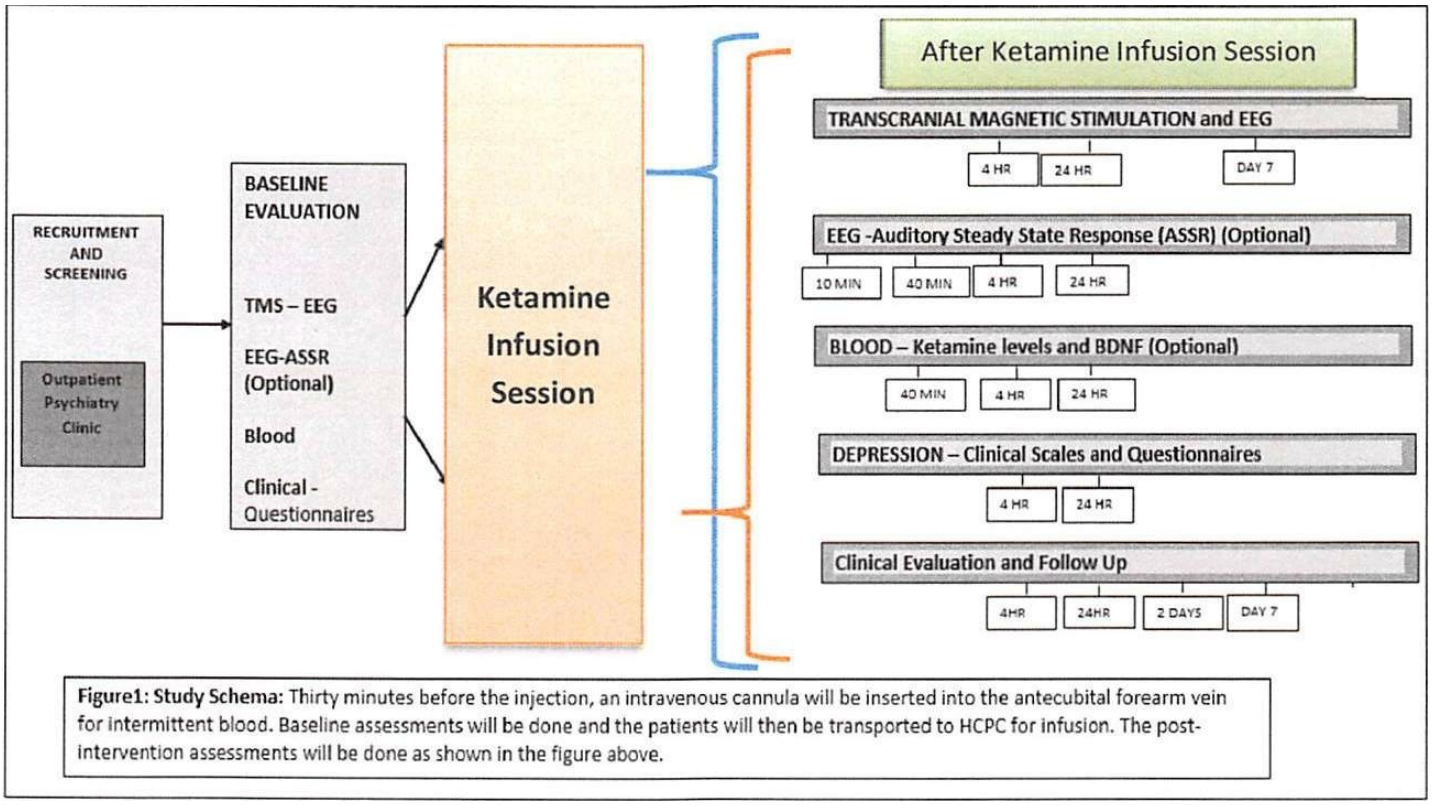

Figure1: Study Schema: Thirty minutes before the injection, an intravenous cannula will be inserted into the antecubital forearm vein for intermittent blood. Baseline assessments will be done and the patients will then be transported to HCPC for infusion. The post-intervention assessments will be done as shown in the figure above.

We will employ an open-label study in which the infusion session, the enrolled TRD patients will receive low dose Ketamine (0.5 mg/kg) over 40 minutes as previous studies [58].

The overall study design is described in Figure1 above.

Ketamine infusion: We will use slow infusions of ketamine over a time period of 40 minutes. Study drug will be provided in syringes, containing clear solutions of 1 ml of (0.5mg/kg) ketamine. The dosage will be calculated using the patient's weight in kilograms. For dosing purposes, 0.5-0.9 round up to the next dose and from .01-0.49, we will round down to the lower dose. An ACLS psychiatrist (Salih Selek) will monitor the patient at HCPC. The administration of ketamine will be initiated by the anesthesiologist. The anesthesiologist will be present during the ketamine administration (40 minutes) and post-procedure (until patient is awake and anesthesiologist deems patient is clinically stable. The infusions will be conducted in our hospital during the mornings in a room set specially for this study. During the infusion, patients will be asked constantly about "how they feel" and will receive constant attendance from a psychiatric nurse and the attending ACLS- trained psychiatrist. ECG and pulse oximetry monitoring will be maintained throughout the infusions to check for hemodynamic changes.

After completion of each 40 minute infusion, patients will rest for 2-4 hours under observation of the clinical nurse before being discharged to a responsible adult who will accompany the patient home after completing study tasks. Standard blood sample measurements may occur before the infusion day. All participants will undergo urine drug tests before each infusion. During the course of infusions, patients will receive treatment-as-usual from their primary psychiatrists as would be the case in a traditional therapeutic setting. There will be no changes in medication regimen throughout the 2-week trial. Participants will be provided with a contact card directing them to the nearest ER/physician in case of emergency. We will make it clear to the patient that all of the study procedures are optional at patients' and the PIs discretion.

Cortical Excitability-TMS procedure: TMS-EEG will be performed at 4 hours, 24 hours and 7 days after the infusion. Patients will be seated in an armchair with elbow semi-flexed; the forearm pronated and fully relaxed. Participants will be given ear plugs for comfort and noise safety. A MagVenture MagPro X100 transcranial magnetic stimulator system with a figure-of-eight coil (diameter=70 mm for each loop) will be placed tangentially over the scalp overlying the primary motor cortex and DLPFC. The stimulator is paired with Localite neuronavigation system which allows precisely localized stimulus application and its integration also allows

external control of the MagPro system. EMG readings may be obtained from the APB muscle. TMS stimulation will be applied to the corresponding region of the contralateral primary motor cortex. The optimal coil position will be determined by moving the coil in 1-cm increments over the motor cortical area while delivering magnetic pulses and by observing maximal contraction of the contralateral APB. The procedure may be repeated for the opposite hemisphere. The main purpose of this study is to examine the cortical excitability measures after Ketamine. Resting motor threshold (RMT) is defined as the stimulation intensity eliciting an MEP greater than 0.5 mV in 5 of 10 trials with a relaxed APB. Intra-cortical facilitation (ICF) is defined as subthreshold conditioning stimulus set at 80% of resting MT calibrated to produce an average MEP of 0.5- to 1.5-mV peak-to-peak amplitude in the contralateral APB. A suprathreshold test stimulus follows conditioning stimulus between 10-20 milliseconds (ms) (55). Conditioning stimuli maybe delivered to left Dorsolateral Prefrontal Cortex (DLPFC) or Broadmann Area 6 or Motor cortex prior to the test stimulus in 1 of 3 random inter-stimulus intervals10 ms (ICF-10), 15 ms (ICF-15), and 20 ms (ICF-20) for ICF measures. The sequence of administration will be counterbalanced to reduce order effects. For ICF, the change in test stimulus MEP amplitude of each inter-stimulus interval was expressed as a percentage of the mean unconditioned MEP amplitude (55). TMS will be applied concurrently with EEG recordings. **EEG/EMG Acquisition and Analysis -** EEG sessions will be conducted in a sound-attenuated room lit with a low-level ambient light. EEG Brain Products Brain Amp MRPlus 64 channel system is used to acquire EEG data. EEG signals are recorded with a 64 channel cap at a high sampling rate (500 Hz) from the entire scalp, with signals amplified by a shielded, MR-compatible amplifier. The software, Brain Vision Recorder, will be used to coordinate EEG recordings with EPrime. Continuous data will be filtered off-line with a .2- to 100-Hz bandpass filter with a 60-Hz ideal notch filter. ASSR Epochs are defined as −500 to 1000 ms relative to stimulus onset adjusted to a −350 to −150-ms prestimulus baseline. Ocular and ECG artifacts will be removed with ICA-based detection and correction methods (EEGLab); [59]. Data will be re-referenced to average reference. EMG data will be obtained using the Brain Amp ExG amplifier which allows for up to 8 simultaneous bipolar recordings at a 5-kHz sampling rate via the same software as EEG data. MEP epochs will be defined as -200 to 300 relative to stimulation. ICF trials will be time adjusted to align the second pulse with single pulse trials, and MEP amplitudes will be determined as the peak to be difference identified between 10 and 50 ms post-TMS pulse. Software and hardware for the analysis of EEG/EMG data, include state-of-the-art analytic and statistical packages (e.g. EEGLab, Brainstorm, Matlab, and SPSS).

OPTIONAL: ASSR/EEG paradigm: Task - Patients will wear ER-3A insert earphones (Etymotic Research, Elks Grove, IL, USA) for auditory stimuli and will be seated ~80 cm from an LCD computer monitor used to present visual stimuli. All stimuli are presented using E-Prime software (Psychological Software Tools, Pittsburgh, PA, USA). Click trains of 500-ms duration will be presented binaurally at 65 ± 5 dB. The click train repetition frequencies will be 40 Hz and presented in the context of an auditory oddball paradigm to ensure participant attention to the stimuli. Standard stimuli will be click trains with individual clicks being 1-kHz carrier frequency whereas Oddball stimuli will be click trains with clicks of 2-kHz carrier frequency. During click train presentation and for 200 ms after click train cessation, the screen remained blank (black). Participants will be then prompted by the appearance of a central fixation cross to respond by button press with either their left index finger for Standard stimuli (110 trials per block) or their right index finger for Oddball stimuli (10 trials per block). Behavioral analyses will be conducted to confirm attention to task but only correct Standard trials will be submitted for EEG analyses.

OPTIONAL: Blood Tests:

Gene Expression and Methylation: The study aims to identify alterations in gene expression and methylation markers induced by the ex vivo treatment of peripheral blood mononuclear cells from symptomatic patients with treatment-resistant depression with ketamine and assess their correlations with clinical response (as measured by the reduction in the Hamilton Depression Rating Scale - HDRS). For that end blood samples will be collected from all subjects immediately before the first administration of ketamine, and peripheral blood mononuclear cells will be isolated with Ficoll-Paque®, according to the manufacturer's instructions. Cell will then be re-suspended in RPMI and plated at 4 x 105/cm2. After recovery for 6 hours, cells will be treated with ketamine or vehicle. RNA and DNA samples will be isolated from peripheral blood mononuclear cells immediately after treatment (2 hours) and interrogated by RNA-sequencing (Ion Proton, Thermo Fisher) and the Infinium MethylationEPIC kit.

(Illumina), respectively, according to the manufacturer's instructions. The reduction in the HDRS scores from baseline to post treatment will then be correlated with the alterations in the markers from both analyses, and the markers that show the strongest correlations with the reduction in HDRS will be selected. Moreover, expression and methylation markers will be compared between responsive and non-responsive patients. This will lead to the identification of a 'treatment response biosignature' incorporating information from gene expression and DNA methylation that can predict the response to ketamine in patients. **BDNF:** Ketamine and metabolites levels will be measured at baseline, 40 mins, 2-4 hours and 24 hours after ketamine infusion and serum BDNF tests will be performed at baseline (before each infusion), 2-4 hours and 24 hours after each infusion. Samples will be stored at Behavioral and Biomedical Sciences Building (BBSB) for analysis. BDNF concentrations were quantitatively determined by enzyme-linked immunosorbent assay (ELISA). A trained personnel will collect up to a **total** of 10 8 cc tubes (about 5.4 tablespoons) of blood from one subject in all the visits combined. A maximum of 4 8 cc tubes of blood (2.5 tablespoons) will be drawn per day from an individual.

Assessment

The protocol consists of three components: clinical and cognitive and neurophysiological assessments.

Clinical assessment

This evaluation will obtain family history and demographic data. Copies of any recent medical evaluations (past medical history, physical exam, etc.) will be requested from treating clinicians and health care providers. We will perform a urine drug screening (UDS) to rule out recent undisclosed use illicit substances. Demographic information is obtained through a standardized form and covers: age, race, gender, education (information both on siblings and their parents), religion, and socioeconomic status. Handedness in all participants will be confirmed with Oldfield's Edinburgh Handedness Inventory

Patients may fill out mood questionnaires rating the severity of the clinical symptoms at various time points at the discretion of the PI.

Self-report questionnaires and Clinical Assessments to assess mood, anxiety and severity of clinical symptoms:

1. Adult (**Brief Dissociative Experiences Scale** [DES-B]—modified): to monitor the dissociative side effects, Scale features: simple, easy to use, new DSM-5 self-rated scale. Frequency: After every injection
2. **Severity Measure for Generalized Anxiety Disorder**—adult: to measure the severity of anxiety. Scale features: simple, easy to use, new DSM-5 self-rated scale. Frequency: At the discretion of the PI
3. **Snaith–Hamilton Pleasure Scale**: to measure the level of anhedonia, Scale features: easy to use, used before in ketamine studies, self-rated scale. Frequency: At the discretion of the PI.
4. **SCID-I Interview**: This is the standard psychiatric research evaluation for ascertainment of psychiatric diagnosis in the context of research studies (Spitzer et al., 2007). It is a comprehensive structured interview for determining axis I diagnosis according to the DSM-IV. Frequency: Before the start of the study
5. **Clinical Global Impression Scale** (CGI): This is a clinical instrument to assess global changes in patients' status in clinical studies. We will use the CGI-BD, for use with BD patients (Guy et al., 1976).
6. **Montgomery-Åsberg Depression Rating Scale** (MADRS): this scale rates depressive symptoms, and its psychometric properties are well-established (Montgomery et al. 1979). Frequency: Before the start of the study and at 4 hours, 24 hours and 7 days after infusion. (Changed based on the PI).
7. **Young Mania Rating Scale** (YMRS): This is a well-established clinical severity rating scale for manic symptoms that has adequate inter-rater reliability and internal consistency (Young et al., 1978). Frequency: Before the start of the study and at 4 hours, 24 hours and 7 days after infusion. (Changed based on the PI).
8. **Positive and Negative Affect Schedule** (PANAS): the PANAS comprises two mood scales, one that measures positive affect (PA) and the other which measures negative affect (NA). Used as a psychometric scale, the PANAS can show relations between positive and negative affect with personality stats and traits. Ten descriptors are used for each PA scale and NA to define their meanings. Participants in the PANAS are required to respond to a 20-item test using 5-point scale that ranges from very slightly or not at all (1) to extremely (5). This scale may be administered prior to and following each infusion to assess acute changes in mood. Frequency Before the start of the study and at 4 hours, 24 hours and 7 days after infusion. (Changed based on the PI).
9. The World Health Organization Quality of Life(WHOQOL)-Brief): this scale is patient-reported and measures the degree of enjoyment and satisfaction experienced by subjects in various areas of daily functioning *(Endicott*

et al., 1993). Frequency: Before the start of the study and at 4 hours and 24 hours after infusion. (Changed based on the PI).
10. **Hamilton Depression Rating Scale** (HDRS): a multiple item questionnaire used to provide an indication of depression, and as a guide to evaluate recovery. Frequency: Before the start of the study and at 4 hours, 24 hours and 7 days after infusion. (Changed based on the PI).
11.**Hollingshead Socioeconomic Status:** This scale is patient-reported and indication of socioeconomic status:Frequency: At the discretion of the PI.
12. Columbia Suicide Severity Rating Scale

Cognitive assessment (Optional)
Patients may complete the BAC-A is based on the Brief Assessment of Cognition in Schizophrenia (BAC-S). The BAC-S has been validated both linguistically and psychometrically in a number of psychiatric populations, including patients with schizophrenia and BD (Cuesta et al. 2011Cuesta et al., 2011; Hill et al., 2013; Kuswanto, Sum, & Sim, 2013; Salgado et al., 2007; Segarra et al., 2011). It has been shown to be as valid and sensitive as a traditional neuropsychological assessment and takes approximately 35 minutes to administer (Keefe et al., 2004; Velligan et al., 2004). Six of the 8 subtests of the BAC-A match those found in the BAC-S. These tests are the Token Motor Task, Symbol Coding, List Learning, Digit Sequencing Task, Category Instances (Animals) and Controlled Oral Word Association Test (F and S-words), Tower of London (Keefe et al., 2004). In addition to these 6 subtests the BAC-A comprises the Emotion Inhibition Test (a modified version of the Emotional Stroop task (LaMonica, Keefe, Harvey, Gold, & Goldberg, 2010; Williams, Mathews, & MacLeod, 1996)) and the affective auditory verbal learning test (Affective interference test). The latter task is similar to the Affective Auditory Verbal Learning Test (AAVLT) (Snyder & Harrison, 1997).

Participants may complete the Effrt task (Treadway et. al 2009) which is an effort-based decision making task using E-Prime software (Psychological Software Tools). Participants may make additional bonus money from this task and it will be added to their compensation.

Subjects may also be asked to do some simple tasks which will involve looking at a computer screen display (figures, symbols, numbers, letters, words or sentences) or listening to sounds through headphones (clicks, beeps, or words). The participant is asked to respond to the stimuli by pressing a response button. Stimuli will be presented on a computer screen using E-Prime (Psychological Software Tools, Pittsburgh, PA). Dependent variables will be reaction time, response, and accuracy collected via keyboard, joystick, button box or similar device and stored on a computer. The task will involve separate trials and trials are organized in blocks so that participants will be allowed to rest in between blocks. In each session, cognitive tasks will be presented on a computer screening using E-Prime (Psychology Software Tools, Pittsburgh, PA).

Neurophysiological assessment (Optional)
TMS -TMS stimulation will be applied to the corresponding region of the contralateral primary motor cortex and left DPFC or Broadmann Area 6 to examine the cortical excitability measures after Ketamine infusion. EEG- EEG will be acquired as participants perform an auditory task. The purpose of EEG is to study gamma band oscillations as a marker of Ketamine's early excitatory effect on cortex. TMS and EEG will take place concurrently.

Summary
We will pre-screen potential participants over the phone to minimize the invitation to non-eligible participants. During the screening visit, we will screen patients for eligibility. Patients will then arrive for their study Day # 2(Baseline), when they will get blood draws (optional), complete questionnaires and undergo TMS-EEG. Patients will come to our inpatient psychiatric hospital facility on the another day (Day # 3) , where they will be given ketamine by the study nurse. During the infusion, patients will be asked constantly about "how they feel" and will be monitored continuously from psychiatric nurse and the attending Anesthesiologist. EKG and pulse-oximetry will be monitored throughout the procedure to check for hemodynamic changes. After the completion of 40 min infusion, patients will rest for two – four hours under the observation of clinical nurse and, reassessed again by psychiatrists at 2 hours after infusion. Patients may be undergoing EEG/ASSR at 10minutes, 40 minutes and at 4 hours after infusion and blood tests after infusion(40 minutes) and 2-4 hours after infusion. Patient will then

have undergo TMS-EEG & EMG at 4 hours. Patient will be discharged to a responsible adult who will accompany the patient home. Patients will be invited again for day #4 (24 hours after infusion), where they will have to complete TMS-EEG & EMG, EEG/ASSR(optional) and blood tests(optional). They will be invited again for just TMS 7 days after infusion. Patients may be contacted every day after the infusion by telephone to check on their physical and mood status and will be scheduled for a clinical visit with the psychiatrists on days 1, 4, and 7for a follow up assessment of mental state.

Study Population

We will enroll 20 patients with Treatment Resistant Depression (TRD) in this study. Participants will be male and female adults, aged 18-60 years, who meet the criteria for TRD. Participants will be recruited without regard to race, religion or ethnicity. Children will NOT be included in this research. Children younger than 18 are excluded as the incidence of TRD is relatively small in individuals less than 18 years old and because the safety and efficacy of the proposed study medication and treatment should be established in adults first, before conducting this type of treatment research in children. No other special classes of vulnerable individuals will be included. Participants will be recruited through flyers and clinic referrals in the Houston area.

We anticipate recruiting equal numbers of male and female patient subjects. Men and women of all ethnic backgrounds will be recruited to participate. It is anticipated that the subject demographic profile will closely mirror the larger population of individuals with TRD from which they are recruited. We will do so by distributing flyers in neighborhoods known to have a high minority population.

Selection Criteria for individuals:

Inclusion criteria –

1. Be between 18-60 years of age
2. Meet criteria for TRD (defined as two or more unsuccessful trials of antidepressants at an adequate dose for at least 4 weeks)

Exclusion criteria –

1. Diagnosed with intellectual disability, eg. Mental retardation, neurodegenerative diseases, eg. Early onset neurocognitive disturbances such as frontotemporal dementia or behavioral disorders, eg. adult onset Attention Deficit Hyperactivity Disorder
2. Diagnosed with Bipolar Disorder (BD)
3. Diagnosed with personality disorders
4. Previously or currently diagnosed with psychosis (schizoaffective disorder –SAD) or schizophrenia – SCZ)
5. Current major medical problems that affect brain anatomy, neurochemistry, or function, e.g., obstructive sleep apnea requiring Continuous Positive Airway Pressure (CPAP), liver insufficiency, kidney insufficiency, cardiovascular problems, systemic infections, cancer, auto-immune diseases, and any brain disorder (seizure disorder, stroke, dementia, degenerative neurologic diseases); history of any brain diseases, including seizures, stroke, meningitis, encephalitis, dementia, degenerative brain diseases, and head injury with loss of consciousness for any period of time
6. Diagnosed specifically with a cardiovascular disorders such as "uncontrolled" hypertension or diastolic BP over 100, Arrhythmias, Chronic Heart Failure, Myocardial Infarction (MI) or suffering from Chronic Obstructive Pulmonary Disease (COPD) or asthma. Cardiac clearance prior to enrolling in the study and medical records from physician will be required per patient's PCP
7. Patients with increased risk of laryngospasm, active upper respiratory infections, respiratory depression, increased intracranial pressure, hyperthyroidism, or porphyria
8. Current substance abuse or dependence. Only patients who achieved stable, full remission for at least 6 months will be included
9. Pregnancy or Breast feeding. All female in reproductive age will undergo pregnancy tests. Female participants will be required to provide evidence of use of contraceptives during the course of the study,
10. Unable to understand the design and requirements of the study

11. Unable to sign the informed consent for any reason.

Sources of Materials: All participants will provide demographic, health and psychiatric history information obtained at screening, and electro-physiological (e.g. EMG-TMS, blood pressure) data obtained during the study sessions. We will also obtain urine at all study sessions to ascertain drug and alcohol use and (in women) pregnancy status. All data collected on paper forms will be stored in locked cabinets, while electronic data is stored on our secure password protected server (maintained by the UTHealth Medical School Information Technology Department). All electronic records will identify study participants only by a study code, and the electronic file linking codes to individual identifiers will be destroyed once data collection is complete.

Potential Risks

For all participants:

1. Diagnostic procedures and questionnaires: Some of the questions asked during the screening may be considered sensitive information, including drug use history and psychiatric history. Answering these questions may be psychologically discomforting to some subjects. There are also risks associated with loss of confidentiality.

2. Psychophysiological monitoring: We will monitor psychophysiological responses using conductive electrodes attached to the skin of participants with an adhesive. There may be mild discomfort or irritation to the participant's skin as a result of cleaning the sites to apply the sensors, but this should be transient. Approximately half of individuals experience slight stinging/irritation lasting around 15 min. A few individuals experience red marks at the site of application that can last up to one day after the application. A very small number of participants with particularly sensitive skin may experience marks that last longer (up to two to three days). All equipment will be appropriately grounded and shielded, and stimulus equipment will be optically isolated from the participant making any electrical hazard to the participant extremely unlikely. All surfaces in contact with the participants' skin are disposable, and all equipment will be thoroughly cleaned between sessions to make risk of infection also unlikely.

Neurophysiology Testing- Participants may experience some mild discomfort during tasks involving presentation of auditory stimuli.

EEG-The EEG (electroencephalograph) is a non-invasive procedure which may cause skin irritation from the placement of recording electrodes in less than 1% of people. Itchiness of scalp or redness because of electrode gel could result and is the same amount of risk as any saline solution. The EEG is administered by trained research staff and can be stopped at any time if the subject becomes uncomfortable.

TMS- The side effects with TMS are very rare. The most common side effects include headaches and ringing in the ears (earplugs are always worn to prevent this). TMS side effects also include a small risk of seizure and convulsion. This is a very rare complication; the reported risk is less than 1 in 1000 for repetitive TMS and even lower in physically healthy patients. This risk is lowered in this protocol as we will not be doing repetitive TMS.

3. Study Medication. Side effects associated with ketamine injection are rare and, if any, of transient duration. The most commonly reported side effects during a 4 hour period after infusion include feeling strange or unreal (58.3%), abnormal sensations (54.2%), blurred vision (50.0%), feeling drowsy or sleepy (48.5%) and headaches (30-40%). Based on previous evidence, we do not expect ketamine to impair cognitive functions such as memory, attention or language. If patients are currently taking certain medications on a daily basis within 24 hours prior to and / or after receiving ketamine, they will not be able to take these medication(s) while receiving a ketamine infusion without clearance or approval of the physicians involved in administering ketamine. Medications include: Sedatives (e.g., clonazepam, lorazepam, alprazolam); Antibiotics (e.g., azithromycin, clarithromycin); Antifungal agents (e.g., ketoconazole); Tramadol. This is due to concerns for potential increased sedation or trouble breathing. The risk of addiction to ketamine for a single –dose study is very rare.

Adequacy of protection against risks

Recruitment and Informed Consent: Participants will be self-referred in response to various study advertisements via flyers. Individuals who call for information will be given a brief description of the study. Those interested will then be asked to answer questions about their disease status. A trained research assistant will conduct this telephone-screening interview. Eligible subjects will be scheduled for an in-person screening visit at the BSBB.

Once eligible for the study, they will be invited for study Day# 1, which will begin with the presentation of the informed consent form. The consent form will detail the requirements of study participation (e.g., # of visits, type of data collected, time commitment, etc.) Subjects will be told that the purpose of the study is to evaluate neurophysiological measures to study the effect of ketamine on TRD. Subjects will be informed that they will have to attend a total of five visits (screening and four study visits). Other information on the consent form will include a full description of study requirements, reimbursement, risks, benefits, alternatives, and the role of the local IRB. All questions will be answered before written consent is requested. Informed consent will be obtained only by the PI or a trained senior member of the staff (e.g. study coordinator).

The research protocol, consent form, and all assessment/advertising materials will be reviewed and approved by the Committee for the Protection of Human Subjects (CPHS) at UTH.

Protections against risk

1. *Risks related to diagnostic procedures/questionnaires:* As noted above, the primary risks related to these procedures are participant discomfort and loss of confidentiality. Regarding participant discomfort, we will make clear that we ask for this sensitive information as part of the consent process. Further, while subjects may be uncomfortable reporting these issues, the risks of serious sequelae are extremely low. Regarding confidentiality, we have rigorous procedures in place to ensure confidentiality of data, including locked cabinets for confidential files, participant coding, secure computer systems, and rigorous training of personnel. Computer systems are secure and strictly monitored by University IT staff. Laboratory staffers are trained in confidentiality of participant information. No information is allowed to leave the lab or to be accessed by a computer outside of the university's secure computer system, and all data are further protected by permissions and passwords given only to necessary research personnel. No information will be published in a form in which the participant can be identified. We will also obtain a Certificate of Confidentiality for this study to provide additional protection for sensitive information.

2. *Risks related to psychophysiological monitoring:* As noted above, precautions are taken to make electrical hazards from equipment unlikely, including correct grounding and optical isolation of the participant. Disease hazards are minimized through disposable electrode collars and thorough cleaning processes. Mild, transient skin irritation from cleaning procedures is common (as described above), but the possibility of more serious skin irritation will be minimized by asking participants about previous allergic reactions to rubbing alcohol or exfoliants and rigorous training in correct skin cleaning procedures for research assistants.

3. Electroencephalography (EEG): The EEG is administered by trained research staff and can be stopped at any time if the subject becomes uncomfortable.

4. *Transcranial Magnetic Stimulation (TMS):* Earplugs are always worn to prevent any headaches or ringing in ears caused by TMS noise. In this protocol, since it will not be repetitive TMS, the risk is very rare.

5. *Neurophysiology Testing:* Patients may stop at any time if they experience any discomfort during tasks involving presentation of emotional pictures or auditory stimuli. The decibel levels of the auditory task will be checked before start of the study and will not fluctuate significantly through the study.

6. *Risks related to study medication:* The following procedures will be taken to safeguard against adverse medication events: (1) careful initial intake evaluation to determine eligibility based on inclusion/exclusion

criteria; (2) thorough physical evaluation prior to infusion, consisting of physical examination, standard laboratory tests, electrocardiogram, urine toxicology screen, pregnancy test and vital signs; (3) Monitoring of concomitant illicit drug use at each visit with self-report, urine and/or breath testing; (4) Review of medication response, adverse events, and medication compliance with the study nurse; (5) Regular evaluation of all medical information. In the event that contraindicated medical conditions and/or other serious adverse symptoms arise after initiation of the medication, medication will be discontinued and the subject will be examined.

7. *Risks related to alternative treatments:* As noted above, we believe that our therapeutic interventions provide treatment that is considerably superior to most, if not all treatment opportunities in the community. Nevertheless, we will refer patients to other facilities upon request or when required by other circumstances. We will also conduct regular literature searches on alternative treatments, and in the event that an alternative treatment emerges with clearly superior efficacy, we would suspend the current study and provide all participants with referrals for this alternative treatment.

8. *Study withdrawal*: The research study and participation is completely voluntary. Participants can withdraw from the study at any time without giving a reason and without any consequence. A decision not to take part or to stop being a part of the research project will not change the services available to them from the UTHSC-H, Department of Psychiatry or the HCPC. The investigators could stop participation if an unfavorable or unexpected reaction is noticed.

9. *Unanticipated Hospitalizations:* If a patient needs to be hospitalized for worsening of symptoms or for their own personal safety, they will be removed from their study participation and their study treatment will end at that time.

10. *Risks of blood draw:* There are minimal risks involved in having the blood draw for the study. The blood sample will be drawn from a peripheral vein, by a trained staff person. The risks involved are related to local bruising but these will be minimized by the use of trained personnel.

Potential benefits of the proposed research to the participants and others

All assessment and services provided in these studies will be free. This particular pseudo randomization is considered for this study so as to offer the benefitting treatment to the majority of study population and will be similar to other recent studies. The treatments should help in TRD and preventing relapse. Subjects will be told if unusual information is discovered during the study that will make a difference in treatment for this or other problems. By taking part in this research subjects will help others with similar problems because this study is likely to identify how the treatment works and could also lead to development of biomarkers for monitoring treatment response.

Reimbursement

Patients will be provided $15 per hour for their participation in the study except for the infusion day, where they will be paid a total of $100 for the day, as well as the 7 day follow-up, where they will be paid $10 for the day. Total reimbursement for patients may go up to $100 plus $15x8 +$10 = $230.

Importance of the knowledge to be gained

Research participation will help the patients in curing TRD and possibly preventing relapse. The current project aims to address this important gap by conducting the systematic investigation of ketamine effect on cortical neurophysiological function in TRD patients by employing EEG and Transcranial magnetic stimulation (TMS) based techniques. The selected medication has shown preliminary evidence of benefit in helping patients with TRD. TRD is highly prevalent and leads to devastating consequences on a personal and societal level. Further, this study will pilot new approaches to evaluating treatments that may lead to more personalized and effective treatments for TRD. The above stated risks are relatively mild in degree and procedures have been designed to minimize their probability. We believe this protocol has an extremely favorable risk/benefit ratio. The psychiatry department has an excellent track record in conducting similar controlled trials with the utmost attention to safety.

Data and Safety Monitoring Plan

The principal investigator will be responsible for the Data and Safety monitoring of the study.

Data monitoring plan
Data will be collected and stored as described above in Sources of Material, and analyzed with primary outcomes as described in the Statistical Plan. Trained research assistants will enter data into an existing, relational database. Allowable input values will be restricted to standardized Access entry forms so as to maintain data integrity. All observations will be double-entered to verify accuracy, with any problems detected discussed with the PI. If necessary, re-training of research assistants will be conducted. Due to the comparatively small and initial nature of the current trial, an interim data analysis is not planned.

Safety monitoring plan
During screening, study applicants will undergo a complete psychological and physical exam to determine their eligibility and safety of their participation in this study, per inclusion/exclusion criteria detailed above. During the treatment phase of the study, participants will be asked about adverse events at each clinic visit and vital signs will be continuously monitored during the infusion and two hours after the infusion.

All adverse events (AEs) occurring during the course of the study will be collected, documented, and reported to the Principal Investigator. The occurrence of AEs will be assessed at baseline and each visit during the treatment phase of the study. The PI, in consultation with the co-investigators, will review any AE's as soon as they are reported. The study investigators will follow all AEs to the point of a satisfactory resolution. A study participant may have their medication discontinued of may be withdrawn from the study if the Study Physician determines it is the best decision in order to protect the safety of a participant. All AEs will be assessed to determine if they meet criteria for an SAE.

Serious adverse events (SAEs), as defined by the FDA, will be evaluated at each visit. Any SAE, whether or not related to study medication, will be reported to the IRB within 24 hours. The initial SAE report will be followed by submission of a completed SAE report within 2 days. In the event that a patient either withdraws from the study or the investigator decides to discontinue a patient due to SAE, the patient will have appropriate follow-up medical monitoring. Monitoring will continue until the problem requiring hospitalization has resolved or stabilized with no further change expected, is clearly unrelated to study medication, or results in death. Outcome of SAEs will be periodically reported to IRB.

Inclusion of Women
The subjects will include males and females, ages 18-65 years old, from various race/ethnic backgrounds, as reflected in the local community in the greater Houston area.

Inclusion of Minorities
Our study will include minority groups in proportions that will be representative of the ethnic/racial composition of the local community in Houston, Texas. Our projected numbers for minority enrollment are detailed in the enrollment table attached.

Inclusion of Children
Children will not be included in the study. The inclusion of children at this early stage would increase substantially the number of subjects needed for the overall project. At this time, we will focus on an adult population (ages 18-65 years old), which will also allow us to limit some of the potential confounding factors to our brain imaging and neurocognitive findings. Depending on our results these investigations will be extended to children and individuals over 65 years old in future studies.

Prisoners and pregnant women
We are aware of the special protections afforded to prisoners per federal regulations. We will not actively recruit people who are incarcerated to participate in this study. We do, however, acknowledge the possibility of research participants in our target population being incarcerated during the study. If an active participant is incarcerated,

for an extended period of time, disenrollment from the study will be necessary. No research interaction or intervention will take place until an incarcerated participant is released from jail. Finally, neither pregnant woman nor neonates will be enrolled in this study.

Power Analysis

Sample size justification: For the current proposal, our power calculation is based on our prior data on ASSR (Komek K et. al 2012) after pharmacological manipulation with effect size estimate of 0.9 and is guided by a previous study which used intravenous antidepressant clomipramine in TRD patients and found decreased motor excitability (increased RMT)(Minelli A et.al 2010) with large effect size estimate of Cohen's d = 1.2. For this project proposal, even if we assume a lower effect size of d=0.9, assuming a two-sided alpha <0.05, a sample of N=15, randomized in a cross-over design, participants provides 95% power to detect a difference between the two groups. Our sample size of 20 subjects would be sufficient event at attrition of 20%.

Statistics

Normality assumptions for continuous variables will be examined. Where appropriate, outliers will be winsorised and log, square root or reciprocal transformations applied to achieve normality if appropriate. If normality cannot not been achieved, either untransformed or dichotomized scores were used. Baseline characteristics will be compared between responders and non-responders with the Mann-Whitney U test for continuous variables and the chi-square test for categorical variables. Changes between two time-points for continuous variables were tested with paired t tests, and associations between continuous variables will be quantified with the Spearman correlation coefficient. Random effects models will be performed to quantify changes in clinical score and its component items over time and to compare temporal differences between eventual responders and non-responders. Splines will be used to determine differences in the pattern of response over time among all patients and to identify the time at which there was no additional improvement in depressive symptoms. Additionally, the relationship between response status between baseline and end of study calculating sensitivity, specificity, and positive and negative predictive values will be reported. Time to relapse for patients who met response criteria at end point will be estimated with the Kaplan-Meier method. Analyses will be performed with IBM SPSS Statistics (version 19; SPSS, Chicago, Illinois) and SAS (version 9.2; SAS, Cary, North Carolina).

Data Handling and Record Keeping

This is a low-risk project in terms of ethical concerns. This study will acquire, use and create individually identifiable health information (known as Protected Health Information or PHI). Confidentiality will be protected at all times by having research records identified by code number only. All research information will be stored in locked files at all times. Only authorized research staff will have access to the information gathered in this study. Only subjects capable to provide consent will be included in the study.

As per the Health Insurance Portability and Accountability Act (HIPAA) all individuals who are eligible and agree to participate in this research study will be required to sign a HIPAA research authorization prior to participation. If an individual refuses to sign the HIPAA research authorization, they will not be able to participate in this study. The HIPAA form is part of the official consent form.

Data will be stored separately from participants' identifiers. Both will be stored on encrypted drives on computers in locked offices. Confidentiality will be protected by having research records identified by code number only. All research information will be stored in locked files at all times. Only authorized research staff will have access to the information gathered in this study. All paper data will be stored on the the 3rd floor of the UT Department of Psychiatry, at the BBSB (room number 3260) in a double locked cabinet. The electronic data will be stored on the L drive, in a subfolder labelled "BPRC", located under the subfolder "UT Center of Excellence on Mood Disorders". An Accounting of Disclosure (AOD) will be created and maintained for any disclosure of individually identifiable information (III) outside the UTHSC-H. The manual spreadsheet will include the date of the disclosure, nature or description of the III disclosed purpose of each disclosure and the name and address of person or agency to which the disclosure was made. The study imparts only minimal risk to included subjects.

Strict monitoring of hemodynamic and respiratory changes as well as psychiatric screening will be performed during each infusion of ketamine.

Ethics

IRB approval will be sought from CPHS for the University of Texas Health Science Center at Houston. Written informed consent will be obtained directly from the subject. Privacy and confidentiality will be maintained with a special number to be used to identify the subject in the study and only the investigator and his research staff will know their name.

Quality control and assurance

No plans to have ongoing third party monitoring. However, we will conduct routine (bimonthly) reviews of consistency, reliability and accuracy of data collected for any paper records. Similar checks for any data in electronic form will also be conducted but in an automated fashion (e.g checking for accurate time/date stamps, file sizes etc.).

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