Protocol Title: Investigation of the Rapid (Next Day) Antidepressant Effects of an NMDA Antagonist: Substudy 2 (Rapid improvement research in bipolar depression) and Substudy 4 (An investigation of predictors and neural correlates of antidepressant response to an NMDA antagonist with Multimodal Imaging and electrophysiology studies)

Abbreviated Title: Investigation of the Rapid (Next Day) Antidepressant Effects of an NMDA Antagonist

Protocol Number: 04-M-0222

Date of This Submission/Version: 11/08/16, Amendment MM

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Human Research Protections Program Investigator and Staff Training:

For this protocol, the following "Just in time" human subjects protection training courses are required for investigators and staff:
N/A

Total requested accrual

Substudy 2:

27 Patients with BP depression

0 volunteers

Substudy 4:

40 healthy volunteers

60 patients with MDD

60 patients with BP depression

Project Uses Ionizing Radiation: ☐ No ■ Yes (*attach RSC/RDRC documentation)*

☐ Medically indicated only

■ Research-related only

☐ Both

IND/IDE ☐ No ■ Yes *(attach FDA documentation)*

Drug/Device/# FDG #23,195

Sponsor: Peter Herscovitch

Durable Power of Attorney ■ No ☐ Yes

Multi-institutional Project ■ No ☐ Yes

Data and Safety Monitoring Board ☐ No ■ Yes

Technology Transfer Agreement ☐ No ■ Yes

Agreement type and number: MTA # P-11-011 Expiration Date: None

Samples are being stored ☐ No ■ Yes

Précis:

Bipolar disorder and major depressive disorder (MDD) are common, severe, chronic and often life-threatening illnesses. Impairment in physical and social functioning resulting from depression can be just as severe as other chronic medical illnesses. Recent preclinical and clinical studies suggest that the glutamatergic system is involved in the mechanism of action of antidepressants. In two separate trials, we tested riluzole (an inhibitor of glutamate release) and found it to have antidepressant properties in patients with MDD and bipolar depression (BD). In another study (Substudy 1), we found that the non-competitive NMDA antagonist (ketamine) was effective in treatment-resistant MDD. Ketamine resulted in rapid, robust and relatively sustained antidepressant effects. Response with ketamine occurred within 2 hours and last approximately 1 week. The current protocol consists of 2 substudies designed to address 2 major questions. The original protocol consisted of 6 substudies. Four of the studies (1, 3, 5 and 6) have met their enrollment quota and sufficient analyzable data has been obtained.

Substudy 1 (Rapid improvement research in MDD)

Does the NMDA antagonist ketamine produce rapid antidepressant effects in patients with treatment-resistant MDD? This substudy is closed.

Substudy 2 (Rapid improvement research in bipolar depression)

Does the NMDA antagonist ketamine produce rapid antidepressant effects in patients with treatment-resistant BD? Patients, ages 18 to 65 years with treatment-resistant BD will, in a double-blind crossover study, receive either intravenous ketamine or saline solution added to a mood stabilizer (lithium or valproate).

Substudy 3 (Rapid and sustained improvement research in unipolar depression)

Does riluzole promote and maintain response in patients with treatment-resistant MDD who have received a single intravenous dose of ketamine? Patients, ages 18 to 65 years, with treatment-resistant MDD who have received a single intravenous dose of ketamine will, in a double-blind study, receive either riluzole or placebo. This substudy is closed.

Substudy 4 (Predictors and neural correlates of antidepressant response to ketamine)

What are the predictors and neural correlates of antidepressant response to ketamine? Patients, ages 18 to 65 years with treatment-resistant MDD and BD will, in a double-blind crossover study, receive either intravenous ketamine or saline solution and multimodal MRI, MEG and polysomnography. Does the antidepressant response to sleep deprivation, measured using a clinician administered depression scale score 2-weeks prior to ketamine infusion predict the antidepressant response to ketamine in patients with MDD and healthy volunteers? Are the neural mechanisms mediating rapid acting antidepressant response to ketamine and sleep deprivation similar? Does relapse from sleep deprivation induce a corresponding change in MRI and MEG measured neurobiology?

Substudy 5 (Neurophysiological Mechanisms of Rapid Antidepressant Response to Ketamine)

Will activity of glutamatergic circuits within the brain change following the onset of ketamine-induced antidepressant effects help predict response to ketamine? Drug-free bipolar and unipolar patients participating in Substudies 2 and 3 will undergo PET scans before and 2-hours following drug infusion (consistent with the timing of onset of antidepressant effects). This substudy is closed.

Substudy 6 (Role of Glutamatergic and GABAergic systems in treatment response to ketamine/riluzole)

Will subjects with treatment-resistant major depression who receive ketamine followed by riluzole show a greater increase in the concentration of GABA, glutamate, glutamine and NAA compared to subjects randomized to placebo? Unipolar patients participating in Substudy 3 will have 1H-MRS scans immediately before both infusions (ketamine, placebo) and at the end of each experimental phase. This substudy is closed.

Primary hypotheses for the active substudies are: Substudy 2) rapid response can be achieved in patients with treatment-resistant BD, and Substudy 4) aims are to examine the predictors and neural correlates of antidepressant response to ketamine.

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List of Abbreviations

Major Depressive Disorder (MDD)

Bipolar Disorder (BD)

1. Introduction/Scientific Rationale

a. Type of Protocol

Substudy 2 is a Phase II proof-of-concept clinical trial. The study is a double-blind acute phase, placebo-controlled crossover study in which the efficacy of a single intravenous (i.v.) dose of ketamine and saline solution (placebo) are compared in patients with bipolar depression.

Substudy 4 is a Phase II mechanistic and pathophysiology study. This substudy is a double-blind, acute phase, placebo-controlled crossover study in where the neural correlates and predictors of response to a single intravenous (i.v.) dose of ketamine will be investigated using multimodal imaging in patients with MDD and BD.

b. Background

Substudy 2:

The treatment of depression was revolutionized about a half-century ago by the serendipitous discovery of monoamine oxidase inhibitors and tricyclic antidepressants. Since then, the availability of a host of newer medications with better side effect profiles has greatly increased our ability to safely treat a significant percentage of patients. However, the newer medications are largely "me too" drugs in as much as they exert their primary biochemical effects by increasing the intrasynaptic levels of monoamines, and as such, there has been limited (if any) progress in developing medications with improved efficacy, or with a faster onset of action. Indeed, the development of more rapidly acting antidepressants is widely viewed as the Holy Grail in neuropsychopharmacology. We recently found (protocol #04-M-0222) that the high-affinity *N*-methyl-D-Aspartate (NMDA) ketamine produces a rapid onset of antidepressant effects (significant at 110 minutes through day 7) in patients with treatment-resistant major depression (see below). We now wish to study the antidepressant effects of ketamine in patients with bipolar disorder on mood-stabilizers using a similar design to protocol #04-M-02222. Should we find a rapid onset of antidepressant effects in patients with bipolar depression (NMDA antagonist is associated with a rapid antidepressant effect), then subsequent studies could be carried out that examine strategies on how to maintain the antidepressant effects achieved with a single-dose of an NMDA antagonist. Furthermore, understanding the mechanism of action of the glutamatergic modulator's antidepressant effect may ultimately lead to further insight into the pathophysiology of mood disorders in general.

Substudy 4:

<u>Background of the current protocol and glutamatergic studies conducted at NIMH</u>

Substudy 4 is one of several that are designed to more precisely determine what aspects of glutamatergic modulation are relevant for acute antidepressant effects in subjects with mood disorders. Protocol #04-M-0222 (Substudies 1-3): An investigation of glutamatergic modulators as pharmacologic strategies to bring about rapid (next day) and sustained antidepressant effects consists of 3 substudies. Substudy 1 (Rapid improvement research in unipolar depression)) was

completed and published and was a crossover single-infusion of ketamine in patients with treatment-resistant major depressive disorder. Substudy 2 (Rapid improvement research in bipolar depression) was recently completed (the imaging component still ongoing) the results of which are in the preliminary studies section, and Substudy 3 (Rapid and sustained improvement research in unipolar depression) is no longer open to enrollment. Studies 2 and 3 had neuroimaging and electrophysiological components (positron emission tomography (PET), magnetic resonance spectroscopy (MRS), magnetoencephalography (MEG), and polysomnography electroencephalogram (EEG) recordings) that provided us with preliminary information used to propose the present substudy. The data collected under Substudy 4 may also be linked with data obtained from the other substudies under this protocol (04-M-0222), from the screening protocol (01-M-0254) and other mood disorder protocols in our program (e.g., brain imaging, DNA, psychophysiology tests, treatment studies, etc.) with the purposes of better understanding the diagnosis, pathophysiology, and treatment response of patients with mood disorders.

c. Overview/Research Questions

Substudy 2

A delay in the therapeutic effects of antidepressants and negative consequences

Mood disorders (recurring major depressive disorder [MDD] and bipolar disorder [BPD] are common, chronic recurring serious mental illnesses that are associated with significant morbidity and often become life-threatening. Medications used to treat depression include a variety of antidepressants. Unfortunately, these medications take weeks to months to achieve their full effects and in the meantime, patients continue to suffer from their symptoms and continue to be at risk of self-harm. It was recently reported that the greatest risk of suicidal behavior is in the first 9 days of starting an antidepressant (Jick et al 2004). Although a significant proportion of patients usually obtain a response within 3-4 weeks, remission rates are usually low at this point and make take several more weeks to become manifest (Entsuah et al 2001). In controlled trials of bipolar depression, it is estimated that approximately 50% of subjects achieve remission by 8 weeks (Calabrese et al 2005; Tohen et al 2003). A more rapidly acting antidepressant medication would have a significant impact on the treatment of bipolar depression and have major health care implications.

Existing antidepressant treatments suffer from a limited efficacy and a slow onset of action. The design of clinical trials for the evaluation of fast-acting antidepressants is also critical, and several strategies have been proposed (Leon 2001; Montgomery et al 2002; Thase 2001). Some studies have suggested that mirtazapine has a more rapid onset of action than SSRIs (Quitkin et al 2001), that venlafaxine is more rapid than other antidepressants (Entsuah et al 2001; Quitkin et al 2001) and that certain augmentation strategies may lead to a more rapid onset of antidepressant effects in patients with unipolar depression (Blier 2001; Jahn et al 2004; Shiah et al 2000). Overall, the data is largely retrospective and has not been designed to examine specifically the rapidity of onset of antidepressant effects with modern designs (Leon 2001; Montgomery et al 2002; Thase 2001). As such, there has been limited progress in developing medications with improved efficacy or with a faster onset of action (Nestler et al 2002). The notion that the ‘onset’ of beneficial drug effects in depression is delayed for 2 or 3 weeks has been challenged recently,

with a growing appreciation that earlier improvements can be detected in eventual drug responders (Frazer and Benmansour 2002). However, it is clear that a finite lag period for true antidepressant effects (that is, beyond the improvement of certain symptoms such as insomnia and anxiety) *does* indeed exist, and furthermore, that optimal drug-induced improvement of depression is quite slow; thus, some studies suggest that about one-fourth to one-third of depressions that do not respond by 4 weeks will do so by week 8 (see Rush and Ryan 2002(Rush and Ryan 2002) for a very salient discussion). Finally, if poor long-term outcome is related to chronicity, a more rapid antidepressant response may also have long-term benefits by minimizing the deleterious psychosocial impact and neurobiological (and potentially structural) effects of sustained or repeated episodes of major depression (Sheline et al 1996).

Can rapid antidepressant effects actually be attained?

Clinical studies

Several clinical observations suggest that a rapid antidepressant effect can, indeed, be achieved in humans. For example, bipolar patients often exhibit rapid changes in their mood-going from depression to mania in a matter of hours (Kramlinger and Post 1996). In addition, many patients with depression experience profound diurnal variation of mood, and may achieve euthymia at particular times of the day. The most convincing evidence comes from sleep deprivation studies, which have shown that ~50% of patients with depression (both unipolar and bipolar) respond positively to one night of sleep deprivation (Wu and Bunney 1990). One might argue that the response to sleep deprivation is only transient and is therefore not a "real" antidepressant response. However, there is ample evidence in the literature and from clinical experience that none of our other somatic antidepressant treatments (including ECT) sustain their effects if they are discontinued immediately after they begin to exert beneficial effects. Thus, although the depressive relapse occurs more rapidly, the fact that most patients relapse when the treatment is stopped is hardly surprising. Furthermore, there is increasing evidence that the antidepressant effect of sleep deprivation can be sustained with various maneuvers including sleep phase advancement or the addition of medications such as lithium or antidepressants (Wirz-Justice and Van den Hoofdakker 1999). We recently found that a single-dose of the NMDA antagonist ketamine resulted in a rapid onset of antidepressant effects (within 110 minutes) but more interestingly, the effects persisted beyond when the treatment was discontinued (up to 7 days improvement).

The role of glutamate system in the pathophysiology of depression

Preclinical evidence

The "monoamine hypothesis" of depression, which was developed for the pharmacological effects of early drug development, no longer provides a satisfactory explanation of the mode of action of all antidepressant agents or of the underlying pathophysiology in depression. Further, none of these prevailing theories of depression (i.e., "serotonergic, noradrenergic or dopaminergic hypothesis") have resulted in a better understanding of the mechanism responsible for a rapid onset of antidepressant action. For example, a single dose of a dopamine agonist (e.g., amphetamine) results in enhancement in mood (not an antidepressant effect) and its effects are

short-lived lasting only a few hours. A growing body of preclinical research suggests that the glutamate system and especially NMDA class of glutamate receptors may be involved in the pathophysiology of major depression and the mechanism of action of antidepressants (Skolnick 1999; Skolnick 2002; Skolnick et al 1996) reviewed in (Zarate et al 2003; Zarate et al 2002). Chronic treatment with antidepressants has been shown to reduce the number of cortical β-adrenoreceptors (Koshikawa et al 1989; Vetulani 1984). NMDA antagonists 1-aminocyclopropanecarboxylic acid (ACPC; a partial agonist at the glycine or co-activator site) and MK-801 (noncompetitive antagonist) for 1 week showed reduced [^{3}H] dihydroalprenolol binding to β-cortical adrenoreceptors (Klimek and Papp 1994; Paul et al 1992). Similar effects were produced by imipramine (Klimek and Papp 1994). Microinjections of glutamate in the prefrontal cortex has been shown to aggravate learned helplessness in rats at 1 and 72 hours, but not at 24 hours, after administration (Petty et al 1985). NMDA receptor antagonists have demonstrated antidepressant effects in many animal models of depression, including the application of inescapable stressors, forced-swim, and tail suspension–induced immobility tests, in learned helplessness models of depression, and in animals exposed to a chronic mild stress procedure (Layer et al 1995; Meloni et al 1993; Moryl et al 1993; Papp and Moryl 1994; Przegalinski et al 1997; Trullas and Skolnick 1990). A single dose of the NMDA antagonist ketamine in male Wistar rats was shown to interfere with the induction of behavioral despair for up to 10 days after its administration (Yilmaz et al 2002). The prolonged beneficial effect of ketamine in this study has been suggested to be due to a long-term change in glutamatergic activation and its consequences. O'Neill and Sanger (O'Neill and Sanger 1999) have shown that a single pretreatment dose with MK-801, an NMDA antagonist, induces an enduring sensitivity to the second administration of the same agent 4, 7, or 14 days later. Conversely, antidepressant administration has been shown to affect NMDA receptor function and binding profiles (Mjellem et al 1993). Also, chronic administration of antidepressants demonstrates adaptive changes in ligand binding at the NMDA receptor glycine site (Nowak et al 1993). Tricyclic antidepressant (TCA) drugs interact directly with the NMDA receptor complex to block the action of NMDA in vitro. An early study (Reynolds and Miller 1988) reported that desipramine and imipramine slowed the dissociation rate of [^{3}H] MK-801 binding in a manner similar to zinc (an NMDA antagonist), which is thought to act noncompetitively at a site outside the channel. TCAs appear to be less potent with the addition of magnesium and L-glutamate (Sills and Loo 1989), and to be selective for the low-affinity state of the PCP binding site. TCAs (imipramine, amitriptyline), fluoxetine, sertraline and citalopram all enhanced the MK-801 induced locomotor effect (Maj et al 1991). Furthermore, the role of glutamatergic dysfunction in depression is further supported by the fact that repeated antidepressant administration regionally alters expression of mRNA that encodes multiple NMDA receptor subunits (Boyer et al 1998) and radioligand binding to these receptors within circumscribed areas of the central nervous system (Skolnick 1999).

Other evidence that the glutamatergic system may have a role in the pathophysiology/treatment of bipolar disorder comes from the effects of lithium and valproate on the glutamatergic system.

Lithium

Nonaka and colleagues (Nonaka et al 1998) found that chronic treatment with therapeutically relevant concentrations of lithium chloride in cultured rat cerebellar, cortical, and hippocampal neurons protected against glutamate-induced excitotoxicity involving apoptosis mediated by NMDA receptors. In the same study, chronic treatment of mice with therapeutically relevant concentrations of lithium (0.7mM) up-regulated synaptosomal uptake of glutamate. The investigators found that the protection in cerebellar neurons is specific for glutamate induced excitotoxicity and can be attributed to inhibition of NMDA receptor-mediated Ca^{2+} influx and is not the result of down-regulation of NMDA receptor subunit proteins (NR1, NR2A, NR2B or NR2C) (Hashimoto et al 2002; Nonaka et al 1998) or the ability to block inositol monophosphatase activity. However, while lithium has been reported not to alter the total protein levels of NR1, NR2A and NR2B subunits of NMDA receptors, a recent study suggests that lithium protection against glutamate excitotoxicity in rat cerebral neurons may be a result of reducing the level of NR2B phosphorylation at Tyr1472 (Hashimoto et al 2002). NR2B phosphorylation has been reported to be positively correlated with NMDA receptor-mediated synaptic activity and excitotoxicity. It is also possible that lithium modulates glutamatergic neurotransmission by affecting the non-NMDA receptor-sensitive channels. Karkanias and Papke (Karkanias and Papke 1999), reported that lithium in frog oocytes (xenopus laevis) produces an increase of inward and outward currents of AMPA receptors and a decrease in the currents of kainate and NMDA receptors. The potentiation induced by lithium was greatest for the GluR3 subtype of the AMPA receptors and least for GluR1.

Valproate

Ueda and Willmore (Ueda and Willmore 2000) recently reported the effect of sodium valproate on glutamate transporter expression in the hippocampus. With a dose of 100 mg/kg/day of sodium valproate given for 14 days, they found an increase in EAAT1 levels and a decrease in EAAT2 levels. Hassel and colleagues (Hassel et al 2001) reported that chronic treatment of rats with sodium valproate (200 mg or 400 mg/kg, for 90 days) leads to a dose-dependent increase in hippocampal glutamate uptake capacity as measured by uptake of [^{3}H]glutamate into proteoliposomes via increasing the levels of the glutamate transporters EAAT1 and EAAT2 in the hippocampus.

In rodent models, valproate has been shown to reduce seizure activity induced by AMPA glutamate receptor agonists (Steppuhn and Turski 1993; Turski 1990). In post-mortem human brain tissue, Künig and colleagues (Kunig et al 1998), showed that therapeutic levels of valproate decreased binding of AMPA to the AMPA glutamate receptors- thus effectively blocking them. In a series of studies from different laboratories which have used various preparations, valproate appears to block synaptic responses mediated by NMDA glutamate receptors as well (reviewed in (Loscher 1999).

In summary, in vitro and animal studies suggest that NMDA antagonists produce neurochemical alterations in the brain similar to antidepressant drugs and that they show an antidepressant-like behavioral profile in some animal models of depression. Furthermore, both lithium and valproate which are primary treatments used in bipolar disorder exert effects on the glutamatergic system.

Clinical evidence

Cerebral spinal fluid (CSF) and plasma levels of glutamate in depressed patients

Several groups have reported higher serum levels of glutamate in depressed patients compared with control subjects (Kim et al 1982; Mauri et al 1998). On the other hand, Maes et al. (1998) found no difference (p=.075) in depressed patients compared to age and sex matched controls, however their study was in treatment-resistant patients and the data was in the same direction as the other studies. Recently, Berk and colleagues (Berk et al 2000) reported that the platelet glutamate receptors may be supersensitive in schizophrenia and depression with psychotic features but not in mania with psychotic features compared to normal controls.

Post-Mortem studies of glutamatergic dysfunction in depression and suicide

In postmortem studies, Nowak et al. (1995) reported that the proportion of high-affinity, glycine-displaceable [^{3}H] CGP-39653 binding to glutamate receptors was reduced in patients who committed suicide compared with control subjects. However, in another study (Holemans et al 1993), the actual number of NMDA receptors in 9 brain regions of 22 suicide victims and age/sex matched controls were compared by studying [^{3}H]MK-801-binding characteristics; no significant differences were found. This time the suicide victims had a firm diagnosis of depression (diagnosis was made retrospectively by a psychiatrist) and had not recently been treated with antidepressant drugs. The problem in comparing these studies is that they each examine different aspects of NMDA receptor function. The “adaptive” changes to NMDA receptor-binding characteristics found in the study by Nowak et al. (1995) have also been found in animal experiments. However, the changes are in the same direction as those caused by antidepressant treatment, so it is not clear whether the post-mortem changes can be attributed to disease state (e.g., depression) or drug treatment. Recently, a significant decrease in the NMDA receptor density (the glycine site on the NR1 subunit) was recently reported in both bipolar and major depressive disorders (Nudmamud-Thanoi and Reynolds 2004). There is also a recent report suggesting that the NR2C subunit of the NMDA receptor in the locus coeruleus in patients with major depression is increased by 61% (Karolewicz et al 2005). The same group also found that neuronal nitric oxide synthetase (nNOS), which is activated as a result of NMDA receptor stimulation, was decreased by 44% in individuals with major depression (Karolewicz et al 2004). Of related interest, a small study on bipolar disorders showed decreased [^{3}H]MK-801 binding in the hippocampal CA3 regions (Pavey et al 2003), and a recent genetic study demonstrated that a polymorphism of the NR1 subunit may confer susceptibility to bipolar disorders and schizoaffective disorders of the manic type (Mundo et al 2003). Additional clinical evidence for a role of the glutamatergic system in depression is summarized in the next section.

In vitro MRS measurement of glutamate in mood disorders

Reduced Glx levels were seen in the anterior cingulate cortex in a mostly medicated population of depressed patients (Auer et al 2000) and in the left amygdala in subjects following a short (3-day) medication free period (Michael et al 2003). The latter abnormality was normalized by electroconvulsive therapy. A similar reduction was also reported in the anterior cingulate

cortex in medication-naïve children with major depression (Mirza et al 2004; Rosenberg et al 2004), but a preliminary study showed no significant change in glutamate/glutamine levels in medication-free patients with later-life major depression (Binesh et al 2004). With regard to bipolar disorders, elevated levels of Glx were found in both the frontal lobe and basal ganglia of depressed bipolar children compared to a control group (Castillo et al 2000) and in the cingulate gyrus in unmedicated adult patients (Dager et al 2004). A recent study by Sanacora et al. showed glutamate levels in the occipital cortex to be significantly elevated, by approximately 15%, in 29 medication-free patients with unipolar major depression, as compared to 28 age- and gender-matched healthy controls (Sanacora et al 2004). In vivo measures of glutamate strongly suggest that regulation of the amino acid neurotransmitter systems are altered in subjects with mood disorders.

Substudy 4

The goals of this application are to replicate our previous substudy, Substudy 1, of ketamine's antidepressant efficacy and to carefully examine neural correlates and predictors of response to a single intravenous (i.v.) dose of ketamine by using multimodal imaging in patients with MDD and BD. A placebo-controlled randomized cross-over trial will be conducted using different magnetic resonance imaging (MRI) techniques to determine whether changes in corticolimbic activity correlate with rapid antidepressant response to ketamine in patients with MDD and BD. MRI techniques used to investigate the neural correlates of antidepressant response to ketamine will include fMRI (rest and task-related activity) and MRS (modulation of GABA and Glx/Glutamate concentration by ketamine). To limit the effects of possible confounders related to chronicity of illness, cumulative stressors, and past drug exposure, all of which could confound our understanding of ketamine's neurophysiological mechanisms of action, we will also investigate the effects of ketamine on functional brain activity in healthy volunteers.

Given the importance of discovering novel biomarkers of treatment response, which could lead to more personalized treatments for depression, we will also assess baseline resting and task-related activity response to emotional and non-emotional stimuli, as well as whether baseline morphometric measures and the integrity of white matter fiber tracts between the ACC and the amygdala predict antidepressant response to ketamine. We will also investigate whether pretreatment concentrations of GABA and Glx/Glutamate in the prefrontal cortex differentiate treatment responders to ketamine from non-responders. Another potential biomarker to be examined is sleep slow wave activity (SWA), an indirect putative measure of synaptic plasticity that preliminary studies indicate correlates with rapid clinical improvement in response to ketamine.

Additionally, we will include a period of total sleep deprivation (40 hours) to examine whether the clinical antidepressant response to total sleep deprivation will predict the clinical response to ketamine treatment in patients with MDD and healthy volunteers. Such a finding would inform us regarding the mechanisms of action of novel rapid acting antidepressants. Importantly, sleep deprivation is a safe and low cost intervention and if shown to be predictive, could be used to infer whether or not someone is appropriate to receive riskier medications such

as ketamine. It is hypothesized that the antidepressant response to both ketamine and sleep deprivation will have common neurobiological mechanisms.

This multimodal approach will also allow us to combine data acquired via several different measures. Assuming that more than one imaging measure will correlate with MADRS score changes in individuals receiving ketamine, multiple linear regression will be used to determine the amount of variance associated with response to ketamine as assessed by the imaging measures. Finally, we will also investigate baseline functional and morphometric MRI measures and compare these measures to those of healthy control subject.

d. Background of Current Aims and Hypotheses

Substudy 2

Clinical trials with drugs that affect glutamatergic neurotransmission in mood disorders

Evidence that NMDA antagonism may be important for antidepressant effects in humans comes from cases series and blinded trials conducted with the non-competitive NMDA antagonist amantadine. Amantadine, an NMDA antagonist has been shown to have antidepressant effects in patients with Parkinson's disease and in unipolar and bipolar patients (Parkes et al 1970; Vale et al 1971). More recently Stryjer and colleagues (Stryjer et al 2003) reported that amantadine augmentation was successful in 8 patients with treatment-resistant depression. Until recently most of the evidence that NMDA receptor antagonists have antidepressant activity was based on very little clinical research (Vale et al 1971) and more on animal models of depression. It has to be acknowledged that some of these drugs with glutamatergic properties also have indirect effects on other systems. Several other recent studies also support the role of glutamatergic dysfunction in major depression. Berman et al. (2000) reported antidepressant effects of a single dose of the NMDA receptor antagonist ketamine (discussed in preliminary studies section). Calabrese and colleagues in their double-blind placebo-controlled study of bipolar depression reported that the antiglutamatergic drug lamotrigine was effective in acute depressive phase, an effect that first became apparent at week 3 of the trial (Calabrese et al 1999). Lamotrigine is now approved by the FDA for the prevention of mood episode relapses in patients with bipolar disorder. Barbosa and colleagues (Barbosa et al 2003) reported that lamotrigine was effective as an augmenting agent to fluoxetine in patients with treatment-resistant major depression. Similarly, the glutamatergic modulator riluzole was found to have significant antidepressant effects when given openly in patients with treatment-resistant unipolar (Zarate et al 2004) and bipolar depression (Zarate et al 2005).

Taking together, the preclinical data and preliminary clinical data indicate that the glutamatergic system may be involved in the pathophysiology of mood disorders and the mechanism of action of antidepressants. This protocol proposes to replicate our study with ketamine in unipolar depression to determine whether indeed the acute administration of the NMDA antagonist ketamine is associated with a rapid antidepressant effect in patients with bipolar depression. Exploring pharmacological strategies that have rapid antidepressant effects within hours or a few days would have an enormous impact on patient care.

The model presented here is *a clinically testable one*, and one that if successful, holds the potential to develop rapidly acting pharmacological agents. In addition, this protocol will

significantly enhance our understanding of the neurobiology of bipolar depression and rapid antidepressant response.

NMDA antagonist, Ketamine

Ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist (Kohrs and Durieux 1998), is a popular agent for emergency department procedural sedation in children with ample experience to support its safety and efficacy (Green et al 1998; Green et al 1998). Similarly, ketamine has been used safely in procedural sedation in adults in doses as high as 2 mg/kg IV. Administration of ketamine and related NMDA antagonists have been shown to have anxiolytic and antidepressant effects in animal models of anxiety and depression (Adamec et al 1999; Aguado et al 1994; Mickley et al 1998; Silvestre et al 1997) (see preclinical studies, Section 1.c) and in humans (Berman et al 2000; Zarate et al in press) (Zarate, Protocol #04-M-0222).

Side effects of ketamine

Ketamine is a general anesthetic for human and veterinary use. It is also known as “special K” related to PCP and is a drug of abuse especially among teens. For that reason, ketamine was placed in Schedule III of 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 good safety profile. In addition, it has been used in psychotherapy and in psychophysiological studies in normal volunteers and patients with severe mental disorders (with similar doses to that of our study) safely for years (Krystal et al 2003; Krystal et al 2002; Krystal et al 2000; Krystal and D'Souza 2001; Krystal et al 1999; Krystal et al 1998; Krystal et al 1994; Krystal et al 2003; Krystal et al 1998; Lahti et al 1995; Lahti et al 2001; Micallef et al 2003). Krupitsky and colleagues (Krupitsky et al 2002) in their ketamine psychotherapy study with single doses of 2.0 mg/kg im and 0.2 mg/kg im, reported no significant adverse events at 2-year follow-up. Ketamine exerts sympathomimetic activity and may produce mild to moderate increases in blood pressure, heart rate, and cardiac output. The reported incidence of perceptual disturbances varies from less than 5% to greater than 30% (Knox et al 1970; White et al 1980). Perceptual disturbances manifested as vivid dreaming, visualization of psychodelic 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 (Green and Johnson 1990). When such reactions occur, they are usually mild and short-lived (Green and Johnson 1990). Carpenter (Carpenter 1999) has systematically collected short-term outcome and potential patients distress data during ketamine challenge interviews from three North American institutions that conducted these studies. These studies suggest that ketamine administration has short-lasting effects on symptoms, usually less than 60 minutes and rarely lasting longer than 2 hours. The increase in psychosis is also generally not distressing to volunteers. Furthermore, in one long-term outcome study of patients who received ketamine during research, no serious adverse events were noted in more than 90 patients exposed to ketamine. This study found no differences between patients who did and did not receive ketamine on any measures of psychopathology, psychiatric care in the 8 month follow-up period

(Lahti et al 2001). There is no evidence in these long-term studies that subjects that were exposed to a ketamine were at greater risk of abusing it on follow-up. In this protocol, in order to minimize the risk of perceptual disturbances that may occur with ketamine, we will exclude patients with a current or past history of psychotic symptoms. Although ketamine is an anesthetic and has respiratory effects, we will use subanesthetic doses of ketamine.

Substudy 4

Primary aims

1. To assess the efficacy of a single dose of i.v. ketamine (0.5 mg/kg) compared with placebo in improving overall depressive symptomatology in patients with MDD or BD

Less than one third of patients with MDD achieve remission with an adequate trial of a standard antidepressant after 10-14 weeks of treatment (Trivedi, Rush et al. 2006). Similarly, and despite adequate trials, many patients with BD do not respond to antidepressants or medications approved for the management of BD, such as quetiapine, lithium, valproate, or the combination olanzapine/fluoxetine (Nierenberg, Ostacher et al. 2006; Sachs, Nierenberg et al. 2007). Notably, accumulating evidence suggests that the glutamatergic system may a useful target for the development of novel therapeutics for mood disorders that work more rapidly and effectively than exiting medications. In the last several years, several controlled studies found that ketamine, a broad-spectrum N-methyl-D-aspartate (NMDA)-receptor antagonist, manifests rapid antidepressant effects (within hours) in patients with depression. Two of these studies were conducted in patients with MDD (Berman, Cappiello et al. 2000; Zhou, Zarate et al. 2006), and one in patients with BD (Diazgranados, Ibrahim et al. in press). Because the study by Berman and colleagues had several limitations, we decided to conduct an adequately powered placebo-controlled randomized trial to assess the efficacy of a single intravenous infusion of ketamine in patients with MDD and, subsequently, patients with BD.

We found that ketamine produced fast, robust, and relatively sustained antidepressant effects in both patients with treatment-resistant MDD and BD (Zhou, Zarate et al. 2006; Diazgranados, Ibrahim et al. in press). However, these studies constitute the only two adequately powered randomized investigations of ketamine's antidepressant efficacy; it should also be emphasized that patients enrolled in our previous studies represented a very severely ill sample of patients who were hospitalized and had failed at least two adequate antidepressant trials during their current depressive episode; therefore, it is unknown whether these findings are generalizable to patients with less severe forms of illness or those in outpatient settings. Finally, the patients with BD enrolled in our BD study, Substudy 2, were receiving monotherapy with either lithium or valproate (Diazgranados, Ibrahim et al. in press); thus, we cannot be certain that some of ketamine's antidepressant effects were not due in part to the concomitant treatment they were receiving. Therefore, there is a critical need to replicate evidence of ketamine's antidepressant effects in an independent sample of patients with MDD and in drug-free patients with BD. Broader treatment-resistance criteria (i.e., requiring as inclusion criterion a failure of only one antidepressant trial during the current episodes or past episodes of depression, instead of two failures during the current episode) and the possibility of conducting Substudy 4 in an outpatient

setting when clinically indicated will help determine whether ketamine's antidepressant effects are more generalizable.

Ketamine

Relevant pharmacology

Initially, ketamine is distributed to highly perfused tissues, including brain, to achieve levels four to five times that in plasma. Ketamine has high lipid solubility and low plasma protein binding (12%), which facilitates rapid transfer across the blood-brain-barrier. The distribution half-life is approximately 10 minutes. Biotransformation of ketamine into multiple metabolites involves N-demethylation by cytochromes p450 to norketamine, an active metabolite with a potency approximately one-third that of ketamine. Norketamine is then hydroxylated and conjugated to water-soluble compounds that are excreted in urine. Elimination occurs primarily via the kidney, with only a small percentage recovered in the urine as unchanged drug (Chang, Savory et al. 1970). The elimination half-life is approximately two hours which is secondary to the combination of rapid clearance and large volume of distribution (Domino, Domino et al. 1984).

Safety

Ketamine is a general anesthetic for human and veterinary use. Ketamine been administered as an anesthetic to several million adults and children over the last few decades and has a good safety profile. Ketamine exerts sympathomimetic activity and may produce mild to moderate increases in blood pressure, heart rate, and cardiac output. The reported incidence of perceptual disturbances varies from less than 5% to greater than 30% (Knox, Bovill et al. 1970; White, Ham et al. 1980). When such reactions occur, they are usually mild and short-lived (Green and Johnson 1990). No strong evidence exists from long-term studies suggesting that subjects exposed to ketamine were at greater risk of abusing it on follow-up. We also have extensive experience with ketamine, having infused (subanesthetic doses) approximately 90 patients with treatment-resistant depression under protocol 04-M-0222. We will continue to use the same dose of ketamine, which was found to be safe in our previous studies.

2. To determine whether functional and structural measures obtained via MRI and MEG predict treatment response to ketamine

Another issue in the treatment of patients with mood disorders is that, to date, no reliable predictors of remission, response, or non-response have been identified. Identifying biomarkers that predict response to treatment would be of enormous benefit to patients with MDD and BD, as it would minimize the lengthy trial-and-error process that currently occurs when trying to find the most optimal treatment. Such predictive biomarkers might reduce the early morbidity and mortality that result from major depressive episodes. Using fast acting interventions such as ketamine allows the investigation of predictors and biological correlates of antidepressant response within a short period of time and concurrently minimizes the effect of confounding factors inherent in long-term treatments (e.g., non-adherence to treatment, substance use, etc.).

In our search for such pre-treatment biomarkers of response in mood disorders (reviewed in (Mayberg 2009)), we found that increased pgACC activity in response to fearful faces positively correlated with rapid antidepressant response to ketamine infusion in MDD patients (Salvadore, Cornwell et al. 2009). In a second study, we implicated the same brain region as a putative biomarker of treatment response to ketamine during a cognitively-demanding task (i.e., the N-back task) showing an opposite effect that was observed during exposure to negative-emotional faces; MDD patients who displayed the least engagement of pgACC with increasing working memory loads showed the greatest symptomatic improvement within four hours of ketamine (Salvadore, Cornwell et al. 2010). In addition, long-range coherence (i.e., functional connectivity) between the pgACC and the amygdala was associated with rapid antidepressant response (Salvadore, Cornwell et al. 2010). These promising results are nonetheless preliminary, given the small sample size (N=11 and N=15, respectively) and lack of a placebo group. Because pgACC activity has also been implicated in the neurobiology of placebo-response (Mayberg 2009), it is unknown whether those putative biomarkers of response are specific to antidepressant response to ketamine, or instead represent general markers of amelioration of depressive symptoms. Therefore, there is a need to replicate and expand our initial findings using a placebo-controlled design and a larger sample size. The present study, Substudy 4, proposes to use fMRI instead of MEG because it is a more widely accepted approach; furthermore, recent, significant advances in data processing and analysis provide more reliable measures of resting-state activity and functional connectivity between brain structures compared to MEG. Moreover, fMRI provides complementary information to MEG, because it measures the Blood Oxygen Level Dependent (BOLD) signal that arises from the magnetic properties of hemoglobin and the manner in which brain metabolism and blood flow are related to changes in neuronal activity, rather than magnetic fields that reflect synchronous firing of several thousands of neurons that are parallel to each other. We also propose to use structural MRI, diffusion tensor imaging (DTI) and MRS in our search for novel putative biomarkers of antidepressant response to ketamine (see **Appendix D** for a full listing of proposed imaging techniques and **Appendix E** for a description of cognitive tasks).

3. To determine whether ketamine modulates task-dependent and resting-state activity in the frontolimbic circuitry and whether changes in activity in the frontolimbic circuitry are correlated with antidepressant response to ketamine

Our preliminary findings indicate that pretreatment activity in the pgACC and the amygdala is a putative biomarker of antidepressant response to ketamine. However, it remains unknown whether the corresponding pretreatment activity in the pgACC and amygdala are relevant not only as potential biomarkers of treatment response but also as direct targets for ketamine's mechanisms of action. Uncontrolled studies in patients with depression suggest that prolonged treatment with conventional antidepressants such as SSRIs and TCAs modulate limbic reactivity to emotional stimuli and increase cortico-limbic connectivity. Specific regions implicated in the mechanisms of action of antidepressants include the pgACC, dorsolateral prefrontal cortex, subgenual cingulate cortex, amygdala, hippocampus, and posterior cingulate (reviewed in (Mayberg 2009)), which overlap with the same regions we implicated as putative predictors of antidepressant response to ketamine. It should be noted also that there is little agreement across studies that investigated the effects of conventional antidepressants regarding the direction of brain activity changes compared

to baseline (i.e., increase vs. decrease) in treatment responders vs non-responders. Possible reasons for these inconsistencies include small sample size, lack of a placebo control group, and methodological heterogeneity across studies (e.g.: resting vs. task-related activity; PET vs. fMRI). Furthermore, findings from our laboratory suggest that the emotional and cognitive demands of the tasks used to investigate predictors of treatment response might also contribute to this heterogeneity (Salvadore, Cornwell et al. 2010). No study has evaluated ketamine's effects on the default mode network (DMN) in patients with mood disorders; however, evidence from a study conducted in healthy volunteers showed that ketamine acutely modulates activity in the ventromedial prefrontal cortex, decreasing BOLD response in the orbitofrontal cortex and subgenual cingulate, while increasing activity in mid-posterior cingulate, thalamus, and temporal cortical regions (Deakin, Lees et al. 2008). Some of the regions implicated in ketamine's acute effects are part of the mood regulating circuitry and a change in their activity might explain ketamine's antidepressant properties.

A critical need thus exists to identify the neural targets of ketamine and to investigate whether changes in activity in the frontolimbic circuitry correlate with antidepressant response to ketamine. Knowing how ketamine modulates different brain activities, levels of amino acid neurotransmitters in the frontolimbic circuitry, and how these are related to clinical outcome, might help guide drug development research in the search of more effective and faster-acting antidepressants. Here, we propose a multimodal imaging approach to identify neural correlates of ketamine's antidepressant properties in patients with MDD and BD. We also aim to investigate the neural effects of a sub-anesthetic single intravenous infusion of ketamine compared to placebo in a sample of healthy humans. This part of the protocol is critical for disentangling the neural mediators of antidepressant response in patients with MDD and BD from the nonspecific neural effects of the drug itself, which can be observed in patients as well as in healthy subjects.

4. To determine if functional and structural measures obtained via MRI and MEG that show treatment effects following ketamine return to baseline with symptom relapse and sustain themselves with response maintenance.

As detailed above, we know that some of the acute effects of ketamine implicate mood-regulating circuitry, and we anticipate that ketamine will induce changes in task-related responses within these regions. In addition to identifying neural targets of ketamine, determining the specificity of such responses also would be critical. Here we plan to reassess functional brain responses 10 to 11 days following treatment response. This allows us the unique opportunity to study the functional brain response observed under relapse in those patients who have not retained the antidepressant response. This design also allows us to evaluate the persistence of functional changes observed under ketamine in patients who retain the antidepressant response.

5. To determine if the antidepressant response and the mediating neural correlates (MEG and MRI) to total sleep deprivation predict the antidepressant response to ketamine and its associated neural mechanisms of action.

The aim is to assess the predictive validity of total sleep deprivation for the rapid acting antidepressant ketamine in patients with major depressive disorder. It is hypothesized that both techniques share overlapping mechanisms of action via plasticity enhancement. Our primary

hypothesis is that patients who respond to total sleep deprivation will demonstrate a similar response profile to that of ketamine. Additionally, we predict that the neural changes associated with response to ketamine will also be found to a similar degree in those who respond to total sleep deprivation, thus establishing a cross methodological profile of rapid-acting antidepressant response in patients with treatment resistant major depressive disorder.

Secondary aims

1. To determine whether ketamine modulates amino acid neurotransmitter concentration in the prefrontal cortex in patients with MDD or BD and whether changes in amino acid neurotransmitters concentration are correlated with antidepressant response to ketamine.

Our preliminary results indicate that pretreatment Glx/Glutamate levels are inversely correlated with the magnitude of antidepressant response to ketamine in patients with MDD (see preliminary studies section); however, we did not measure GABA or Glx/Glutamate after ketamine administration; therefore it is unknown whether ketamine modulates the concentration of amino acid neurotransmitters and whether those measures might be used as surrogate markers of treatment response. Substudy 4 proposes to use high field (7T) MRS to quantify prefrontal concentrations of GABA, Glutamate and Glx before and after the administration of ketamine or placebo in patients with MDD, BD, and healthy controls and to investigate the link between changes in amino acid neurotransmitters and clinical improvement.

2. To determine whether baseline measures of slow wave activity (SWA) predict clinical response to ketamine in patients with MDD or BD and whether changes in SWA following ketamine or placebo are correlated with rapid antidepressant response to ketamine.

Recent evidence suggests that sleep EEG might provide electrophysiological measures (e.g., SWA, slow wave slope) that are indirect indices of synaptic plasticity in humans. For example, high-density EEG studies have shown that manipulations leading to synaptic potentiation in local cortical circuits (e.g., rotation learning, high-frequency TMS) lead to a local increase in SWA during subsequent sleep(Huber, Ghilardi et al. 2004; Esser, Huber et al. 2006), while manipulations leading to synaptic depression (e.g., arm immobilization) lead to a local reduction in SWA (Huber, Ghilardi et al. 2006). Our preclinical studies suggested that ketamine's antidepressant effects are mediated by enhancing AMPA receptor signaling (Maeng, Zarate et al. 2008); notably, synaptic potentiation is known to involve AMPA trafficking, which enhances AMPA throughput. Therefore, it is possible that enhanced glutamatergic throughput of AMPA relative to NMDA receptors after ketamine treatment may result in increased synaptic potentiation. The overall hypothesis to be tested in this protocol is that the rapid antidepressant effects of ketamine are caused by enhanced AMPA throughput in cortical structures, thus increasing synaptic plasticity. In our preliminary studies we found that ketamine increases SWA amplitude, and that this correlates with antidepressant response (see preliminary studies section). Here, we hypothesize that a single dose of ketamine will increase SWA more than placebo, and that this increase will be correlated with clinical response.

3. To determine whether baseline functional and structural measures obtained through the MRI and MEG are altered in patients with MDD and BD compared to healthy control subjects.

We plan to use MEG, fMRI and structural MRI (VBM, DTI, Magnetization transfer ratio MTR imaging, and MRS) to investigate functional and morphometric abnormalities in patients with MDD and BD compared to healthy controls. Given the possibility of enrolling a large sample of drug-free patients with MDD and BD and healthy control subjects to investigate the clinical efficacy and the neurobiology of antidepressant response to ketamine, this protocol represents a unique opportunity to understand the pathophysiology of depression using pre-ketamine (i.e., baseline) functional and structural imaging measures. We also plan to utilize high field (7T) MRI to characterize subtle volumetric changes in patients with MDD and BD, potentially identifying predictors of clinical response. Findings from functional and structural imaging studies in depression are summarized in **Appendix D**.

4. To determine whether ketamine will increase plasma/serum brain-derived neurotrophic factor (BDNF), and/or decrease vascular endothelial growth factor (VEGF) mRNA levels in peripheral leukocytes compared to placebo and/or baseline levels.

5. To determine the mechanisms of action of total sleep deprivation in treatment refractory depression.

Utilizing MRI we aim to acquire measures of neural function following both sleep deprivation and a typical night's sleep. These measures will contribute to our understanding of the underlying mechanisms by which sleep deprivation produces an antidepressant response. Furthermore, we aim to use electrophysiological imaging techniques (EEG and MEG) to investigate the change from rapid remission to relapse that often occurs during the recovery sleep period. Changes will inform us regarding the neurobiological correlates of relapse in major depressive disorder. Baseline images acquired using MRI will also be used to predict the antidepressant response to sleep deprivation. Finally, we will collect functional magnetic resonance imaging activity related to a reward decision-making task and cognitive emotional tasks before and after sleep deprivation to examine the cognitive and neurobiological consequences associated with response to sleep deprivation.

e. Preliminary Studies

Substudy 2

Clinical data suggests that indeed, glutamatergic modulators may have antidepressant effects in humans. We first tested the glutamatergic modulator riluzole (an inhibitor of glutamate release and AMPA modulator [FDA approved for ALS]) and found it (doses 100-200 mg/day) to have antidepressant properties (onset of action usually after 3 weeks) in patients with treatment-resistant major depression (Zarate et al 2004) and bipolar depression (Zarate et al 2005).

<u>A 6-week open-label study with riluzole in treatment-resistant DSM-IV major (unipolar) depression (Protocol #02-M-0034)(Zarate et al 2004)</u>

Nineteen patients received riluzole at a mean daily dose of 168.8 ± 27.2 mg. The mean age of onset of the illness was 23.9 ± 12.6 years, mean number of lifetime episodes of depression was

8.5 ± 11.3, and the mean duration of the current episode of depression was 5.4 ± 3.7 months. Most were stage II or greater of treatment-resistance.

Sixty-eight percent (N=13) of subjects completed the 6-week trial. Significant improvement in the Montgomery-Asberg depression rating scale (MADRS) scores occurred on weeks 3 through 6 for all patients (p=0.007). Clinical Global Impression-Severity (CGI-S) and Hamilton Anxiety Rating (HAM-A) scales also improved significantly in weeks 3 through 6 (p=0.04).

The most common adverse events during the trial were headache (58%), gastrointestinal distress (nausea or vomiting) (43%), decreased salivation (47%), constipation (32%), and tension/inner unrest (26%). No serious adverse events were noted.

An 8-week open-label study with riluzole in treatment-resistant DSM-IV bipolar depression (Protocol #03-M-00092) (Zarate et al 2005)

Fourteen patients with bipolar depression entered the study. The mean duration of the current episode of depression was 7.7 ± 5.8 months. Past treatments included lithium (n =11), selective serotonin-reuptake inhibitors (n=11), valproate (n=6), atypical antipsychotics (n=6 [olanzapine 4, quetiapine 2]), lamotrigine (n=4), bupropion (n=4), mirtazapine (n=4), venlafaxine (n=4), other antidepressants (n=4), stimulants (n=4), carbamazepine/oxcarbazepine (n=3), topiramate (n=2), and ECT (n=2).

Eight subjects (57%) completed the 8-week trial. Patients received riluzole at a mean daily dose of 171.4 ± 42.6 mg. The mean serum lithium level during the study was 0.81 ± 0.17 mEq/L. Significant improvement occurred at weeks 5 through 8 for the MADRS (p=.0007). Seven out of 10 MADRS items (apparent sadness, reported sadness, inner tension, concentration difficulties, lassitude, inability of to feel, and suicidal ideation) improved significantly. YMRS scores did not change significantly during the study. The response and remission rate at week 8 was 50% (7/14).

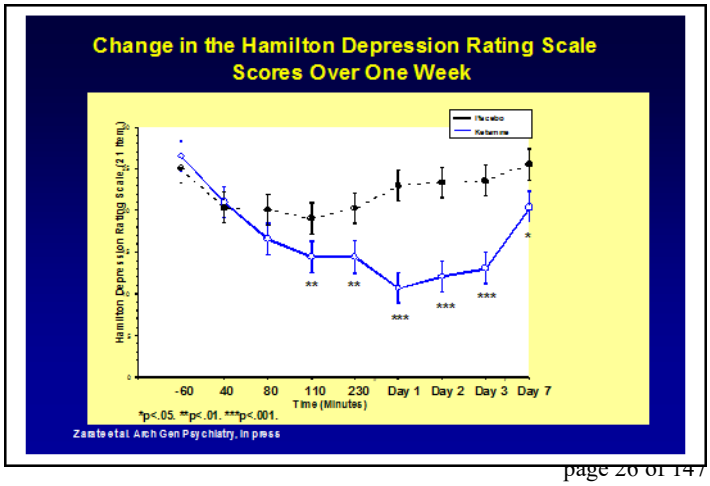
The most common adverse events during the trial were fatigue (n = 4), decreased salivation (n = 4), reduced sleep (n = 4), nausea (n = 3), diarrhea (n = 3), weight loss (n = 3), and blurred vision (n = 3). Two subjects had asymptomatic LFTs that were likely related to study drug. These tests returned to normal shortly after the study medication was discontinued. Such laboratory changes have been described to occur with riluzole treatment (Wagner and Landis 1997). No serious adverse events were noted.

Although the exact mechanism of action of riluzole is unknown, it has been reported that riluzole inhibits glutamate release through inactivation of voltage dependant sodium channels (Benoit and Escande 1991; Hebert et al 1994) and P/Q-type calcium channels (Wang et al 2004). Riluzole also is an AMPA potentiator and increases expression of BDNF. A single intraperitoneal injection caused a rise in BDNF localized in dentate granule neurons, the hilus, and the stratum radiatum of the CA3 regions. And repeated injections of riluzole resulted in prolonged elevation of hippocampal BDNF and were associated with increased numbers of newly generated cells in the granule cell layer in rats (Katoh-Semba et al 2002), regions that are implicated in depression. In terms of AMPA receptor trafficking, our group (Du et al. unpublished) found that riluzole, similar to lamotrigine, increased cell surface expression in hippocampal neurons of GluR1 and GluR2, while the antimanic agent valproate caused significantly less cell surface expression of the same receptors compared to placebo. It is noteworthy that –although riluzole shows similarities in

its mechanism of action with lamotrigine (i.e, inhibits release of glutamate) – 3 of 4 patients in our bipolar depression study, who had previously failed to respond to lamotrigine, subsequently responded to riluzole (Zarate et al 2005). While differences in the underlying neurobiology of these patients' current episode of depression compared to previous ones (when they received lamotrigine) cannot be ruled out, the findings suggest that riluzole may modulate the glutamatergic system in a non-identical manner to lamotrigine. Indeed, it has been reported that riluzole exerts its modulatory effects on the glutamatergic system not only by attenuating glutamate release, but also by increasing its clearance from the synaptic space through enhancement of the reuptake process (Frizzo et al 2004). Furthermore, riluzole has also been postulated to directly act postsynaptically at ionotropic glutamatergic receptors, notably at AMPA receptors (De Sarro et al 2000). The latter observations are noteworthy since preclinical studies have demonstrated that AMPA receptor potentiators increase BDNF expression (Legutko et al 2001; Mackowiak et al 2002), and show efficacy in animal models of depression (Li et al 2001).

Contrary to memantine a low-to-moderate affinity NMDA antagonist which is devoid of antidepressant effects (Zarate et al 2006), we found that the NMDA antagonist ketamine produced a rapid, robust and relatively sustained antidepressant effect in patients with treatment-resistant major depression.

Ketamine, a dissociative anesthetic, is a noncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist (Kohrs and Durieux 1998). It is a popular agent for emergency department procedural sedation in children with ample experience to support its safety and efficacy (Green et al 1998; Green et al 1998). One of the side effects associated with ketamine are psychotomimetic side effects which has led it to being misused among teenagers. There is no evidence that ketamine causes physical dependence in humans (Britt and McCance-Katz 2005) and a recent study found no evidence that repeated, albeit limited, exposures to ketamine increased the risk of more severe or more protracted psychosis, perceptual changes resembling dissociation, severe emotional distress, or euphoria in healthy subjects (Cho et al 2005). Administration of ketamine and related NMDA antagonists have been shown to have anxiolytic and antidepressant effects animal models of anxiety and depression (Adamec et al 1999; Aguado et al 1994; Mickley et al 1998; Silvestre et al 1997) (see preclinical studies, **Section 1.c**) and in humans (Berman et al 2000). We recently found that the non-competitive NMDA antagonist ketamine produces significant antidepressant properties in patients with treatment-resistant major depression. In this double-blind placebo-controlled crossover study (NIMH 04-M-0222; Zarate et al. 2006), we found that a single intravenous dose of ketamine (0.5 mg/kg over 40 minutes) in patients with treatment-resistant major depression resulted in a rapid onset of antidepressant effects within hours (figure 1). Using only those who completed both phases of the study, a linear mixed model with the HDRS showed significant main effects for drug ($F=58.24$, $df=1,203$, $p<.0001$) time ($F=9.48$, $df=8,203$, $p<.0001$) and an



interaction between drug and time (F=4.15, df=8,203, p<.001). Simple effects tests indicated significant improvement on ketamine over placebo at 110 minutes through 7 days. The effect size for the drug difference was very large (d=1.46, 95% C.I. 0.91-2.01) after 24 hours and large (d=0.68, 95% C.I. 0.13-1.23) after 1 week. Thus far, 12/17 (71%) met response criteria (50% decrease in Hamilton depression rating scales [HDRS] scores), and 29% achieved remission (≤7 HDRS) at 24 hours following the infusion of ketamine. Six (35%) subjects maintained response to ketamine for at least 1 week; 2 of these maintained response at least 2 weeks. By contrast, no subject on placebo responded at 1 or 7 days. BPRS positive symptoms (Kane et al 2001) were worse on ketamine than placebo only at 40 minutes (drug: F=4.23, df=1,200, p=.04; time: F=9.31, df=8,200, p<.0001; drug x time: F=6.89, df=8,200, p<.0001). Similarly, YMRS scores were worse (higher score) on ketamine than placebo at 40 minutes only (drug: F=3.08, df=1,201, p=.08; time: F=3.54, df=8,201, p<.001; drug x time: F=4.68, df=8,201, p<.0001). Mild perceptual disturbances occurred in most patients which in all cases lasted less than 1 hour. No serious adverse events occurred; overall the study mediation was well tolerated. The most common side effects of ketamine were perceptual disturbances, confusion, elevations in blood pressure, euphoria, dizziness, and increased libido.

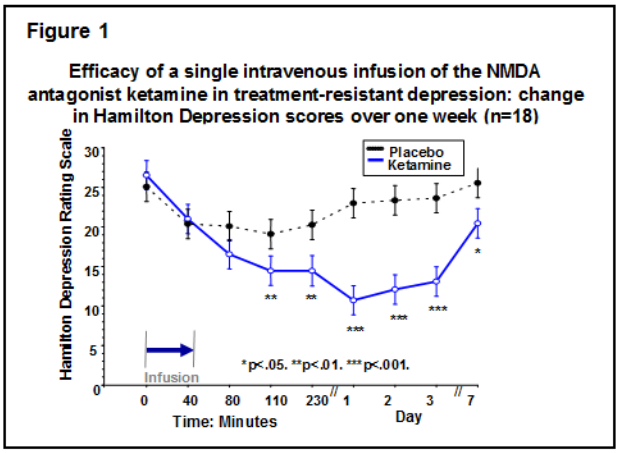
The fact that the antidepressant effects of a single dose of ketamine do not subside as soon as the drug has been cleared from the body suggests that NDMA antagonism brings about downstream signaling changes in critical circuits responsible for modulation of mood. It is thus our working hypothesis that a direct blockade of NMDA receptors will bring about rapid antidepressant effects. In the present study, we propose to expand our previous findings on the efficacy of glutamatergic modulators in patients with severe recurring mood disorders by testing a specific, new mechanism whereby we use an NMDA antagonist to bring about rapid antidepressant effects in patients with bipolar depression. The model presented here is *a clinically testable one*, and one that if successful, holds the potential to develop rapidly acting pharmacological agents.

Substudy 4

Clinical Studies with the NMDA antagonist ketamine

The NMDA antagonist ketamine produces rapid, robust, and relatively sustained antidepressant effects in patients with treatment-resistant MDD

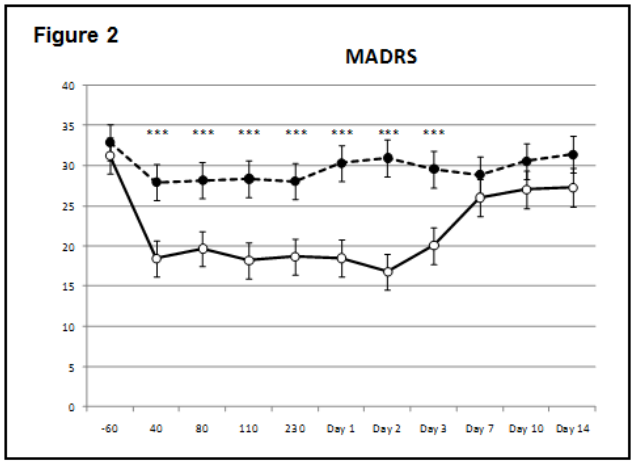
Figure 1



We found that ketamine has significant antidepressant properties in patients with treatment-resistant MDD. In this double-blind, placebo-controlled crossover study (NIMH 04-M-0222, Study 1; (Zhou, Zarate et al. 2006)), a single intravenous dose of ketamine (0.5 mg/kg over 40 minutes) resulted in a rapid onset of antidepressant effects within hours (**Figure 1**). Significant improvement resulted from ketamine over

placebo at 110 minutes through Day 7. The effect size for the drug difference was very large (d=1.46, 95% C.I. 0.91-2.01) after 24 hours and large (d=0.68, 95% C.I. 0.13-1.23) after one week. 71% of patients met response criteria (50% decrease in Hamilton depression rating scales [HDRS] scores), and 29% achieved remission (≤7 HDRS) 24 hours after ketamine infusion. Six (35%) subjects maintained response to ketamine for at least one week.

The NMDA antagonist ketamine produces rapid, robust, and relatively sustained antidepressant effects in patients with treatment-resistant bipolar depression



Our recent double-blind placebo-controlled crossover study (NIMH 04-M-0222, Substudy 2) (Diazgranados, Ibrahim et al. in press) similarly found evidence of ketamine's rapid antidepressant properties in patients with treatment-resistant bipolar depression. We found that depression rating scale scores in treatment-resistant BD patients who received ketamine improved far more significantly than scores for patients receiving placebo; this improvement occurred as early as 40 minutes post-infusion. In fact, this difference was statistically significant for four different efficacy scales: the Montgomery Asberg Depression Rating Scale (MADRS), the HDRS, the self-rated Beck Depression Inventory (BDI), and the depression subscale of the Visual Analogue Scale (VAS). In all cases, this significant difference occurred from 40 minutes through Day 3 (**Figure 2**).

Imaging studies

Increased anterior cingulate cortical activity in response to fearful faces: a neurophysiological biomarker that predicts rapid antidepressant response to ketamine

Our first imaging study investigating moderators of treatment response to ketamine in patients with treatment-resistant MDD used MEG to investigate whether pre-treatment ACC activity would be a putative biomarker of rapid antidepressant response (Salvadore, Cornwell et al. 2009). We elicited ACC activity in drug-free MDD patients and healthy controls by rapidly presenting fearful faces, a stimulus known to activate rostral regions of the ACC, among other structures. Sources of magnetic activity were localized using spatial-filtering analyses (Hillebrand, Singh et al. 2005). We found that subjects who showed the highest engagement of the pgACC to repeated exposures to fearful faces were more likely to respond to ketamine 230 minutes after infusion ($r = 0.68$; $p<0.05$; **Figure 3**). We also found that amygdala response to fearful faces was negatively correlated with the antidepressant response observed 230 minutes after ketamine infusion ($r = -0.72$, $p<0.05$; **Figure 4**), corroborating previous studies with conventional antidepressants (Saxena, Brody et al. 2003; Langenecker, Kennedy et al. 2007).

Figure 3. Nonparametric correlation between increased ACC activity and change in depressive symptoms 230 minutes after ketamine infusion

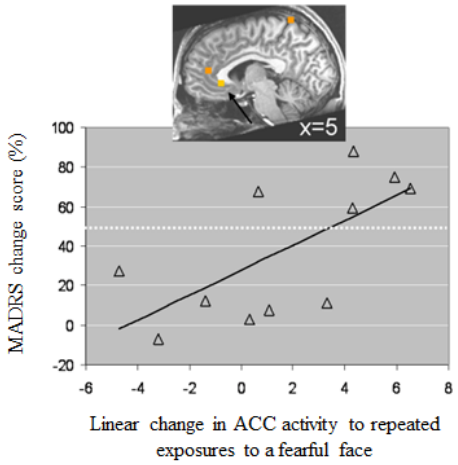
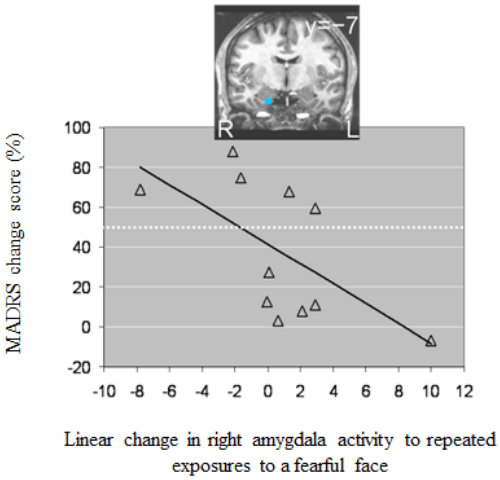


Figure 4. Nonparametric correlation between decreased right amygdala activity and change in depressive symptoms 230 minutes after ketamine infusion



Anterior cingulate desynchronization and functional connectivity with the amygdala during a working memory task predicts rapid antidepressant response to ketamine

In a subsequent study (Salvadore, Cornwell et al. 2010), we demonstrated that pgACC activity in a non-emotional context has can predict antidepressant response, just as it does in an emotionally-arousing context. Fifteen drug-free MDD patients were administered the N-back working memory task one to three days before receiving a single ketamine infusion. Notably, functional abnormalities have been previously documented in patients with MDD using the N-back task not only in task-dependent regions such as the dorsolateral prefrontal cortex, but also in regions not typically activated by this task, such as the pgACC (Rose, Simonotto et al. 2006; Schoning, Zwitserlood et al. 2009).

We found that pre-treatment pgACC beta desynchronization during N-back task performance predicted antidepressant response to ketamine in patients with MDD (Salvadore, Cornwell et al. 2010) ($r = 0.82$; $p = 0.0002$; FDR < 5%; **Figure 5**). Because higher beta desynchronization with increasing memory load reflects greater ACC engagement, our results imply that the pgACC shows opposite changes in activity in emotional versus nonemotional cognitive task contexts in MDD patients who respond favorably to ketamine. Therefore, high pgACC activity in response to emotionally-salient stimuli, but low pgACC activity in response to increased cognitive demands, predicted antidepressant response to ketamine.

Figure 5.

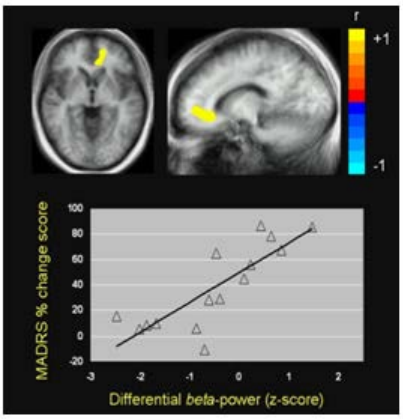
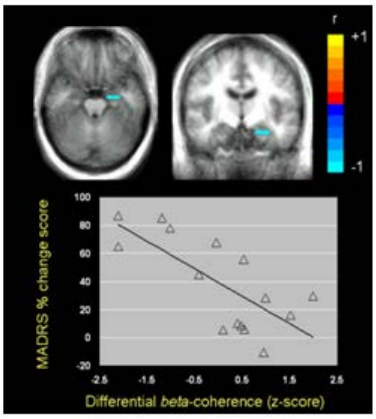


Figure 6.



Moreover, we found that subjects who showed the lowest source coherence between the pgACC and the left amygdala were the most likely to respond to ketamine (Salvadore, Cornwell et al. 2010) (**Figure 6**). Decreased functional connectivity between those two regions might reflect decreased subcortical activation and therefore less need for cortical control over the amygdala in those who respond to ketamine. Indeed, subjects with the highest coherence had the lowest treatment response. Thus, these data might indicate that ketamine responders show functional integrity of the corticolimbic mood regulating circuit, which is not actively engaged during cognitively demanding tasks, but that non-responders display abnormal activation of mood regulating circuitry even in the absence of emotionally-arousing stimuli.

Glutamate but not GABA levels in the dorsomedial/dorsal anterolateral prefrontal cortex (PFC) predict rapid antidepressant response to ketamine: preliminary findings

Several MRS studies suggest that patients with MDD show abnormalities in GABA and Glx (a peak formed by the overlapping spectra of glutamate and glutamine) levels in different brain areas, which might be modulated by antidepressant treatment (Sanacora, Gueorguieva et al. 2004; Hasler, van der Veen et al. 2007). In the search for putative predictors of rapid clinical improvement to ketamine, we used proton magnetic resonance spectroscopy (^{1}H-MRS) to investigate whether prefrontal levels of GABA, glutamate (Glu) and the ratio Glx/Glu (a putative surrogate of glutaminergic activity) correlated with the decrease in depressive symptoms observed after a single intravenous infusion of ketamine in patients with MDD. Our preliminary results with 13 patients suggest that pre-treatment GABA or Glutamate were not correlated with antidepressant response in either of the two regions of interest (p>0.1), while pretreatment Glx/Glu ratio in the dorsolateral/anterior medial prefrontal cortex was negatively correlated with clinical improvement to ketamine ($r_s(10) = -0.64$, p <0 .03). A lower Glx/Glutamate ratio could be attributable to a lower intracellular pool of glutamine.

These preliminary findings provide empirical support for the use of MRS to investigate ketamine's effects on amino acid neurotransmitters.

Sleep studies

Ketamine, sleep, mood and synaptic plasticity

In collaboration with the University of Wisconsin we are testing the hypothesis that clinical improvement after ketamine treatment is associated with α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) mediated plasticity by measuring both SWA and mood response using an open-label design.

All night sleep recordings were obtained from 24 patients on the night before ketamine infusion (BL), and the first (N1) and second (N2) nights after ketamine infusion. Half of the patients were treated with riluzole after the infusion and before N1 as part of an ongoing study. Preliminary analyses of the first 25 patients indicates a trend (p=.053) in the correlation between ketamine's ability to rapidly (within 24 hours) improve mood (MADRS ratings) and to increase SWA on the first night after ketamine infusion. The correlation between clinical improvement and SWA is consistent with the hypothesis that ketamine's antidepressant response is linked to plasticity changes in cortical structures.

Plasma/Serum biomarkers

Plasma/serum BDNF and VEGF mRNA levels in peripheral leukocytes

Brain-derived neurotrophic factor (BDNF) is up-regulated in the hippocampus by antidepressant treatments, and centrally administered BDNF can produce antidepressant-like effects in rodent behavioral models of depression (Duman and Monteggia 2006). Chronic administration of antidepressant drugs or electroconvulsive therapy regulates BDNF and trkB mRNA in the rat brain; it has further been suggested that these interventions could promote neuronal survival and protect neurons from the stress-related damage (Nibuya, Morinobu et al. 1995). Recent research suggests that serum BDNF concentration is reduced in depression and that successful antidepressant treatment leads to increased serum BDNF concentrations (Hellweg, Ziegenhorn et al. 2008). However, we recently found that BDNF plasma levels were not correlated with acute antidepressant response to ketamine (Machado-Vieira, Yuan et al. 2009), but it is important to note that study had no control group. Vascular endothelial growth factor (VEGF) is another neurotrophic factor whose signaling has been implicated in the behavioral actions of antidepressant treatment (Greene, Banasr et al. 2009). In addition, we will be collecting blood for the purpose of transcriptional profiling, metabolomics, proteomics, and the measurements of inflammatory measures including cytokines and kynurenine pathway metabolites. See **Appendix J** for an expanded discussion of plasma/serum neurotrophic biomarkers.

2. Study Objectives

a. Primary objectives

1. To assess the efficacy of a single dose of i.v. ketamine (0.5 mg/kg) compared with placebo in improving overall depressive symptoms in patients with MDD or BD.

Hypothesis: Subjects with MDD (currently experiencing a major depressive episode) or bipolar depression randomized to a single i.v. dose of an NMDA antagonist or placebo will have a superior response to the NMDA antagonist.

2. To determine whether functional and structural measures obtained via MRI and MEG predict treatment response to ketamine.

   Hypothesis 1: Pre-treatment pgACC and amygdala activity during task-related and resting-state activity as measured by fMRI and MEG will predict the magnitude of clinical improvement to ketamine—but not to placebo—in patients with MDD or BD.

   Hypothesis 2: Pre-treatment integrity of the white matter fibers (i.e., fractional anisotropy, diffusion coefficient, magnetization transfer ratio) connecting the ACC to the amygdala will be correlated with the magnitude of clinical improvement to ketamine—but not to placebo—in patients with MDD or BD.

   Hypothesis 3: Pre-treatment concentration of amino acid neurotransmitters (GABA, Glx/Glutamate) in the prefrontal cortex will be correlated with the magnitude of clinical improvement to ketamine—but not to placebo—in patients with MDD or BD.

3. To determine whether ketamine modulates task-dependent and resting-state activity in the frontolimbic circuitry and whether changes in activity in the frontolimbic circuitry are correlated with antidepressant response to ketamine

   Hypothesis 1: Ketamine administration will increase pgACC activity and decrease amygdala activity to emotional faces more than placebo. A similar effect will be observed in the default-mode network activity recorded under resting conditions. Ketamine will also decrease pgACC activity during the N-back task more than placebo.

   Hypothesis 2: The magnitude of change in task-dependent and resting state activity within the pgACC and the amygdala will be correlated with the magnitude of antidepressant response after ketamine infusion. These correlations will be specific to antidepressant response to ketamine but not to placebo.

4. To determine if functional and structural measures obtained via MRI and MEG that show treatment effects following ketamine return to baseline with symptom relapse and sustain themselves with response maintenance.

   Hypotheses 1: Ketamine induced changes in rest or task-related activity as measured with fMRI and MEG will revert to baseline levels in the patient subgroup experiencing symptom relapse by 10 or 11 days post infusion.

   Hypothesis 2: Ketamine induced changes in rest or task-related activity as measured with fMRI and MEG will persist in the patient subgroup showing maintenance of response to ketamine by 10 or 11 days post infusion.

5. To determine whether response to sleep deprivation predicts response to ketamine in treatment resistant major depressive disorder and whether the mediating neurobiological changes that occur following response are common to both treatments.

   Hypothesis 1: Total sleep deprivation (40 hours) will produce an antidepressant response in a portion of depressed patients and healthy volunteers. The magnitude of response to sleep deprivation, measured as the change in depression rating score from baseline on the MADRS, will predict response to ketamine in a subsequent placebo controlled infusion study. Greater response to sleep deprivation will be predictive of better response to ketamine.

   Hypothesis 2: Response to sleep deprivation and ketamine infusion will produce similar alterations in glutamatergic activity in the pre-genual cingulate region.

   Hypothesis 3: Response to sleep deprivation will produce an alteration in reward related decision-making and the underlying neural activity, as measured by fMRI.

   Hypothesis 4: Response to sleep deprivation will produce an alteration in emotional processing and the underlying neural activity, as measured by fMRI.

   Hypothesis 5: Baseline EEG slow-wave sleep activity will predict response to total sleep deprivation as well as ketamine response.

b. Secondary objectives

1. To determine whether ketamine modulates amino acid neurotransmitter concentration in the prefrontal cortex in patients with MDD or BD and whether changes in amino acid neurotransmitters concentration are correlated with antidepressant response to ketamine.

Our preliminary results indicate that pretreatment Glx/Glutamate levels are inversely correlated with the magnitude of antidepressant response to ketamine in patients with MDD (see preliminary studies section); however, we did not measure GABA or Glx/Glutamate after ketamine administration; therefore it is unknown whether ketamine modulates the concentration of amino acid neurotransmitters and whether those measures might be used as surrogate markers of treatment response. Substudy 4 proposes to use high field (7T) MRS to quantify prefrontal concentrations of GABA, Glutamate and Glx before and after the administration of ketamine or placebo in patients with MDD, BD, and healthy controls and to investigate the link between changes in amino acid neurotransmitters and clinical improvement.

2. To determine whether baseline measures of slow wave activity (SWA) predict clinical response to ketamine in patients with MDD or BD and whether changes in SWA following ketamine or placebo are correlated with rapid antidepressant response to ketamine.

Recent evidence suggests that sleep EEG might provide electrophysiological measures (e.g., SWA, slow wave slope) that are indirect indices of synaptic plasticity in humans. For example, high-density EEG studies have shown that manipulations leading to synaptic potentiation in local cortical circuits (e.g., rotation learning, high-frequency TMS) lead to a local increase in SWA during subsequent sleep(Huber, Ghilardi et al. 2004; Esser, Huber et al. 2006), while

manipulations leading to synaptic depression (e.g., arm immobilization) lead to a local reduction in SWA (Huber, Ghilardi et al. 2006). Our preclinical studies suggested that ketamine's antidepressant effects are mediated by enhancing AMPA receptor signaling (Maeng, Zarate et al. 2008); notably, synaptic potentiation is known to involve AMPA trafficking, which enhances AMPA throughput. Therefore, it is possible that enhanced glutamatergic throughput of AMPA relative to NMDA receptors after ketamine treatment may result in increased synaptic potentiation. The overall hypothesis to be tested in this protocol is that the rapid antidepressant effects of ketamine are caused by enhanced AMPA throughput in cortical structures, thus increasing synaptic plasticity. In our preliminary studies we found that ketamine increases SWA amplitude, and that this correlates with antidepressant response (see preliminary studies section). Here, we hypothesize that a single dose of ketamine will increase SWA more than placebo, and that this increase will be correlated with clinical response.

3. To determine whether baseline functional and structural measures obtained through the MRI and MEG are altered in patients with MDD and BD compared to healthy control subjects.

We plan to use MEG, fMRI and structural MRI (VBM, DTI, Magnetization transfer ratio MTR imaging, and MRS) to investigate functional and morphometric abnormalities in patients with MDD and BD compared to healthy controls. Given the possibility of enrolling a large sample of drug-free patients with MDD and BD and healthy control subjects to investigate the clinical efficacy and the neurobiology of antidepressant response to ketamine, this protocol represents a unique opportunity to understand the pathophysiology of depression using pre-ketamine (i.e., baseline) functional and structural imaging measures. We also plan to utilize high field (7T) MRI to characterize subtle volumetric changes in patients with MDD and BD, potentially identifying predictors of clinical response. Findings from functional and structural imaging studies in depression are summarized in **Appendix D.**

4. To determine whether ketamine will increase plasma/serum brain-derived neurotrophic factor (BDNF), and/or decrease vascular endothelial growth factor (VEGF) mRNA levels in peripheral leukocytes compared to placebo and/or baseline levels.

5. To determine the mechanisms of action of total sleep deprivation in treatment refractory depression.

Utilizing MRI we aim to acquire measures of neural function following both sleep deprivation and a typical night's sleep. These measures will contribute to our understanding of the underlying mechanisms by which sleep deprivation produces an antidepressant response. Furthermore, we aim to use electrophysiological imaging techniques (EEG and MEG) to investigate the change from rapid remission to relapse that often occurs during the recovery sleep period. Changes will inform us regarding the neurobiological correlates of relapse in major depressive disorder. Baseline images acquired using MRI will also be used to predict the antidepressant response to sleep deprivation. Finally, we will collect functional magnetic resonance imaging activity related to a reward decision-making task and cognitive emotional tasks before and after sleep deprivation to examine the cognitive and neurobiological consequences associated with response to sleep deprivation.

6. To determine the effect of ketamine on suicidal ideation in a larger meta-analysis across multiple sites.

Our research into ketamine was one of the first its kind to describe a reduction in suicidal thoughts after ketamine infusion. These initial studies led to a burgeoning interest in ketamine as a potential antisuicidal agent. However, as suicidal patients are often excluded from depression clinical trials, it has been difficult to assemble a sufficient sample to explore the potential impact of ketamine on suicidal thoughts. We have collaborators who are willing to send coded data from Non-NIH clinical trials in ketamine. This data will be combined with data acquired under this protocol to conduct a larger meta-analysis on ketamine's potential as an antisuicidal agent. Data and samples will only be accepted if the participants gave consent for such sharing and use or if the IRB at the institution providing the samples or data waived consent. We hope that the expanded sample size will enable us to conduct a more robust evaluation of ketamine's ability to reduce suicidal ideations.

3. Subjects

a. Description of study populations

Substudy 2

Patients, ages 18 to 65 with a diagnosis of bipolar disorder I or II, currently depressed without psychotic features will be recruited into this study.

Substudy 4

Male and female patients, ages 18 to 65 years with a diagnosis of MDD, currently depressed or in a current major depressive episode of BD without psychotic features will be recruited into this substudy. In addition, healthy volunteers will also be recruited into this substudy.

The proportion of ethnic minorities (vs. Caucasian) in the total sample will be consistent with the ethnic/racial proportions of the Washington D.C. and Montgomery county areas. We appreciate that minority groups tend to be underrepresented in research samples for mood disorders. Therefore, we make every effort to recruit minority patients, in order to ensure that the subject sample represents the community. Although it is not known whether racial differences will affect responses to pharmacotherapy, Substudy 4 will assess such effects; however, the size of the racial subsamples may be too small to identify statistically meaningful differences.

Anticipated ethnic/racial distribution of sample areas

	Black/ African-American	Asian	Caucasian	Native American/ Alaskan	Hispanic/ Latino	Other/Two or more	Total
District of Columbia	60%	2.7%	30.8%	0.3%	7.9%	6.2%	100%

Montgomery County	15.1%	11.3 %	64.8 %	0.3 %	11.5%	8.8%	100%

b. Inclusion criteria

General patient inclusion criteria

1. Male or female subjects, 18 to 65 years of age.
2. Each subject must have a level of understanding sufficient to agree to all required tests and examinations and sign an informed consent document.
3. Subjects must fulfill DSM-IV criteria for Bipolar I or II depressed without psychotic features, based on clinical assessment and confirmed by a structured diagnostic interview, SCID-P.
4. Subjects must have an initial score of at least 20 on the MADRS at screen and at baseline of study phase I.
5. Subjects must have failed to respond in the past to an adequate dose and duration of at least one antidepressant (SSRI, bupropion, or venlafaxine) during a depressive episode (as defined in Thase et al., 2000).
6. Current depressive episode of at least 4 weeks duration.

Additional inclusion criteria for substudy 2 (patients with MDD)

1. Subjects must take VPA or lithium (valproate 50-125 μg/ml or lithium 0.6-1.2 mEq/L) for at least 4 weeks prior to Visit 2 and for the entire duration of the study. If the subject is not taking lithium or VPA, the research physician may start them on lithium or VPA at the NIH.

Additional inclusion criteria for substudy 4 (patients with MDD or BD)

1. Age of onset less than 40 years of age.
2. Subjects with MDD must fulfill DSM-IV criteria for Major Depression single episode or recurrent without psychotic features based on clinical assessment and confirmed by a structured diagnostic interview (SCID-P).
3. Subjects with bipolar disorder must have a YMRS of 12 or less at baseline for Phase I.
4. A failed adequate trial of ECT would count as an adequate antidepressant trial.
5. In women of childbearing age, a negative pregnancy test within 24 hours of MRI.

Inclusion criteria for healthy control subjects (Substudy 4 only)

1. Age 18-65 years.
2. Written informed consent completed.

c. Exclusion Criteria

General patient exclusion criteria

1. Current or past diagnosis of Schizophrenia or any other psychotic disorder as defined in the DSM-IV.

2. Subjects with a history of DSM-IV drug or alcohol dependency or abuse (except for nicotine or caffeine) within the preceding 3 months.
3. Female subjects who are either pregnant or nursing.
4. Serious, unstable illnesses including hepatic, renal, gastroenterologic, respiratory, cardiovascular (including ischemic heart disease), endocrinologic, neurologic, immunologic, or hematologic disease.
5. Subjects with uncorrected hypothyroidism or hyperthyroidism.
6. Subjects with one or more seizures without a clear and resolved etiology.
7. Treatment with a reversible MAOI within 4 weeks prior to study phase I.
8. Treatment with fluoxetine within 5 weeks prior to study phase I.
9. Treatment with any other concomitant medication not allowed (Appendix A for Substudy 2; Appendix G for Substudy 4) 14 days prior to study phase I.
10. No structured psychotherapy will be permitted during the study.
11. .Current NIMH employee/staff or their immediate family member.

Additional exclusion criteria for substudy 2 (patients with MDD)

1. Previous treatment with ketamine or hypersensitivity to amantadine.

Additional exclusion criteria for substudy 4 (patients with MDD or BD)

1. Subjects who currently are using drugs (except for caffeine or nicotine), must not have used illicit substances in the 2 weeks prior to screen and must have a negative alcohol and drug urine test (except for prescribed benzodiazepines) urine test at screening.
2. Presence of any medical illness likely to alter brain morphology and/or physiology (e.g., hypertension, diabetes) even if controlled by medications.
3. Clinically significant abnormal laboratory tests.
4. For imaging procedures, Presence of metallic (ferromagnetic) implants (e.g, heart pacemaker, aneurysm clip).
5. Subjects who, in the investigator's judgment, pose a current serious suicidal or homicidal risk, or who have a MADRS item 10 score of >4.

Exclusion criteria for healthy control subjects (Substudy 4 only)

1. Current or past Axis I diagnosis
2. Presence of metallic (ferromagnetic) implants (e.g., heart pacemaker, aneurysm clips).
3. Presence of medical illness likely to alter brain morphology and/or physiology (e.g., hypertension, diabetes) even if controlled by medications.
4. Treatment with any of the exclusionary medications detailed in **Appendix G** 14 days prior to Phase 1 of the Substudy 4.
5. Current or past alcohol or substance abuse or dependence diagnosis (except for nicotine or caffeine).
6. Presence of psychiatric disorders in first-degree relatives.
7. Female subjects who are either pregnant or nursing.

8. Current NIMH employee/staff or their immediate family member.

d. Rationale for General Inclusion and Exclusion criteria

Severity criteria: Only subjects with a certain severity of depression will be included to increase the likelihood of finding the desired results.

Exclusion of women who are pregnant, plan to become pregnant or are breast-feeding: The study medication, ketamine is generally considered unsafe for use during pregnancy and breast-feeding.

We are not excluding comorbid anxiety disorders. Exclusion of patients with comorbid anxiety disorders would affect the generalizability of our findings since a substantial percentage of patients with treatment-resistant bipolar disorder may have these comorbid diagnoses. Instead, we will exclude patients with this comorbid diagnosis only if it is believed to be of clinical significance. Allowing participation by patients with histories of comorbid anxiety disorders broadens the inclusion criteria to more closely approximate patients seen in real world settings.

Use of the mood stabilizers lithium or valproate for at least 4 weeks prior to randomization is to minimize the possibility that the improvement in depressive symptoms is due to the use of a mood stabilizer rather than ketamine. Also, the mood stabilizers would help protect against the switch into hypomania/mania.

Subjects with bipolar disorder must have previously failed to respond to at least one antidepressant trial (adequate dose and duration) for a depressive episode and a prospective trial of either lithium or valproate for the current depressive episode. This is a prerequisite so that the population tested is treatment-resistant.

Definition of treatment-resistance

All subjects are required to have previously failed one adequate antidepressant trial and a prospective trial of either lithium or valproate. Adequacy of antidepressant trials will be determined with the Antidepressant Treatment History Form (Sackeim 2001).

4. Study Design and Methods

a. Study overview

Substudy 2 overview

This is a randomized, double blind, placebo-controlled, crossover single site inpatient experimental study that will assess the efficacy and safety of i.v. 0.5 mg/kg of ketamine, an NMDA antagonist given as a single dose in patients meeting diagnostic criteria for Bipolar I or II depression (without psychotic features), according to DSM-IV and confirmed by the Structured Clinical Interview for the Diagnostic Manual of Mental Disorders, Fourth Edition, Patient Version (SCIP-P). Randomization will be performed at a 1:1 ratio into two groups. Patients who complete the single-dose of i.v. ketamine will then be blindly crossed over to the second treatment condition (after 2 weeks) for or an equal period of time and exact same design as the first phase.

Subjects will receive either a single intravenous infusion of ketamine hydrochloride (0.5 mg/kg) or saline solution over 40 minutes in a randomized order. To avoid carry-over effects between the different test sessions, there will be an interval of 14 days. Approximately 27 subjects with bipolar depression will be recruited for this study.

Study phases

Study Phase I (Days –28 to –14):

Screen and taper off of medications (Days –28 to –14):

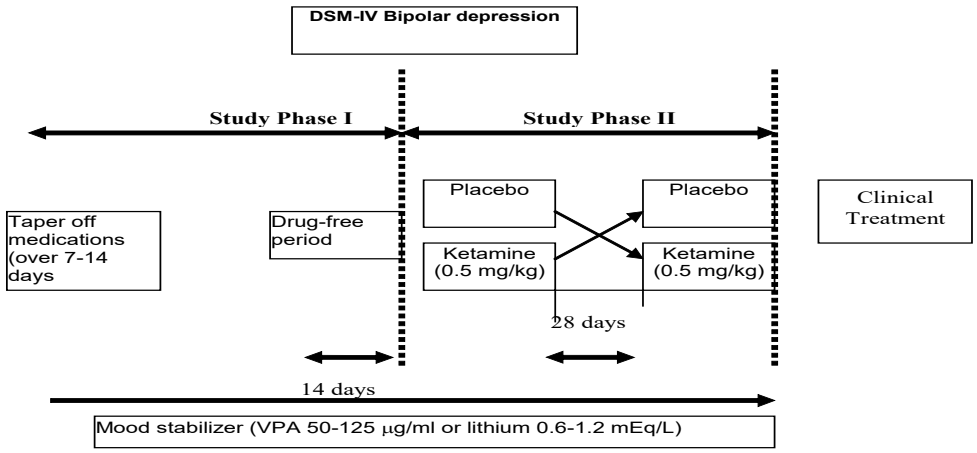
After consenting to the study (**Section 14**), patients will undergo a screening that will consist of laboratory tests, patient history, psychiatric and physical examinations (**Section 4.c**). Medications will be tapered off during this period of time. Medications allowed and not allowed are listed in **Appendix A**. Patients are expected to meet all inclusion and exclusion criteria before the taper off of medications. Subjects who are not taking medications will enter the drug-free period directly. All volunteers must have a score of ≥20 on the MADRS at screening and baseline of Study Phase I. All subjects must be taking therapeutic levels of valproate or lithium (VPA 50-125 μg/ml or lithium 0.6-1.2 mEq/L) for the entire duration of the study (Study Phase I and II).

Drug-free period (Day –14 to day –1):

Except for lithium or valproate, subjects will begin a 2-week drug-free period of all other psychotropic medication prior to the administration of ketamine. Subjects, who do not have a score of ≥20 on the MADRS by the end of Study Phase I, will be excluded and will receive standard treatment.

Study Phase II (Day 0 to 28): In this study phase, subjects will be randomized to receive either ketamine 0.5 mg/kg or a saline solution (both plus valproate 50-125 μg/ml or lithium 0.6-1.2 mEq/L) administered intravenously over a period of 40 minutes. All patients who have a MADRS ≥20 by the time of the treatment condition 2 will crossover. All subjects who discontinue the study or who complete study phase II will then receive either clinical treatment or offered to participate in another research protocol.

Schematic Figure of Study Design



Sample stratification

There will be no sample stratification.

Sample size justification

Based on our preliminary data in unipolar depression (protocol #04-M-0222), response rates to ketamine were 86% compared to (0%) for the placebo condition at 24 hours. Using a more conservative estimate of 70% response to ketamine and 15% to placebo, then at least 19 patients with bipolar depression will need to be recruited for the double-blind crossover study. This calculation is based on 90% power and a two-tailed alpha equal to .05. If you assume that 30% of subjects will either not complete the drug free period or will drop out during the crossover double-blind portion of the study, then a minimum of 27 bipolar depressed patients are necessary to complete the study.

a.

Justification for the study design and procedures: discussion of design and control

Study Phase I Subjects will have a 14-day washout prior to entering the next study phase.

Study Phase II is designed to assess the antidepressant efficacy of a single dose of i.v. ketamine.

Justification for a drug free period

Subjects will be drug free for two weeks (except for therapeutic levels of lithium or valproate) prior to the first treatment condition. This drug-free period was chosen to reduce the risk of a carry-over effect. Such a carry-over effect from previous treatments could make the interpretation of study results (e.g., response and side effect rates) difficult to interpret.

Justification for using a placebo arm

By using a placebo control instead of an active comparator, we will be able expose the fewest subjects to a experimental condition and to placebo and at the same time address our hypothesis

of whether a rapid antidepressant response can be achieved with a single intravenous dose of ketamine in patients with major depression. Over two dozen placebo-controlled trials with ketamine have been conducted.

Duration of the study

The duration of Study Phase I including the 14-day drug-free period is 4 weeks. Study phase II is 4 weeks long. Thus, the total duration of the study is approximately 8 weeks. Subjects will be required to be hospitalized during Phase II of the study (28 days). However, if it is clinically warranted (e.g., severe depression), subjects may need to be hospitalized during the entire duration of the study.

Justification for using lithium or valproate

Mood stabilizers are being used to decrease the risk of switch into hypomania/mania with ketamine, although there has been no case reported of ketamine induced mania. In our unipolar depression study, subjects who received ketamine had a short-lived increase in YMRS scores (scale used to measure manic symptoms such as euphoria) lasting only 40 minutes post-infusion. In animals, there was a study reporting an interaction between ketamine and lithium and rats (increased head bobbing and walking times) (Rubin and Wooten 1982). The relationship of these findings to humans is unclear. Further complicating the interpretation of the results in this study was that the rat's weight on lithium was 75 to 85% that of non-lithium treated animals. There have been no reported drug interactions between ketamine and lithium in humans (the latter drug is not metabolized in liver). One study examining the combination of ketamine with valproate did not find any significant motor impairment in the chimney test or memory deficit in the passive avoidance task in rats. In the same study, ketamine did not affect the free plasma concentrations of valproate (Borowicz and Czuczwar 2003).

In summary, although lithium and valproate have been reported to have glutamate effects, patients entering this study will have been required to have failed to respond to 4-weeks of therapeutic levels of either of these mood-stabilizing agents. The presumed effects of glutamate would be expected to be in the same direction of lithium and valproate (i.e., downregulate NMDA throughput).The data summarized in this section suggests that it would be safe to use ketamine in patients medicated with lithium and valproate and that their use in combination would not likely affect our ability to address our hypothesis. For example, we found robust antidepressant effects of riluzole, another glutamatergic modulator (riluzole) when it was combined with lithium.

Substudy 4 overview

This randomized, double blind, placebo-controlled, crossover single site inpatient/outpatient substudy will investigate the efficacy and safety of i.v. 0.5 mg/kg of ketamine, an NMDA antagonist given as a single dose, in patients meeting diagnostic criteria for MDD without psychotic features or BD depression without psychotic features, according to DSM-IV criteria and confirmed by the Structured Clinical Interview for the Diagnostic Manual of Mental Disorders, Fourth Edition, Patient Version (SCIP-P). Randomization will be performed at a 1:1 ratio into two groups. Patients who complete the single-dose of i.v. ketamine/placebo portion of Substudy 4 first

will have a two-week drug-free period before crossing over to the second treatment condition. This second treatment condition will take an equal period of time and have the exact same design as the first phase.

Furthermore, to investigate the neural effects of ketamine that are specific to antidepressant response and are not necessarily related to the initial effects of the drug, we also plan to enroll 40 age- and gender-matched healthy control subjects. Subjects will receive either placebo (saline solution) or a single intravenous infusion of ketamine hydrochloride (0.5 mg/kg) over 40 minutes in a randomized order. To avoid carry-over effects between the different test sessions, there will be an interval of 14 days between phases. Approximately 60 subjects with MDD, 60 subjects with BD, and 40 age- and gender-matched healthy controls will be recruited for Substudy 4.

Patients who consent will have a multimodal MRI and MEG scan in the drug-free period before receiving the intravenous infusion of either ketamine or saline solution. They will repeat the MEG procedure 4-5 hours after each of the two infusions, and will repeat the MRI procedure between the day of infusion to two days following the infusion. Patients who show an antidepressant response (50% reduction in MADRS) following infusion 1 or infusion 2 will undergo an interim assessment using the same MRI and MEG scan battery to assess relapse (if the response is lost) or sustained response (if the response is retained) 10 to 11 days post-infusion, depending on availability of scanning resources. Non-responders will undergo the interim MRI and MEG scan sessions at 10 to 11 days post-infusion as scan-time allows (all patients will receive identical compensation to remove any financial incentive to respond). Healthy controls will undergo the interim MRI and MEG scan sessions as scan-time allows (healthy subjects will be compensated for the number of scans they are asked to participate in).

In a subset of 26 patients and 26 healthy volunteers, total sleep deprivation (TSD) for 40 hours (7am – 11pm the subsequent day) will be investigated at the behavioral and neural level as a rapid acting antidepressant prior to Phase II of this study. Patients who participate in this optional component of the substudy will require an additional 7 days for their drug free period. In the evening prior to the sleep deprivation period, participants will undergo a baseline 3T MRI (within 2 days of TSD) and a high field 7T MRI scan (within 2 days of TSD) to acquire images of the brain prior to sleep deprivation. This will be followed by one 7T scan after the sleep deprivation period toward the end of the 40 hour period, but before recovery sleep. During the MRI scan participants will play a simple reward-learning task and also perform a cognitive emotional task whereby emotional faces will be presented, while functional images are acquired. Additionally, two MEG scans and two sleep EEGs will be acquired to examine the electrophysiology of response and relapse. During the MEG scan participants will wear an MEG compatible EEG cap and leads. Total sleep deprivation will occur approximately 1 week following the beginning of the drug-free period.

Feasibility of the study

Placebo-controlled studies to evaluate the neural effects of new experimental treatments are critical for drug development and to individualize treatment to each patient's needs (Mayberg 2009). I.V. sub-anesthetic doses of ketamine have been shown to have robust antidepressant effects even in the most treatment-refractory patients with MDD; however, the biological

mechanisms underlying ketamine's rapid antidepressant properties are still unknown. Substudy 4 will address this key gap in our knowledge. We are aware that this protocol will require a major effort by the research subjects as well as medical and non-medical staff. However, it will represent a unique opportunity to investigate the functional/pathophysiological mechanisms underlying rapid antidepressant response in drug-free patients with mood disorders. Furthermore, the large sample size will allow us to integrate different biological variables in order to identify biomarkers of response and antidepressant efficacy. Assuming that more than one imaging measure correlates with change in depressive symptomatology in patients receiving ketamine, multiple linear regression will be used to determine the amount of variance explained in ketamine response by the imaging measures.

The pilot studies that led to this proposal highlight our ability to perform several research procedures (imaging, electrophysiological, and peripheral chemical) and to successfully coordinate the medical and non-medical staff required for the successful integration of different aspects of this research protocol. We have studied over 90 drug-free treatment-resistant depressed patients; our staff is adept at keeping patients safe while drug-free and administering multiple procedures. For example, in addition to receiving traditional clinical care, patients who participated in protocol 04-M-0222 (Studies 2 and 3) underwent repeated fluorodeoxyglucose PET imaging scans, repeated MRS scans, repeated MEG scans, and repeated polysomnography EEG recordings around the time of ketamine infusion while simultaneously being assessed for antidepressant response and side effects.

During the sleep deprivation period, subjects will sign a record sheet (every hour) and conduct a psychomotor vigilance test (PVT) every 2 hours while under constant observation by the research or nursing staff in daytime lighting conditions. The nursing staff on the 7 Southeast unit has extensive experience in handling adults with severe psychiatric illnesses and they are adept at maintaining patient safety and comfort. Furthermore, the nursing staff has recently completed a sleep deprivation study with patients with major depressive disorder. Technical assistance and a clinical staff member will attend all imaging scans.

Study design

Substudy 4 will consist of two phases, Phase I and Phase II. The second phase will have two subphases, Subphase IIA and Subphase IIB.

Study Phase I (Day -35 to -1):

Medication tapering (Day -35 to Day -21):

Having signed protocol 01-M-0254, patients and control subjects will have undergone a screening consisting of laboratory tests, psychiatric and medical history, and psychiatric and physical examinations prior to consenting for Substudy 4 of protocol 04-M-0222. Medications will be tapered off during period I (see **Appendix G** for a list of allowed and excluded medications). Patients are expected to meet all inclusion and exclusion criteria before the taper off of medications. All patients must have a score of ≥20 on the MADRS at screening, in order to be eligible to enter Substudy 4, and also at the baseline of Subphase IIA. Patients who do not have a

score of ≥20 on the MADRS by the end of Study Phase I will be excluded and will receive standard treatment.

Drug-free period (Day -21 to Day -1):

Patients enrolled in the (optional) total sleep deprivation (TSD) portion of the study will begin a three-week drug-free period prior to the first ketamine infusion. Healthy control subjects will be free of any of the exclusionary medications (see **Appendix G**) during this 2-week period. Patients and healthy volunteers who are enrolled in the TSD period will begin wearing the actiwatch from the beginning of this period. Those enrolled in TSD will have their adaptation EEG within three days before the baseline sleep night, baseline sleep within two days of sleep deprivation and the day after total sleep deprivation commencement. Subjects enrolled in TSD will not have a subsequent adaptation sleep EEG prior to Phase II. Subjects enrolled in TSD will have a short clinical MRI scan before day -15, a 3T MRI and a 7T within three days prior to sleep deprivation and a 7T following sleep deprivation. Subjects enrolled in TSD will not have a subsequent clinical MRI. During each MRI scan, participants may perform reward-learning and cognitive emotional tasks. Subjects enrolled in TSD will have a baseline MEG within the three day period prior to sleep deprivation and the day after sleep deprivation, day -13. TSD will occur on the night of day -14; participants will be kept awake for 40 hours by interacting with staff. Subjects not enrolled in the sleep deprivation study will begin the medication taper at day -28 and the drug-free period at day -14 and then continue as usual with the protocol. At the end of Phase I of Substudy 4, before entry into Phase II, patients and controls will undergo a 120 minute 3T MRI scan battery, a 60-90 minute 7T scan battery, and 90 minutes of MEG. The 15 minute clinical MRI scan battery may be scheduled at the end of the screening visit, and the baseline MRI and MEG scans prior to the first study infusion. Alternatively, the screening battery may be acquired either with the baseline MRI scan, or in a separate scan session before the first infusion. An adaptation sleep EEG recording will be obtained for all subjects participating in this Substudy 4.

Study Phase II (Day 0 to 28):

During Phase II, patients and controls will be randomized to receive either ketamine 0.5 mg/kg or a saline solution administered intravenously over a period of 40 minutes. All patients except those who have 50% or greater decreases in MADRS scores from baseline two weeks after the first infusion will crossover. All controls will crossover to the second treatment condition. Phase II will last approximately four weeks. During this phase, patients who initially underwent MRI and MEG screening will receive two additional 3T MRI, 7T MRI, and MEG scan batteries and EEG sleep recording after each infusion of either ketamine or placebo. MEG imaging will occur on the same day as the infusion at approximately 230 minutes post-infusion. MRI imaging will occur between the day of infusion to 2 days post infusion. EEG sleep studies will be conducted during the nights immediately before and after the infusion. Healthy controls will also repeat the MRI and MEG scans and EEG sleep recordings in the same manner after each infusion.

Interim MRI and MEG scanning sessions also will occur 10 to 11 days after infusions in patients who show a significant antidepressant response following infusions (50% reduction in MADRS scores). Subjects will be categorized as Response/relapse if they show less than 25% reduction from baseline MADRS scores for 2 consecutive days between infusion and interim

scanning, and subjects will be categorized as response maintenance if they retain a 50% reduction from baseline MADRS scores until day 10 or 11 post infusion. The same MRI and MEG scan batteries that are acquired in post-infusion sessions will be obtained. Subjects who did not respond to the infusion will receive the interim MRI and MEG scanning batteries as scan time allows. In addition, an interim sleep EEG session will occur 6-7 days after infusion in all responders, and in non-responders as scan time allows. All patients regardless of response will receive identical compensation to remove any financial incentive to respond. Healthy subjects will also receive interim scans as scan time allows, and healthy subjects will be compensated for the number of scans they are asked to participate in.

Justification for the duration of the study

Phase I and Phase II of Substudy 4 will last four weeks each, for a total study duration of eight weeks. However, for those enrolled in the sleep deprivation portion, Phase I will last 5 weeks with a one week extension to the drug free period making the total study duration nine weeks. This extension to the drug-free period was chosen to reduce the risk of a carry-over effect. Such a carry-over effect from previous treatments could make the interpretation of study results (e.g., response and side effect rates) difficult to interpret. The period between the two infusions is two weeks, because our previous studies found that one-third of subjects still met response criteria by Day 7. A longer time frame between infusions would thus allow subjects to return closer to baseline. We used this design in our recently completed ketamine in bipolar depression study, Substudy 2. The duration of the Substudy 4 is similar length in length to this previously conducted study with ketamine in bipolar depression. Our past experience using ketamine in a variety of studies suggests that the proposed length of time of Substudy 4 is the shortest duration that would permit us to addresses our hypotheses.

Justification for the route and dose of ketamine used in Substudy 2 and 4

The subanesthetic dose of ketamine was selected on the basis of previous published reports describing its safety (Domino, Chodoff et al. 1965; Corssen and Domino 1966; Krystal, Karper et al. 1994; Lahti, Koffel et al. 1995; Krystal, Karper et al. 1998; Krystal, Petrakis et al. 1998; Carpenter 1999; Krystal, D'Souza et al. 1999; Krystal, Bennett et al. 2000; Krystal and D'Souza 2001; Krystal, Anand et al. 2002; Krystal, Weiner et al. 2003; Krystal, Petrakis et al. 2003). Notably, this dose has also been associated with antidepressant effects (Berman, Cappiello et al. 2000; Zhou, Zarate et al. 2006; Diazgranados, Ibrahim et al. in press). We will use the same dose of ketamine—0.5 mg/kg infused over 40 minutes—used in our previous studies (Zhou, Zarate et al. 2006; Diazgranados, Ibrahim et al. in press).

Justification for using a crossover design

The advantage of using a crossover design is that we will minimize many confounding factors, as each subject will serve as his own control (within group comparison). This type of design will also substantially increase the statistical power of the trial, thus exposing fewer study subjects to the experimental procedure. Most of the disadvantages traditionally present in crossover designs studies such as duration of the study, carry-over effects, and not having

sufficient time between interventions to return to baseline are addressed in the design of this protocol. The durations of the substudies are not increased significantly with this type of design (specifically, Phase II is no longer than 28 days) (Swayze, Andreasen et al.), the carry-over effects are minimized because the half-life of ketamine is less than two hours, and because the intervening time until the crossover takes place is 14 days, and 3) almost all patients are expected to have lost most of the improvement in depressive symptomatology (i.e., to have MADRS scores within five points of baseline) by the time of the second treatment condition. All patients who crossover to the second treatment condition will undergo the same procedures as those done for the first treatment condition.

Justification for including healthy control subjects

This protocol proposes to investigate the neural effects of sub-anesthetic ketamine compared to placebo in a sample of healthy humans in addition to those with mood disorders. This part of the protocol is critical for disentangling the neural mediators of antidepressant response in patients with MDD and BD from the nonspecific neural effects of the drug itself, which can be observed in patients as well as in healthy subjects.

Subanesthetic doses of ketamine have been extensively used in human research in the past two decades to investigate putative models of cognitive dysfunction and psychosis. A recent review of all the studies conducted at Yale University from 1995 to 2005 involving the administration of sub-anesthetic doses of ketamine in healthy subjects concluded that ketamine appears to present an acceptable risk for carefully screened populations of healthy human subjects in the context of clinical research programs (Perry, Cramer et al. 2007). That study investigated 450 subjects who received at least one dose of active ketamine (cumulative number of infusions: 833), and found that mild transient mental status changes occurred in less than 2% of the subjects, most of which resolved shortly after the infusion ended (i.e., within minutes after the discontinuation of ketamine). One subject reported nightmares, insomnia, and difficulty concentrating that lasted three to four days after the test procedure but resolved completely within two weeks. In a follow-up lasting up to six months, no long-term side effects were observed; similarly, no case of substance abuse was observed among the subjects who received sub-anesthetic intravenous ketamine. In addition, over three dozen published, double-blind, placebo-controlled studies have been conducted with ketamine in healthy volunteers in the past several decades, corroborating its safety profile.

Justification for a drug free period in patients with BD

Research into BD has evolved considerably in the last several years. Previously, short-term (<8week) studies were designed as add-on studies for a number of reasons: 1) because the drugs tested in the past were standard antidepressants (e.g., TCAs, MAOIs), which confer a greater risk of manic switch when used alone compared to adjunctively to a mood stabilizer, and 2) data were lacking regarding the risk of manic switch in patients taken off their mood stabilizer and randomized to eight weeks of placebo. Recent data suggest that tapering off concomitant medications (including mood stabilizers) and using placebo monotherapy (without a mood stabilizer) in BD-I can be done safely. Such improvements in research design have helped to more precisely determine whether a drug, when given alone, truly has antidepressant effects. In three

large bipolar depression studies [study 1: N=195 patients [129 active drug, 66 placebo], study 2: N=833 patients [456 active drug, 377 placebo], and study 3: N=542 patients [361 active drug, 181 placebo]) concomitant medications were tapered off and subjects were randomized to either active drug or placebo; these studies each lasted seven to eight weeks (Calabrese, Bowden et al. 1999; Tohen, Vieta et al. 2003; Calabrese, Keck et al. 2005). No significant differences were found in the number of subjects worsening or in the rates of manic switch between subjects randomized to active drug vs. placebo. The switch rates in these studies on placebo were low (4% to 6%) and were not significantly different of the switch rates associated with lamotrigine, olanzapine, and quetiapine treatment (all of which are being used as mood stabilizers). These data suggest that the short-term risks in BD-I patients (i.e., worsening symptoms, switching into mania) randomized to placebo do not significantly differ from those of subjects randomized to active drug (mood stabilizing agents). Our own studies investigating the antidepressant efficacy of ketamine in patients with MDD and BD (Zhou, Zarate et al. 2006; Diazgranados, Ibrahim et al. in press) found fairly similar time of onset and course of antidepressant response (lasting one week or more), euphoria, and psychotomimetic effects (both very mild and lasting no more than 80 minutes) after receiving only one dose of ketamine; this response was nearly identical for all subjects in two different studies involving two different disorders.

Because Substudy 4 is designed to carefully examine whether the glutamatergic modulator ketamine is effective in treating MDD and BD and to investigate pathophysiological correlates of response, it is imperative that we not use a concomitant mood-stabilizing agent (e.g., lithium, valproate, lamotrigine, atypical antipsychotics) as these agents have diverse effects on multiple neurotransmitters and intracellular signaling pathways. Such effects would not permit us to address our hypotheses. Notably, our preliminary study using ketamine adjunctively with mood stabilizer monotherapy in patients with bipolar depression (Substudy 2), found that ketamine did not significantly increase the risk for mood switch compared to placebo (Diazgranados, Ibrahim et al. in press). Our newer understanding of BD research methodology suggests that subjects with BD can be safely included in short-term placebo monotherapy research studies. In addition, a significant number of subjects with MDD and BD do experience short-term benefits after ketamine infusion.

b. Recruitment

Substudy 2 Recruitment:

Patients, ages 18 to 65 with a diagnosis of bipolar disorder I or II, currently depressed without psychotic features will be recruited into this study. The NIMH is a very large research center that provides a full range of psychiatric research services, including inpatient care and outpatient treatment at the Experimental Therapeutics and Pathophysiology Branch (ETPB). Our goal is to enroll on average 1-2 patients per month with the diagnosis of bipolar depression at NIMH over a period of 2 years. All outpatients or inpatients within the community referred to us with a diagnosis of unipolar depression will be evaluated for entry into the study based on the criteria outlined in the Study Design and Methods section listed above. NIH employees will not be directly recruited by or through their supervisors to participate in this study. NIMH employees and their immediate family members cannot participate in this protocol. The sample size chosen

for this preliminary study of 27 with the diagnosis of bipolar depression is the sample size necessary in order to obtain 19 patients to test our hypothesis.

Substudy 4 Recruitment

Our goal is to enroll approximately two patients per month with MDD, two patients per month with BD, and 3 healthy controls per month over a period of three to four years. All outpatients or inpatients within the community referred to us with a diagnosis of MDD or BD will be evaluated for entry into Substudy 4. NIH employees will not be directly recruited by or through their supervisors to participate in this study. NIMH employees and their immediate family members cannot participate in this protocol. Patients with MDD and BD will be free of psychotropic medication for at least two weeks prior to scanning (five weeks for fluoxetine and three weeks for aripiprazole). In addition, women of childbearing age must have a negative pregnancy test on the day of each scan, prior to participating in MRI imaging. Standard MRI safety criteria will be applied (e.g., excluding subjects with pacemakers, cochlear implants, surgical clips or metal fragments in the eyes or body parts). Subjects with anatomical brain abnormalities found on a routine clinical MRI will be excluded (see pg. 9 for a full list of inclusion and exclusion criteria)

NIMH recruits patients centrally through The Office of Human Subjects Protection/NIMH. From this source alone, on a monthly basis there are approximately 270 phone screens of patients with mood disorders of which approximately 30 are referred for in person screens for research studies. Approximately 15 patients with mood disorders are randomized per month into different clinical treatment studies.

Participants may be recruited under screening protocol, 01-M-0254: “The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Volunteers”, or through IRB approved advertisements under this protocol. The methods we use under this protocol and under protocol 01-M-0254 include:

a. Non-Paid Advertisements
    a. Internal NIH media (e.g. NIH Record)
    b. NIH Internet (e.g. http://patientinfo.nimh.nih.gov/)

b. Paid Advertisements:
    a. Local print publications (e.g. Washington Post Express, The Gazette)
    b. National print publications (e.g. Washington Post)
    c. Public transit advertisements (e.g. posters on Washington Metropolitan Area Transit Authority Metrobuses)
    d. Radio advertisements (e.g. WMZQ, WASH, WLZL/CBS Radio)

c. Brochures:
    a. Mental Health Clinics, (e.g. Threshold Services, Adventist Potomac Ridge Behavioral Health, Northern VA Mental Health Institute), Hospitals (e.g. Suburban Hospital, Walter Reed National Military Medical Center), and local Clinicians
    b. Professional Conferences (e.g. American Psychiatric Association and American College of Neuropsychopharmacology annual conferences), Symposiums (e.g.

Challenging Depression: New Insights into Research and Treatment (Suburban Hospital)), and other professional settings (e.g. Latino Behavioral Health Institute, Frederick Providers Council, DC Department of Mental Health)

c. Mental Health Advocacy Conferences (e.g. NIMH Outreach Partnership Annual Meeting, Maryland's Annual Suicide Prevention Conference)
d. Self-Help and Advocacy Groups (e.g. local NAMI and DBSA chapters, Jewish Social Service Agency, Active Minds)

d. NIH Referrals:
   a. Investigators from other NIH inpatient units and outpatient clinics, including those from the NIMH, may refer patients to either study

e. Community Resource Listings:
   a. Community Calendars, Newsletters, electronic and print- twenty-five word text summary

f. Fliers:
   a. Flyers may be distributed throughout the NIH campus and/or on community bulletin boards. Posting on bulletin boards will occur only with permission from management of said facility. Potential locations of the bulletin boards include local grocery stores, public libraries, and local colleges and universities.

g. ListServs:
   a. With the permission of listserv administrators, we will distribute information regarding our studies. We will not post/send directly to the listserv. Rather, an email with information about our study information attached will be sent to the administrator of the listserv which will include the following disclaimer:

      "You are receiving this message because your email address is included in the above listserv. The purpose of this message is to inform you of our NIMH research studies. The moderator of the listserv has permitted its use for this distribution."

h. Brochures and other IRB-approved recruitment materials or study information may be given directly to individuals interested in our research in both hard copy and electronic formats, depending on request. Associate Investigators and members of the NIMH Marketing & Community Relations Unit will distribute recruitment materials to individuals/groups, such as mental health clinics, hospitals, self-help and advocacy groups, and local clinicians. Clinicians who are contacted will be provided with information to disseminate to patients as they see fit. We will explain to them that individuals interested in participating in our studies will need to initiate contact with our group and that we will <u>not</u> make this initial contact.

<u>Gender and minority issues</u>

The proportion of ethnic minorities (vs. Caucasian) in the total sample will be consistent with the population proportions in Washington D.C. and the Montgomery county area. Collaborations with Howard University in Washington D.C. will help us recruit representative samples from the

African American and Hispanic population and will aid exploratory analyses of ethnic effects on outcome. We appreciate that minority groups tend to be underrepresented in research samples of bipolar depression. Therefore, we will try particularly hard to recruit minority patients to assure that the subject sample represents the community. Although it is not known whether racial differences will affect responses to pharmacotherapy, we will explore for such effects. However, the size of the racial subsamples may be too small to identify statistically meaningful differences.

Anticipated ethnic/racial distribution of sample areas

Sample Areas	Black/ African-American	Asian	Caucasian	Native American/ Alaskan	Other/Two or more*	Total	Hispanic/ Latino**
District of Columbia	60%	2.7%	30.8%	0.3%	6.2%	100%	7.9%
Montgomery County	15.1%	11.3 %	64.8 %	0.3 %	8.8%	100%	11.5%

* Combines the categories of Pacific Islander, other race and two or more. * *The Hispanic/Latino category is determined separately in Census 2000 and is not directly comparable with other categories. Source: U.S. Census Bureau, Demographic Profiles: Census 2000

The newly launched Hispanic Research Program ETPB/NIMH has significantly enhanced our ability to recruit minorities. The Hispanic Research Program has been able to recruit up to 29% of Hispanics in to an ongoing Phase IIa study. The proportion of females to males will be approximately 1 to 1 for bipolar depression at our site.

c. Screening

Consent will be obtained before any study procedures, including screening procedures, are done.

Subjects will be screened under screening protocol 01-M-0254: **"**The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Volunteers." Psychiatric history and diagnosis of a depressive episode will be made using the SCID-P (First, Spitzer et al. 2001) and the DSM-IV Diagnostic Criteria. Patients who meet DSM-IV criteria for the above diagnoses (without psychotic features) must have a score of ≥20 on the MADRS at study baseline, and at baseline of Phase II. We will require that all subjects have physical examinations and specific laboratory tests as outlined in the Schedule of Events (**Table 1).** These tests include: physical exam, electrocardiogram (ECG), supine and standing vital signs, complete blood cell counts (CBC), electrolytes, thyroid functioning, fasting blood sugar, liver function tests, urinalysis and toxicology screening. A pregnancy test will be done in women of childbearing age. Subjects must also have a negative human immunodeficiency virus (HIV) test prior to Phase I of Substudy 4. Results of these tests will identify patients who should be excluded because of active medical problems or substance abuse that might affect clinical phenomenology or make participation in the protocol unsafe.

d. Study Procedures

Substudy 2 study procedures

Assessments

Table 1. Schedule of Events**Time**	**Procedure**
Study Phase I (Day –28 to 0)	Informed consent, demographics, psychiatric examination, physical examination, SCID, vital signs (blood pressure, pulse [supine and standing], temperature), weight, ECG, labs: CBC, electrolytes, thyroid function tests, fasting blood glucose, liver function tests, urinalysis, and toxicology screening, pregnancy test and HIV. Screening and taper off of medications; MADRS
Day –14 to –1	Drug-free period (except for lithium or valproate)
Study Phase II (Day 0-14)	
Day 0 8:00 AM –80 minutes –60 minutes	 Insert IV line in antecubital region; begin oximetry, pulse and blood pressure monitoring. Urine pregnancy test. MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS (baseline ratings); ketamine levels (ketamine and norketamine) and plasma neurochemicals*
0 minutes	Infusion of ketamine 0.5 mg/kg or saline solution over 40 minutes
+40 minutes	End of infusion MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
+80 minutes	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
+110 minutes	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
+230 minutes	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*; end oximetry, pulse and blood pressure monitoring
Day 1 or 24 hours, 8:00 AM	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
Day 2 or 48 hours, 8:00 AM	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
Day 3 or 72 hours, 8:00 AM	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
Day 7 8:00 AM	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
Day 10 8:00 AM	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*

Day 14 8:00 AM −80 minutes −60 minutes	Insert IV line in antecubital region; begin oximetry, pulse and blood pressure monitoring. Urine pregnancy test. Subjects with ≥20 on the MADRS crossover; MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
0 minutes	Infusion of ketamine 0.5 mg/kg or saline solution over 40 minutes
+40 minutes	End of infusion MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
+80 minutes	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
+110 minutes	HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
+230 minutes	HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*; end oximetry, pulse and blood pressure monitoring
Day 15 or 24 hours, 8:00 AM	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
Day 16 or 48 hours, 8:00 AM	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
Day 17 72 hours, 8:00 AM	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
Day 21	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
Day 24	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals*
Day 28 End of Study	MADRS, HDRS, BDI, VAS, BPRS, YMRS, CADSS; ketamine levels (ketamine and norketamine) and plasma neurochemicals* Physical examination, vital signs (blood pressure, pulse [supine and standing], temperature), weight, ECG, labs: CBC, electrolytes, liver function tests.

Abbreviations: BDI = Beck Depression Inventory; BPRS = Brief Psychiatric Rating Scale; CADSS = Clinician-Administered Dissociative States Scale; CBC=complete blood count; ECG=electrocardiogram; HDRS = Hamilton Depression Rating Scale-17 item; MADRS = Montgomery-Asberg Depression Rating Scale; *Neurochemicals include: Brain derived neurotrophic factor (BDNF), norepinephrine, metanephrine, cortisol, ACTH; VAS = Visual analogue scales score; YMRS=Young mania rating scale.

Follow-up assessments

The follow-up assessments are outline on Table 1 (Schedule of Events). Researchers will conduct clinical assessments which include: the MADRS, HDRS-17 item, the Beck-Depressive

Inventory (BDI), the visual analogue scale (VAS), the Brief Psychiatric Rating Scale (BPRS), and the Young Mania Rating Scale (YMRS). The MADRS, HDRS, VAS, BDI, scales will be used to collect information on depressive symptoms. The YMRS will be used to collect information on hypomanic/manic symptoms (see **Appendix B** for description of the scales).

Definitions of response

Change from baseline to endpoint in the MADRS total score will serve as the primary efficacy measure for the double-blind, crossover phase of the study. Secondary efficacy assessments include the HDRS, BDI, VAS, BPRS, and YMRS.

In addition to indicating simple change in severity, the MADRS total score will be used to dichotomize patients into response versus nonresponse categories at the end of Study Phase II. A responder will be defined as any patient who demonstrates a 50% or greater decrease in MADRS total score from baseline to endpoint.

Pharmacotherapy

Ketamine administration

After completing Study Phase I, subjects will receive double-blind ketamine or a saline solution (placebo). Fourteen days later, subjects will crossover to the other arm. The ketamine and saline solution will be infused through intravenous tubing in the forearms. The infusions will contain either ketamine 0.5 mg/kg or saline solution that will be administered over the course of 40 minutes. The dose and method of administration of ketamine is reviewed in **Section4.a**. No repeat dose of ketamine will be permitted, as Study Phase II is a single-dose study. Subjects who are unable to tolerate the dose of study medication will be discontinued from the study (not permitted to crossover to the other treatment condition).

Medications allowed and not allowed are in **Appendix A.**

Lithium and Valproate Initiation and Titration

All subjects will openly receive either lithium or VPA which will be kept within the therapeutic serum level (lithium 0.6-1.2 mEq/L and VPA 50-125 μg/ml) during the entire duration of the study (Study Phases I and II). Lithium and VPA levels will be monitored weekly and at the discretion of the investigator and kept and adjusted if need be to remain within therapeutic levels. If a subject is not taking lithium or VPA at the time of Visit 1, they will be started on lithium by the research physician at NIMH to participate in this study. The subject must be on therapeutic levels of lithium or VPA for at least 4 weeks prior to Visit 2.

e. Substudy 4 study procedures

Duration of the study

The duration of Phase I, including the 14-day drug-free period is four weeks. Subjects participating in the TSD study will have an additional 7 days added to the drug-free period, totaling 21-days. Phase II is also four weeks long. Thus, the total duration of Substudy 4 for

patients is approximately eight to nine weeks, depending on sleep deprivation participation. For healthy control subjects who do not undergo a medication tapering, or drug-free period or TSD study, the study will last approximately 4 weeks. For healthy volunteers undergoing TSD, total study duration will last roughly 6 weeks.

Number of visits and commitment of subjects

Schedule of Events (**Table 1**), lists the assessments and procedures carried out at baseline and those carried out at various points during Phases I and II of Substudy 4. The assessments and their timing are based on knowledge gleaned from our previous studies.

Genome Sequencing

Individuals who consent to have genome sequencing will be given the opportunity to be informed of certain incidental findings, confirmed in a CLIA-certified lab at the NIH by approved genetic counselors appointed by the Principal Investigator. Informing participants of all findings would be neither practical nor responsible, since most findings are difficult or impossible to interpret in terms of individual health (Kaye et al. 2010). However, some findings might convey important clinical information to participants and their families. There is no consensus in the human genetics field about which findings should be reported (Wolf et al. 2008; Cho 2008). However, some ethicists (Beskow & Burke, 2010) have proposed a "duty to rescue" standard which kicks in "…when, in the course of research, an investigator discovers genetic information that clearly indicates a high probability of a serious condition for which an effective intervention is readily available." This standard seems reasonable and is consistent with common medical practice. Accordingly, we report to participants mutations that are known to cause diseases of major health significance that are preventable, or would benefit from early detection and treatment. An example would be mutations in BRCA1 that are known to cause breast cancer, where early detection could be of great benefit.

Mutations that fulfill these criteria only in the homozygous (or hemizygous) state are present in almost everyone, so in that case it makes sense to report recessive mutations that are common enough to have an impact on reproductive decisions. A 1% allele-frequency threshold has been adopted by other genome sequencing studies as a middle-ground position between reporting all rare recessive mutations (which is impractical and may cause undue worry in participants), and reporting no recessive mutations, even though reporting might help participants avoid, for example, relatively common illnesses such as cystic fibrosis or sickle cell disease in their offspring

We will not inform participants of all genetic markers that may have health implications or that only predispose to disease, since these are very difficult to interpret in the context of current medical evidence and practice. Many hundreds of such markers have been identified by genome-wide association studies, but none has strong predictive value for an individual's health.

If we find a mutation that fulfills the reporting criteria, participants who indicated in their consent form that they wish to be notified will be contacted by mail and telephone and told that "they might carry a gene change with implications for their health" and that we will need to confirm this with a second DNA collection and testing in a CLIA-certified lab before we can

provide specific information. If the CLIA results confirm our initial findings, we will offer participants an appointment with a genetic counselor to review the findings and their implications. We will also monitor participants' psychiatric status throughout this process and arrange for appropriate treatment, if necessary.

We will inform all participants that genome sequencing has limitations. For example, not all mutations that cause diseases will be detected. We will warn participants that the failure to detect any mutations may falsely reassure them that they are not at risk for disease. We will also warn them that knowing that they carry particular mutations may be distressing to them and to their relatives, should they decide to share the information with them.

After being provided with all of this information and discussing it with the consenting clinician, participants will be given the opportunity to 1) opt out of the genome sequencing portion of the study; 2) request that they not be notified of reportable mutations that we may detect in their DNA.

Blood and/or Saliva collection

Under the screening protocol 01-M-0254, blood and/or saliva was collected to provide material for DNA analysis. If adequate sample volumes exist they will be used for the Genomic Sequencing procedure. If it is determined that more blood and/or saliva are needed then additional samples will be collected.

Blood sample:

An additional 30 ml of blood will be drawn. This procedure can be performed at the NIH or it can be arranged for you to have blood drawn at a doctor's office or lab near your home.

Saliva sample:

Additional saliva will be collected using a kit. The kit will be mailed to the participating subject with instructions on how to collect the saliva. Alternatively, subjects may provide the saliva sample when they are at the NIH**.**

Screening and drug free period

Psychiatric history and diagnosis will be made using the SCID-P (First, Spitzer et al. 2001) and DSM-IV Diagnostic Criteria. Healthy controls will be assessed using the SCID-NP (First, Spitzer et al. 2002). The presence of psychiatric disorders in first-degree relatives will be assessed using the Family Interview for Genetic Studies (FIGS) (NIMH 1992)/The Family History Screen(Weissman, Wickramaratne et al. 2000). Patients who meet DSM-IV criteria for MDD or BD (without psychotic features) must have a score of ≥20 on the MADRS at screen and at the baseline of Subphase IIA of Substudy 4. All participants will undergo physical examinations and specific laboratory tests as outlined in the Schedule of Events (**Tables 1 & 2**). Rating scales obtained during screening and the drug free period is listed in Table 1. Results of these tests will identify patients who should be excluded because of active medical problems or substance abuse that might affect the clinical phenomenology or make participation in the protocol unsafe.

A subset of 26 patients with MDD and 26 healthy volunteers will participate in the optional TSD study during this period. Participants will participate in three extra MRI scans at time points relevant to the sleep deprivation period: two baseline images within three days prior to sleep deprivation and a post sleep deprivation image (day -13). The MRI scans may consist of high-resolution images of the whole brain and specific structures (e.g. hippocampus), resting state functional images, proton magnetic resonance spectroscopy images from the prefrontal cortex and a reward related decision making task, cognitive emotional face processing and simple non-emotional cognitive tasks in conjunction with fMRI acquisition. A MEG scan including cognitive and cognitive emotional tasks and a resting state scan may be acquired at baseline during this period and following the night of sleep deprivation; during this post-sleep deprivation scan, participants will be requested to try to fall asleep. During the MEG scan participants may wear an MEG compatible EEG cap and leads.

Polysomnography will also be performed during the drug free period to examine the effects of sleep deprivation on sleep and its relationship to depression. Actigraphy will be obtained throughout the study (days –21 through to the end of day 28). The Stanford Sleepiness Scale (SSS) will be collected following TSD and recovery sleep. The SSS is a measurement of sleepiness and takes only a few minutes to administer.

Standard clinical rating scales administered during Phase II will also be administered during Phase 1 around the time of the sleep deprivation period (see Table 1 for details). Participants will be woken up on the day of the sleep deprivation period at 7am to conduct ratings and then again following the brief recovery sleep period inside the MEG following sleep deprivation and the subsequent morning following recovery sleep in bed. No caffeine, alcohol, tea or cigarettes will be permitted during the sleep deprivation period. Participants will complete a simple psychomotor vigilance test every two hours during the sleep deprivation period. Participants will be permitted to watch TV, read books and use their computer during the sleep deprivation night.

f. End of participation

Provision of care to research subjects

Concomitant clinical care

All subjects will be admitted and treated on the 7 Southeast/Mood Disorders Research Unit. The nursing staff on the 7 SE has extensive experience in handling adults with severe psychiatric illnesses and they are adept at maintaining patient safety and comfort.

Reasons for withdrawing from the study

At the end of the study or when a patient withdraws from the study prematurely, the efficacy measures and evaluations will be repeated. In addition, a physical exam, vital signs, and laboratory measurements will be obtained. Patients will also be assessed for adverse experiences. Patients may withdraw at any time or be dropped from the study, at the discretion of the investigator, multidisciplinary team, or CORE team member. Other reasons for stopping the study include developing medical contraindications to the assigned medication, intolerable adverse reactions, a worsening of mood based on rating scales (see below), onset of psychotic, manic, or

other symptoms that require pharmacological treatment, or if in the clinician's judgment the patient has worsened to such a degree that further participation would put the patient at risk. The multidisciplinary team will meet three times a week to coordinate treatment efforts, to monitor the progress and safety of a patient, and to determine whether further participation would put the patient at risk. The multidisciplinary team may discontinue a patient from further participation in a study for any of the reasons discussed in this section. Patients may also be withdrawn at any time if the subject requests to be withdrawn from the study. Subjects will be discontinued from the study if they develop mania, psychosis, or other symptoms that require pharmacological treatment. Subjects removed from the study will receive standard treatment at NIMH as long as they agree to receive treatment. However, subjects not agreeing to receive treatment will then be transferred to a local hospital for further treatment in which case NIH will not cover the costs associated with that hospitalization.

Transfer of care/continuity of care

All subjects who complete the trial or discontinue a substudy because of lack of response or side effects will be either invited to participate in other research protocols or will receive short-term treatment as clinically appropriate with standard medication for up to three months after the end of the study4. After receiving short-term clinical care, subjects will be referred for appropriate long-term follow-up care. The guideline we will use when discharging patients back to their referring clinician after completion of a study4 will not be "clinical stability;" rather, we will consider a patient ready to be referred back to their treating clinician to *continue treatment* when they are improved (based on clinical judgment), not a danger to self or others, and with an appropriate after care plan (e.g., adequate psychosocial support system, treating psychiatrist in place).

Information to be shared with subjects or their health care providers

A clinical summary will be provided to the patient and treaters in the community. This summary will include medical and psychiatric assessments performed, laboratory tests, current medications, and recommendations for further clinical management.

5. Management of Data and Samples

a. Storage

For medical records: Clinical data will be managed according on NIH's Clinical Center's policy (http://www.cc.nih.gov/participate/patientinfo/legal.shtml).

For research data: Data will be coded, no individual will be identified by name, and the data will be stored in a locked filing cabinet stored in the principal investigator's locked offices (Mark O. Hatfield CRC, Unit 7SE, Rooms 7-3445, 7-5541, 7-5561, and 7-5563) to protect subject anonymity.

For stored samples: Biological samples are kept in freezers by the NIMH/ETPB with no patient identifiers. Information on processing and storage of samples is on record with the Clinical and Scientific Director's offices at NIMH. Results will be published as group data without the use of characteristics that would identify individual subjects. Samples and date will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only Substudy 2 and 4 investigators will have access to the samples and data.

For imaging: Imaging data will be stored in a secure server offsite.

We obtained a Material Transfer Agreement (MTA # P-11-011) for plasma samples along with corresponding depression rating scores and other demographic and clinical information, collected from human participants under this protocol (04-M-0222) to be transferred to Associate Investigator Coral Barbas, Ph.D, at San Pablo-CEU University, in Madrid, Spain. No personally identifiable information will be provided to Dr. Barbas. Dr. Barbas will only use the Human Material for the Research Project, with the goal of analyzing the Ketamine metabolites in relation to the corresponding data.

b. Data (including genomic data) and sample sharing plan

This study requires submission of genomic data under the NIH GDS policy. Genomic data will be submitted to the following NIH-designated repositories: dbGAP

Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained in the original consent form.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations. If genomic data is submitted to an NIH-designated repository under the NIH GDS policy, information on whether the repository has open or restricted access will be provided at the time of continuing review.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

We may also receive coded data and/or samples from outside investigators, institutions, or databases for analysis under this study. Data and samples will only be accepted if the participants gave consent for such sharing and use or if the IRB at the institution providing the samples or data waived consent. We will make no attempt to decode the data and/or samples.

6. Additional Considerations

a. Research with investigational drugs or devices

N/A

b. Gene therapy

N/A

7. Risks and Discomforts

a. Risks of study Participation by Phase

Level of risk:

This research involves more than a minimal risk to subjects.

General

By agreeing to participate in this study, subjects will be temporarily forgoing the opportunity to receive routine clinical care in the community. This will be clearly explained to all patients, along with the treatment strategies that are generally used in patients with MDD and BD. If subjects discontinue treatment, we will provide short-term treatment at NIMH as long as they remain on a voluntary basis. However, if subjects do not remain on a voluntary status, then they may have to be transferred to a local hospital for further treatment. If subjects are hospitalized elsewhere, NIH will not cover the costs associated with that hospitalization.

Risks of sharing genomic data

Risks of sharing genomic data may include possibility of reidentification.

Screening and Evaluation

The risks and discomforts of the screening and baseline evaluations are minimal. No discomfort is expected to be associated with the physical examination or the clinical interview. Venipuncture or intravenous lines may be associated with the momentary discomfort of the needle stick, as well as a small risk of hematoma (bruise) formation is a potential risk. Subjects may potentially find the discomfort of being asked personal questions distressing.

Drug-Free Period

There may be a worsening of symptoms as the patient's concomitant medications are discontinued. There may be a worsening of depressive symptoms, including suicidal ideation, as the patient's concomitant medications are being discontinued. Subjects may not receive effective antidepressant treatment for as long as 45 days if they participate in Substudy 4 and 52 days if subjects participate in the sleep deprivation portion of Phase 1.

Treatment

Hospitalization may be somewhat upsetting to patients. It is essential to ensure their safety while being withdrawn from concomitant medication. There may be a worsening of symptoms as the patient's concomitant medications are being discontinued. However, this risk should be minimized as substudy 2 uses an add-on design in that all patients will be required to take at least one mood stabilizer lithium or VPA (within therapeutic range) for the entire duration of the study. There may be a worsening of depressive symptoms including suicidal ideation as the patient's concomitant medications are being discontinued. Subjects may not receive effective antidepressant treatment (other than lithium or valproate) for as long as 45 days if they participate in this study.

Ketamine administration (Study Phase II)

Relevant pharmacology

Peak plasma concentrations of ketamine have been reported to occur within 1 minute following intravenous administration (Domino et al 1984; Grant et al 1981). Initially, ketamine is distributed to highly perfused tissues, including brain, to achieve levels four to five times that in plasma. Ketamine has high lipid solubility and low plasma protein binding (12%), which facilitates rapid transfer across the blood-brain-barrier. The distribution half-life approximates 10 minutes. Biotransformation of ketamine into multiple metabolites involves N-demethylation by cytochromes p450 to norketamine, an active metabolite with an anesthetic potency approximately one-third that of ketamine. Norketamine is then hydroxylated and conjugated to water-soluble compounds that are excreted in urine. Elimination is primarily by the kidney, with only a small percentage recovered in the urine as the unchanged drug (Chang et al 1970). The elimination half-life is approximately 2 hours which is secondary to the combination of rapid clearance and large volume of distribution (Domino et al 1984).

Toxicity

Ketamine is a general anesthetic for human and veterinary use. It is also known as "special K" related to PCP and is a drug of abuse especially among teens. For that reason, ketamine was placed in Schedule III of 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 good safety profile. In addition, it has been used in psychotherapy and in psychophysiological studies in normal volunteers and patients with severe mental disorders (with similar doses to that of our study) safely for years (Krystal et al 2003; Krystal et al 2002; Krystal et al 2000; Krystal and D'Souza 2001; Krystal et al 1999; Krystal et al 1998; Krystal et al 1994; Krystal et al 2003; Krystal et al 1998; Lahti et al 1995; Lahti et al 2001; Micallef et al 2003). Krupitsky and colleagues (Krupitsky et al 2002) in their ketamine psychotherapy study with single doses of 2.0 mg/kg im and 0.2 mg/kg im, reported no significant adverse events at 2-year follow-up. Ketamine exerts sympathomimetic activity and may produce mild to moderate increases in blood pressure, heart rate, and cardiac output. The reported incidence of perceptual

disturbances varies from less than 5% to greater than 30% (Knox et al 1970; White et al 1980). Perceptual disturbances manifested as vivid dreaming, visualization of psychodelic 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 (Green and Johnson 1990). When such reactions occur, they are usually mild and short-lived (Green and Johnson 1990). Carpenter (Carpenter 1999) has systematically collected short-term outcome and potential patients distress data during ketamine challenge interviews from three North American institutions that conducted these studies. These studies suggest that ketamine administration has short-lasting effects on symptoms, usually less than 60 minutes and rarely lasting longer than 2 hours. The increase in psychosis is also generally not distressing to volunteers. Furthermore, in one long-term outcome study of patients who received ketamine during research, no serious adverse events were noted in more than 90 patients exposed to ketamine. This study found no differences between patients who did and did not receive ketamine on any measures of psychopathology, psychiatric care in the 8 month follow-up period (Lahti et al 2001). There is no evidence in these long-term studies that subjects that were exposed to a ketamine were at greater risk of abusing it on follow-up. In this protocol, in order to minimize the risk of perceptual disturbances that may occur with ketamine, we will exclude patients with a current or past history of psychotic symptoms. Although ketamine is an anesthetic and has respiratory effects, we will use subanesthetic doses of ketamine.

Magnetic Resonance Imaging Procedure

No known hazards are associated with exposure to magnetic waves during MR imaging, though the potential risk of heart rhythm disturbances exists for patients with a history of heart rhythm abnormalities or those who have certain types of pacemakers. A substantial risk to persons who have metallic objects inside their bodies exists, as the magnet in the scanner can cause these to move. Pregnant women should not undergo MRI because of the possible harmful effects to the fetus. People with claustrophobia may find this procedure uncomfortable as it involves having one's head confined to a relatively small space. A small amount of discomfort may be associated with having to be still for the 120 minutes required for the MR scan, possibly resulting in mild muscle stiffness and fatigue. In addition, very high field magnets (above 3 Tesla) may cause additional side-effects of peripheral nerve stimulation. This is experiences as twitching in the muscles, or a tingling sensation. Additionally, some subjects report dizziness, mild nausea, headache, a metallic taste in their mouth, or a sensation of flashing lights. All of these sensations are associated with moving too rapidly in the magnetic field. These sensations are not considered harmful, and desist upon cessation of scanning. On very rare occasions, subjects may experience some eye discomfort. There have been no biological hazards to humans discovered due to high static magnetic fields. Finally, the MRI scanning procedure is loud, and may affect hearing.

Magnetoencephalography

No known risks are associated with MEG scans.

Polysomnography

No medical risks are associated with the sleep test. There may be slight discomfort while the electrodes are attached to the scalp, and subjects may not like the smell of the paste or the glue remover. The conductive gel sometimes causes mild scalp irritation. Subjects may be uncomfortable sleeping in the sleep lab with the electrodes and monitors in place. These mild problems are addressed by monitoring the scalp for signs of irritation, and by allowing a night for the patient to accommodate to the new sleep room if needed.

Sleep Deprivation

Sleep deprivation is likely to cause some discomfort. Omitting one night of sleep was found to be a bearable discomfort in many previous sleep deprivation studies. Extended sleep and bed rest following the sleep deprivation phase will allow ample time for recovery. Sleep deprivation could provoke seizure activity in individuals with seizure disorders. Subjects with one or more seizures without a clear and resolved etiology are excluded from the protocol. Subjects do not receive any benefit from the sleep deprivation intervention, and sleep deprivation may cause irritation and induce hypomanic or manic symptoms in participating patients. Patients with bipolar disorder will not participate in this substudy. The nursing staff on the 7 Southeast Unit has extensive experience in running sleep deprivation protocols with patients with severe psychiatric illnesses and will minimize any risks to patients. Any subject requiring treatment for manic or psychotic symptoms will be discontinued from the sleep deprivation study.

Saliva Collection

There is no risk associated with a saliva collection.

Blood Draw

There may be some discomfort and bruising at the site of needle entry. There is a very small risk of fainting. Infection in the area of the needle insertion is rare.

b. Procedures to minimize risks

Taper off medications

Subjects will have all their medications tapered off over a period of one to two weeks. All subjects will be either inpatients or outpatients (subjects will have to be hospitalized 1 to 3 days before and the day of each of the 2 infusions) during Substudy 4. Should hospitalization be required, patients will be admitted to the Mood Disorders Research Unit at NIMH. The outpatient and inpatient nursing staff have extensive experience handling adults with severe psychiatric illnesses, and are adept at maintaining patient safety and comfort.

Drug-Free Period

All subjects will be hospitalized during the drug-free period and during Study Phases I and II of Substudy 2. Subjects may participate in Substudy 4 either as inpatients or outpatients. Subjects may choose, or the clinician may feel, that it is in the best interest of the subject to be hospitalized during the drug-free period and during Study Phases I and II. The nursing staff of the 7 Southeast Unit has extensive experience handling adults with severe psychiatric illnesses and are adept at maintaining patient safety and comfort. In order to determine whether a patient should be withdrawn from their medications to participate in the study, the following criteria will need to be met: a) patients must meet criteria for at least moderate depression (see inclusion/exclusion criteria), and will not have fully responded to their current medication; and b) patients must indicate that they have not significantly benefitted from their current medication regimen. We will verify with the treating clinician that this is indeed the case.

Sleep Deprivation

Sleep deprivation may cause irritation to participating patients. The nursing staff on the 7 Southeast Unit has extensive experience in running sleep deprivation protocols with patients with severe psychiatric illnesses and will minimize any risks to patients. Any subject requiring treatment for manic or psychotic symptoms will be discontinued from the sleep deprivation study. In order to minimize the possibility of these symptoms, subjects with bipolar disorder will not be included in the sleep deprivation portion of the study. To prevent the possibility of seizures evoked by sleep deprivation, subjects with seizure disorders will not be included in the study.

Patients will be told to immediately inform their research physician if they develop suicidal ideation or symptoms of mania, or a worsening of depressive symptoms. In addition, for outpatients, we will have a treatment contract. These precautions are likely to be highly effective in minimizing risks.

Double-blind study period

Subjects may participate in Substudy 4 either as inpatients or outpatients (subjects will have to be hospitalized 1 to 3 days before and the day of each of the 2 infusions). Hospitalization may be somewhat upsetting to some inpatients. Outpatients will be informed that if their clinical condition deteriorates during the washout phase of Substudy 4, they may be hospitalized at the NIH. Subjects may be hospitalized at the NIMH if their symptoms worsen, including suicide risk. In this case, or if subjects discontinue treatment, they will receive standard short-term clinical treatment at the NIMH as long as they remain on a voluntary basis. However, if subjects do not remain on a voluntary status, then they may be transferred to a local hospital for further treatment. If subjects are hospitalized elsewhere, NIH will not cover the costs associated with that hospitalization.

Patients will be told to immediately inform their research physician if they develop suicidal ideation or symptoms of mania, or a worsening of depressive symptoms. Subjects will be required to identify someone who knows them well to contact the research physician on their behalf in the event suicidal thoughts or manic symptoms develop. Patients with bipolar depression who participate as outpatients will be ask to sign a treatment contract (**Appendix K**).

We have also included safety measures such as discontinuation of the study drug and transition to standard clinical treatment should subjects worsen to a sufficient degree. These precautions are likely to be highly effective in minimizing risks.

Ketamine administration

Oximetry, pulse, and blood pressure monitoring will occur immediately before and during the 230 minutes after ketamine administration. Subjects are monitored for potential reactions that may occur with the administration of ketamine (Study Phase II). A clinician with advanced medical training (i.e. trained in Advanced Cardiac Life Support (ACLS) and conscious sedation of anesthetic agents (i.e. ketamine and ketamine-like drugs)) will be present throughout the administration of ketamine so that adverse reactions can be evaluated and treated promptly. Subjects will be discontinued from the study if they develop manic, psychotic, or other symptoms that require treatment with medications. If needed, diazepam, or a similar sedative, may be used to address serious untoward effects from ketamine or other symptoms reported by participants. Medications would be given at a clinically indicated dosing regimen if a patient were to experience serious anxiety, agitation, or other untoward effects. A fully equipped emergency medical cart is located nearby in the unlikely case of an untoward reaction or development of another health related problem. Some evidence suggests that excessive noise and stimulation during recovery from ketamine sedation might be associated with emergence reactions (or psychomimetic effects). Thus, ketamine will be administered in a well-monitored location with muted lighting, noise, and physical contact for 230 minutes after the procedure. Benzodiazepines are not permitted during Substudy 4 as this drug may affect our results.

MRI imaging

The potential risks associated with MRI will be minimized in the following manner:

1. Claustrophobia from magnetic resonance imaging will be reduced by explaining the nature of the procedure in detail prior to enrollment in the study.
2. Earplugs will be given to each subject to wear during the scan to minimize discomfort and prevent any adverse effects on hearing resulting from the scanning procedure.
3. A possible history of any intraocular, intra aural, intracranial, or intra-thoracic metal will exclude the subject from the study.
4. Personnel trained in emergency procedures will be present throughout the MRI study in case medical emergencies arise.
5. Menstruating females will undergo either serum or urine pregnancy testing to rule out pregnancy no more than 24 hours prior to each MRI session before the scanning procedures are initiated.
6. A power meter will be utilized to ensure that experimental sequences fall within the accepted guidelines for RF energy exposure. Applied RF power will be limited so that the specific absorption averaged over the head does not exceed 3W/kg for 10 minutes.

7. In order to minimize the occurrence of high field related sensations while scanning on the 7T scanner, subjects will be instructed to move slowly while in the scan room, and the table on the 7T scanner moves significantly slower than those in the 3T scanners.
8. If subjects experience any discomfort in their eyes, or desire to stop the scan for any other reason, the scanning will be stopped immediately.

8. Subject Safety Monitoring

a. Consent Procedures

The Human Subjects Protection Unit (HSPU) will monitor the informed consent process, which may then, in certain circumstances trigger the need for a formal independent capacity assessment. After receiving complete disclosure about the research and opportunity to fully review the consent form, potential participants will be given the opportunity to ask questions. If they choose to take part in the study, they will be asked to sign the consent form.

b. Reasons for discontinuation from the study

At the end of the substudy, or when a patient discontinues the study prematurely, the efficacy measures and evaluations will be repeated. In addition, a physical exam, vital signs, and laboratory measurements will be obtained. Patients will also be assessed for adverse experiences. Patients may withdraw at any time or be dropped from the substudy at the discretion of the investigator, multidisciplinary team, or Human Subjects Protection Unit (HSPU) team member.

c. Reasons for withdrawing a patient from the study at any time include

1. Clinically significant abnormal lab tests or adverse events inconsistent with continuation in the substudy.
2. Clinical judgment of the investigator, the multidisciplinary team, or HSPU team member. The multidisciplinary team will meet three times a week to coordinate treatment efforts, to monitor the progress and safety of a patient, and to determine whether further participation would put the patient at risk. The multidisciplinary team may discontinue a patient from further participation in a study for any of the reasons discussed in this section.
3. Withdrawal of consent and/or patient decision.
4. Psychotic, manic, or other symptoms that require pharmacological treatment.
5. Subjects who have a score >4 on item 10 of the MADRS (which measures suicidal ideation).

9. Outcome Measures

a. Primary outcome

The primary aim will be accomplished by assessing the efficacy of a single dose of i.v. ketamine (0.5 mg/kg) compared with placebo in improving overall depressive symptomatology in patients with bipolar depression. The primary assessment of efficacy is the reduction from baseline of the MADRS between groups after covarying the baseline total MADRS scores.

b. Secondary outcome variables

Secondary efficacy assessments include the HDRS, BDI, VAS, BPRS, and YMRS. In addition to indicating simple change in severity, the MADRS total score will be used to dichotomize patients into response versus nonresponse categories at the end of Study Phase II. A responder will be defined as any patient who demonstrates a 50% or greater decrease in MADRS total score from baseline to endpoint.

Putative biomarkers of response:

1. Task-dependent and resting-state activity in the ACC and the amygdala.
2. GABA and Glx/Glutamate ratio.
3. Gray matter volume and integrity of white matter fibers connecting the ACC and the amygdala
4. fMRI task-dependent and resting-state activity changes in the ACC and the amygdala.
5. Correlations between fMRI task-dependent and resting-state activity changes in the ACC and the amygdala.
6. Differential neural effects of ketamine vs placebo in patients with MDD, BD and healthy controls
7. Baseline and changes of SWA between ketamine vs. placebo.
8. Baseline and changes of peripheral neurotrophic factors and other neurochemicals between ketamine vs. placebo.

10. Statistical Analysis

a. Substudy 2 statistical analysis

Analysis of data/study outcomes

(Specific Aim 1) To assess the efficacy of a single dose of i.v. ketamine (0.5 mg/kg) compared with placebo in improving overall depressive symptomatology in patients with treatment-resistant bipolar depression.

The primary aim will be accomplished by assessing the efficacy of a single dose of i.v. ketamine (0.5 mg/kg) compared with placebo in improving overall depressive symptomatology in patients with bipolar depression. The primary assessment of efficacy is the reduction from baseline of the MADRS between groups after covarying the baseline total MADRS scores. A linear mixed model with a first order autoregressive covariance structure and restricted maximum likelihood estimation will be used with time and drug as repeated measures factors. Bonferroni

corrected simple effects tests will be used to follow up omnibus effects. Also, treatment order will be added to the model in a secondary analysis to understand potential differences in the effects due to the timing of the drug treatment. Additional secondary analyses will be conducted in the same manner as the original analysis for the 17 item HDRS, VAS, BPRS, and YMRS.

Power analysis

All p values will be evaluated as significant at $p<.05$, two-tailed.

A subject will be considered a responder if the MADRS total score has decreased by 50% or more from baseline to endpoint. Response rates will be analyzed using a McNemar test given the repeated measures nature of the data.

For efficacy, accumulating data will be examined after approximately 60% of the data has been collected to make a decision about the validity of the null hypothesis. If the accumulated data provide evidence that supports reliable rejection of the null hypothesis, the study may be terminated.

b. Substudy 4 statistical analysis

Analysis of data/study outcomes

Clinical Response

The primary outcome measure will be change in MADRS total score. A linear mixed model with restricted maximum likelihood estimation will be used to examine changes in depressive symptoms over the course of the trial under an intent-to-treat approach where all participants with at least a baseline and one post-baseline measure will be included. Within-subjects factors will include time with baseline and all other points, treatment, and phase. The interaction between time and treatment will be included along with the fixed intercept. The random intercept and a random effect for subject will be included if they are a significant addition to the model. Schwarz's Bayesian criteria will be used to determine the best fitting variance-covariance structure, where the structure with the lowest value will be used. Bonferroni post hoc tests will be used to examine differences between levels of significant effects.

Criteria for significance

Significance will be evaluated at $p\leq.05$, two-tailed. The Kolmogorov-Smirnov test will be used to examine whether the distribution of the outcome measure is normal. If the data are not normally distributed, transformations will be used to improve the fit. Secondary analysis will include examination of other clinical scales as well as sleep measures with the original statistical model. The criteria for significance will be adjusted using a Bonferroni approach based on the number of secondary measures examined with the linear mixed model. In addition, a between subjects factor for diagnostic type (MDD vs. BD) and the interaction of that factor with time, treatment, and time by treatment will be added to the original statistical model to examine difference in response by diagnosis. Clinical response will be determined by the proportion of change in MADRS from baseline to Day 1. Patients achieving a 50% decrease or better will be

considered responders. The proportion of responders in each treatment phase will be compared using McNemar's Test where only participants with results for both phases can be included. Symptom relapse will be determined 10 to 11 days post-infusion by evaluating patients who experience a clinical response and subsequently retain a 25% improvement from baseline or less for 2 consecutive days.

Correlates and Predictors of Treatment Response

Pearson correlations will be used to examine the effects of ketamine on the proportion of change from baseline on the MADRS and baseline demographic, imaging, and sleep and neurochemical measures, as well as changes in imaging, sleep, and neurochemical measures. Given the diverse issues under investigation as mediators and moderators, adjustment for multiple comparisons will be performed separately for baseline and change measures. Hochberg's corrected Bonferroni procedure will be used for each set of correlations. A similar set of Pearson correlations will be used with placebo data. For any region showing a significant relationship between ketamine and placebo, correlations will be compared to determine whether the relationship is stronger for on one of the treatment phases. This will help tease apart factors related to general change vs those specific to ketamine.

Assuming more than one imaging measure is correlated with change in depression on ketamine, multiple linear regression will be used to determine the amount of variance explained by ketamine response on imaging measures. Only regions with significant Pearson correlations will be included. Further, tolerance levels will be examined to determine whether multicollinearity problems exist within the regression models and regions with the greatest overlap with other regions will be removed one by one until all tolerance levels reach a reasonable range (approximately 0.6). This procedure will be performed separately for baseline and change variables.

A secondary analysis will include similar procedures examining differences between responders and non-responders to ketamine. Given the categorical nature of the response variable, Student's *t*-tests will be used instead of Pearson correlations and logistic regression will be used instead of linear regression. Procedures for imaging data analysis are described in further detail in **Appendix H**.

Analysis of ketamine's effects in patients compared to healthy control subjects

A linear mixed model will be used with imaging and sleep measures to compare patients and controls. The model will be similar to the one used for clinical changes with the addition of between-subjects patient-control factors and interactions with time, treatment, and time by treatment. Baseline group differences will be examined *a priori* and baseline values may be used as covariates when those differences appear substantial.

Analysis of surrogate neurobiological markers

Pearson correlations (for continuous measures) and logistic regression (for categorical measures) will be used to evaluate candidate biological markers for independent association with outcomes.

c. All Substudies: Meta-Analysis of Suicidal Ideation across Multiple Sites

A linear mixed model will be used to determine the effects of ketamine on suicidal ideation over time. Predictors of antisuicidal response will be assessed using Pearson correlations (for continuous measures) and logistic regression (for categorical measures).

d. Power analysis

Though our previous studies show very large clinical effects of ketamine (d>1.0), recommendations for providing an appropriate sample size suggest favoring effects indicating the minimum level of effect necessary to provide a clinically meaningful change. Since other anti-depressant trials frequently show significant and moderately sized effects (d=.5), a moderate effect size should provide a high standard for showing a clinically meaningful difference. We chose a moderate effect size to power the Substudy 4 in order to ensure a substantial amount of change was needed to show a significant effect and to provide sufficient power to detect an effect that may be substantial, even if not as large as the prior study.

The same effect size would require enough of a prediction to explain at least 9% of the variation in ketamine response with MRS and fMRI data. Our previous data with magnetoencephalography showed much better predictions than this, so the moderate effect size should lead to sufficient power to detect a meaningful relationship while an even larger relationship is expected.

Our previous study (Zhou, Zarate et al. 2006) of ketamine vs placebo in patients MDD observed an effect size (Cohen's d) of 1.46 24 hours after infusion. Because one of the primary aims of the present study is to replicate this finding, we will use a more conservative estimate of effect size to reflect a level of change closer to the minimum level of change needed to be clinically meaningful. Thus, using d=0.5 in a crossover study, a minimum of 34 patients would be required to have 80% power to detect the effect with $p \leq .05$, two-tailed. If 15% of patients drop out of the study, six more patients would need to be recruited for a total of 40. Because MDD and BD patients will both be recruited and because these groups may respond differently to ketamine, the estimate of sample size applies to these groups separately (in addition to healthy controls). If there is a moderate difference in response (Cohen's d =0.5) between these groups, the study will have 78% power to detect that difference.

Based on previous research in unipolar subjects (Wu et al., 1999) we estimate with 95% power that a sample of 20 patients is necessary to detect a significant effect of sleep deprivation on levels of depression. However, given the treatment resistant nature of our population, an additional 6 participants are requested, thus the total sample will be 26. Furthermore, in order to detect a Pearson correlation coefficient value of 0.5 at 80% power we calculate that 26 subjects are necessary.

An additional goal is to examine correlations between imaging and sleep measures and changes in depressive symptoms. To detect a moderate correlation (r=0.3) with 80% power and p≤.05, two-tailed, a minimum of 82 patients will be necessary. With 15% drop out, another 13 patients would be necessary (95 patients total). Further, one aim of the study is to determine how much of the variance in depressive symptom changes can be explained through imaging measures. If as many as five variables are included in a linear regression, then 120 patients will provide 80% power to predict as little as 10% variance. Predicting more variance will bring additional power. To successfully examine group differences, a total of 40 healthy controls will be recruited. To allow additional power in the patient groups for examining multivariate relationships with imaging and response, 60 MDD and 60 BD patients will be recruited.

11. Human Subjects Protection

a. Subject selection

Age criteria: Subjects older than 65 are excluded in order to eliminate age-related changes in brain structure and function from confounding analyses.

Medical comorbidities criteria: Subjects with diabetes or hypertension are excluded because they may exhibit changes in brain structure and function.

b. Justification for exclusion of children

We are excluding minors as we feel preliminary safety and efficacy data on the usage of ketamine for psychiatric indications should be obtained in adults before proceeding to minors.

c. Justification for exclusion of other vulnerable subjects

Exclusion of women who are pregnant, plan to become pregnant, or are breast-feeding: Ketamine is generally considered unsafe for use during pregnancy and breast-feeding.

We are not excluding comorbid anxiety disorders. Exclusion of patients with comorbid anxiety disorders would affect the generalizability of our findings, given that a substantial percentage of patients with MDD or bipolar depression may have these comorbid diagnoses. We will exclude patients with this comorbid diagnosis only if it has been the focus of treatment in the past six months. Our most recent data with ketamine in BD found that this agent had significant and rapid anti-anxiety effects as early as 40 minutes after administration, and these lasted at least three days.

Subjects with MDD or BD must have previously failed to respond to at least one antidepressant trial (adequate dose and duration) for a major depressive episode. This prerequisite ensures that subjects have previously received treatment.

Substance abuse/dependence criteria: We could have used more stringent criteria to exclude patients with a history of substance dependence, but opted not to do so because our findings would be less generalizable and also because recruitment would be severely hampered; a large proportion of mood disorders subjects have such a history.

Other: General MRI exclusion criteria will apply, such as the presence of ferromagnetic implants.

NIMH employees and their immediate family members cannot participate in this protocol.

d. Justification of sensitive procedures

Justification for a drug free period: Subjects will be drug free for two weeks prior to the first treatment condition (ketamine administration). This drug-free period was chosen to reduce the risk of a carry-over effect. Such a carry-over effect from previous treatments could make study results difficult to interpret.

Justification for using a placebo arm: By using a placebo control instead of an active comparator, we will be able expose the fewest subjects to experimental conditions and to placebo and at the same time address our hypothesis of whether a rapid antidepressant response can be achieved with a single intravenous dose of ketamine in patients with major depression. A placebo condition would also help us interpret study results.

e. Safeguards for vulnerable populations

All patients will be assigned a Clinical Research Advocate (CRA) from the HSPU who will monitor subjects from initial consent until study completion. After receiving complete disclosure about the research and the opportunity to fully review the consent form, potential participants will be given the opportunity to ask questions. If they choose to take part in Substudy 2 or 4, they will be asked to sign the consent form.

For NIH employees participating in research, neither participation, nor the refusal to participate, will have an effect, either beneficial or otherwise on the subject's employment at the NIH. NIH employee subjects who wish to participate in this study will receive a copy of the "NIH Information Sheet on Employee Research Participation". NIH employees will not be directly recruited by or through their supervisors to participate in this study.

This study collects sensitive medical information. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

f. Qualifications of investigators

Carlos A. Zarate, M.D. is Chief of the Experimental Therapeutics and Pathophysiology Branch Research Unit at the National Institute of Mental Health. Dr. Zarate has a strong track record in recruiting and systematically studying both treatment-refractory and non-refractory unipolar and bipolar disorder patients. He may obtain informed consent.

Lawrence Park, M.D. is a psychiatrist and staff clinician with advanced medical training (Advanced Cardiac Life Support (ACLS). Dr. Park will be working with mood disorder patients and will assist in providing clinical care, assist in consenting subjects for research protocols, conducting research procedures, assisting in data analysis and administering and monitoring the study drug.

Sarah Lisanby, M.D. is Director of the Division of Translational Research (DTR) at the NIMH. Previous to her appointment at the NIMH, she had been serving as Chair of the

Department of Psychiatry and Behavioral Sciences at Duke University School of Medicine. Dr. Lisanby will be working with study participants and will assist in providing clinical care, conducting research procedures, and assisting in research/data analysis. She will not obtain consent.

Bruce Luber, Ph.D. is Associate Research Professor of Psychiatry and Behavioral Neuroscience in the Department of Psychiatry at Duke University. Dr. Luber has experience conducting clinical research involving the use of ketamine. Dr. Luber will be working with study participants and will assist in the administration of rating scales, questionnaires, and research tasks and in research/data analysis. He will not obtain informed consent.

Wallace Duncan, Ph.D. is a research psychologist with extensive experience collecting and analyzing EEG and polysomnography data. He will not obtain informed consent.

Andrew Mannes, M.D. is a staff anesthesiologist with experience in pain management and pain studies. Dr. Mannes may be involved in administering and monitoring the infusion of study drug. He will not obtain informed consent.

Mark J. Niciu Jr. M.D. Ph.D. is a clinical research fellow at the NIMH with advanced medical training (Advanced Cardiac Life Support (ACLS) and conscious sedation) who will be working with mood disorder patients and will assist in consenting subjects, providing clinical care and research procedures, potential research/data analysis, and administering and monitoring the infusion of study drug.

Erica Richards, MD, PhD is a staff clinician in the NIMH Office of the Clinical Director. She will be working with mood disorder patients and will assist in consenting subjects, providing clinical care, and potential research/data analysis.

Marc Lener, M.D. is a clinical research fellow in the NIMH ETPB. Dr. Lener will be working with research participants and will assist in consenting subjects, providing clinical care and research procedures, potential research/data analysis, and administering and monitoring the study drug. Dr. Lener has advanced medical training in Advanced Cardiac Life Support (ACLS).

Lorie Shora, RN, MSN, FNP/CHP is a Nurse Practitioner in the ETPB. She is Board certified by the American Nurses Credentialing Center as a Family Nurse Practitioner. Ms. Shore is state licensed in Maryland, Virginia, and the District of Columbia and is ACLS certified. She has extensive experience providing comprehensive primary and mental health care services. Ms. Shore will provide clinical care, perform research procedures, conduct research/data analysis, and administer and monitor the study drug. Ms. Shora is authorized to obtain informed consent.

Yumi Yi, CRNP, is a licensed Nurse Practitioner in the ETPB. Ms. Yi is Board certified by the American Nurses Credentialing Center as a Psychiatric-Mental Health Nurse Practitioner. She is a credentialed clinician at the NIH Clinical Center and is ACLS certified. Ms. Yi has several years of experience providing mental health care services and working with psychiatric patients in both inpatient and outpatient settings. She will provide clinical care, perform research procedures, conduct research/data analysis, and administer and monitor the study drug. Ms. Yi is authorized to obtain informed consent.

Bashkim Kadriu M.D. is a clinical research fellow in the NIMH ETPB. Dr. Kadriu will be working with research participants and will assist in consenting subjects, providing clinical care and research procedures, potential research/data analysis, and administering and monitoring the study drug. Dr. Kadriu has advanced medical training in Advanced Cardiac Life Support (ACLS).

Níall Lally, M.Sc. is an NIMH clinical fellow and Ph.D. student at the University College of London. Mr. Lally has expertise in conducting functional brain imaging studies. He will be working with mood disorder patients and healthy individuals. Mr. Lally will be involved in experimental design, data collection, data analysis, and manuscript preparation. He may not obtain informed consent.

Only Drs. Zarate, L. Park, Niciu, Richards, Lener, and Kadriu, and Lorie Shora, RN, MSN, FNP/CHP and Yumi Yi, CRNP will be consenting patients for this study.

Nancy Brutsche, M.S.N. is a research nurse with the Experimental Therapeutics and Pathophysiology Branch at NIMH. She will not obtain informed consent.

David Luckenbaugh, M.A. is the senior statistician for the Experimental Therapeutics and Pathophysiology Branch at NIMH. He will not obtain informed consent.

Paul Carlson, M.D. is a collaborator who will help with the imaging data analysis. He will not obtain informed consent.

Allison Nugent, Ph.D. is experienced in collecting and analyzing imaging data in patients with mood disorders. Dr. Nugent will serve as the Lead Associate Investigator on this protocol. She will not obtain informed consent.

Samuel Wilkinson, MD is a fellow in Interventions for Functional Disabilities in Psychiatric Disorders (Director Morris Bell, PhD) at Yale University in New Haven, CT. He has research interests in clinical implementation of therapeutic interventions, including relapse prevention following ECT as well as sustaining antidepressant effects of ketamine. Dr. Wilkinson will be involved in manuscript preparation. He will not obtain informed consent and will not have access to PII.

Gerard Sanacora, MD, PhD completed an NIH sponsored Medical Scientist Training Program at the State University New York at Stony Brook, earning his Ph.D. in Physiology and Biophysics in 1992 and his M.D. degree in 1994. He then moved to Yale University where he completed his internship at Yale-New Haven Hospital, the Clinical Neuroscientist Training Program Residency in the Department of Psychiatry, and an NIH funded Neuroimaging Scientist Training Program Fellowship. He is currently an Associate Professor and the Director of the Yale Depression Research Program. Dr. Sanacora's work is concentrated largely on elucidating the pathophysiological mechanisms associated with mood and other neuropsychiatric disorders. Much of his recent research has focused on identifying the contributions of the amino acid neurotransmitter systems (GABA and Glutamate) to the neurobiology of mood disorders and the mechanism of antidepressant action. In addition, he is involved in several early phase clinical trials designed to test the clinical efficacy of newly developed therapeutic agents. Dr. Sanacora will be involved in manuscript preparation. He will not obtain informed consent and will not have access to PII.

Jessica Ihne, Ph.D. is a post-doctoral research fellow in the Experimental Therapeutics and Pathophysiology Branch (ETPB) of the NIMH. Dr. Ihne's research interest is in the neurobiology of individuals with mood disorders, and she has considerable experience in fMRI analysis. She will assist with imaging procedures and research/data analysis, but will not obtain consent.

Elizabeth Ballard, Ph.D. is a Clinical Psychologist who will be working with mood disorder patients and healthy volunteers and will assist in psychological evaluations, ratings, and research/data analysis. Dr. Ballard will not get informed consent.

Laura Waldman, L.C.S.W. is a licensed social worker in the NIMH ETPB. She will be working with research participants and will assist in psychological evaluations, ratings, and research/data analysis, and recruitment and selection of study participants. Ms. Waldman will not get informed consent.

Nadia Hejazi, MD, is a Neurologist in the NIMH ETPB with extensive experience in neuroscience research. Dr. Hejazi will be involved in PSG, psychological interviews, administration of rating scales and questionnaires, and research/data analysis. Dr. Hejazi will not obtain consent.

Alex J. Noury M.A. will assist with protocol coordination, administrative issues, and regulatory compliance. Mr. Noury will not obtain consent.

Peter Herscovitch, M.D. will be a consultant regarding imaging data. He will not obtain informed consent.

Giacomo Salvadore, M.D. will be involved in imaging data analysis. He will not obtain informed consent.

Irving W. Wainer, Ph.D. is Senior Investigator Bioanalytical Chemistry and Drug Discovery Section of the National Institute of Aging. He will collaborate on analysis of ketamine metabolites and cytochrome polymorphisms. He will not obtain informed consent.

Nancy Brutsche, M.S.N, and Rezvan Ameli, Ph.D. will help with recruitment, administrative issues (e.g. IRB documentation), and clinical ratings regarding this protocol. They will not get informed consent.

Libby Jolkovsky, M.S. will help with recruitment, administrative issues (e.g. IRB documentation), and clinical ratings regarding this protocol. Due to her involvement in coordinating genetics testing, Ms. Jolkovsky is authorized to get consent via Genome Consent and the Informed Written Consent by Telephone Script (Protocol, Appendix L). She may not obtain consent for clinical trial procedures.

Rodrigo Machado-Vieira MD, PhD, is a Psychiatrist and Staff Scientist of the Experimental Therapeutics and Pathophysiology Branch. He will be involved in the identification of new neural subtrates and biochemical/metabolic predictors. Dr. Machado-Vieira will be also evaluating peripheral markers associated with the clinical efficacy of agents in proof-of-concept trials, analyzing data and writing scientific articles originating from this protocol. He will not obtain informed consent.

Craig Marquardt, BS is a doctoral candidate in the Clinical Science and Psychopathology Research Program of the University of Minnesota's Department of Psychology. Mr. Marquardt has training in high-density EEG/ERP and affective processing. He will be involved in the interpretation and analysis of MRI and MRS data. Mr. Marquardt will not get informed consent.

Stephen Robinson, PhD, trained in Physiology and Biophysics, is with the MEG Core Facility in the NIMH. Dr. Robinson has expertise in functional neuroimaging using MEG. He will be involved in the interpretation and analysis of MRI and MRS data. Dr. Robinson will not get informed consent.

Ioline Henter will help with administrative issues related to regulatory requirements. She will not obtain informed consent.

Coral Barbas, Ph.D. is a Professor in Analytical Chemistry in the Pharmacy Faculty at San Pablo-CEU University in Madrid Span, and Head of the Center for Metabolomics and Bioanalysis. Dr. Barbas will be analyzing research bloods and will not be providing clinical care nor consenting subjects.

Jennifer Evans, Ph.D. is a post-doctoral research fellow in the Experimental Therapeutics and Pathophysiology Branch (ETPB) of the NIMH. Dr. Evans' research interest is in the neurobiology of individuals with mood disorders, and she has over 10 years of experience in f/MRI data acquisition and analysis. She will assist with imaging procedures and research/data analysis. Dr. Evans will not get informed consent.

Joanna Szczepanik, Ph.D., currently a doctoral candidate in neuroscience at UMD-College Park, is trained in clinical psychology. Dr. Szczepanik has extensive experience performing assessments in psychiatric populations. She will assist in psychological evaluations, ratings, administering cognitive tests/tasks, and research/data analysis. Dr. Szczepanik will not obtain informed consent.

The Principal Investigator has verified that all individuals working on this protocol required to take HRPP training under OHSRP SOP 25 (Training requirements for the NIH Human Research Protections Program) have completed all required training.

12. Anticipated Benefit

There is no direct benefit to subjects participating in this protocol. However, results from our two previous placebo-controlled studies found that ketamine was effective in the short-term for treatment-resistant patients with MDD and BD. These substudies may yield generalizable knowledge about developing new treatments for mood disorders.

13. Classification of Risk

a. Adults

This research involves more than a minimal risk to subjects and may yield generalizable knowledge about developing new treatments for mood disorders.

b. For adults without consent capacity

N/A

c. For children

N/A

d. Overall risk and benefit consideration

The risks are reasonable in relation to anticipated benefit.

14. Consent Documents and Process

a. Designation of those obtaining consent

Study investigators designated as able to obtain consent in **Section 11.f** above, will obtain informed consent (Drs. Zarate, L. Park, Niciu, Lener, Bashkim, and Richards, and Lorie Shora, RN, MSN, FNP/CHP and Yumi Yi, CRNP will obtain informed consent). Due to their involvement in coordinating genetics testing, Ms. Jolkovsky is authorized to get consent via Genome Consent and the Informed Written Consent by Telephone Script (Protocol, Appendix L). She may not obtain consent for clinical trial procedures.

All study investigators obtaining informed consent have completed the NIMH HSPU "Elements of Successful Informed Consent" training. The Human Subjects Protection Unit (HSPU) will monitor the informed consent process, which may then, in certain circumstances trigger the need for a formal independent capacity assessment.

Study participants who enrolled in this protocol prior to the approval of Amendment 36 may be contacted by telephone to invite them to participate in the genome sequencing project. Telephone consent will be obtained using the "Informed Written Consent by Telephone" script, which describes the steps for obtaining consent and submitting to medical records. If interested, they will be sent two copies of the genome consent form and a pre-paid envelope for returning the form.

b. Consent procedures

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing.

c. Consent documents

The consent form contains all required elements.

The following consent forms are submitted with this protocol:

- Substudy 2: Ketamine-BP: Consent Form
- Substudy 4: Ketamine-MOA: Healthy Volunteer Consent
- Substudy 4: Ketamine-MOA: Depressed Consent Form
- Genome Sequencing Consent Form

15. Data and Safety Monitoring

a. Data and safety monitor

This protocol will be reviewed by the NIMH-IRP Data and Safety Monitoring Board. The DSMB meets two to three times per year. The board will review study accrual and progress, adverse events related to the study, and safety and outcome data. Specific data elements required by the DSMB will be established at the first meeting the protocol is reviewed. The DSMB will have the authority to require changes in the study design, or to stop all or part of any study based on accumulating safety data.

b. Data and safety monitoring plan

The principle investigator will prepare a report to the DSMB for initial review. Thereafter the reporting schedule will be determined by the DSMB based on study accrual and safety needs of the study. Serious adverse events and unanticipated problems will be reported to the DSMB within 7 days if life threatening; otherwise, within 15 days. In the event of a serious medical emergency, the medically responsible physician has the authority to break the blind however, the DSMB and the Clinical Director will be notified immediately.

c. Criteria for stopping the study or suspending enrollment or procedures

1. New findings emerge that indicate it would not be safe to continue studying ketamine.
2. The Combined Neuroscience IRB safety subcommittee decides to stop the study for safety reasons.

16. Quality Assurance

a. Quality assurance monitor

Quality assurance will be monitored by the PI and research team and the Intramural Research Program Auditing Committee (IRPAC), coordinated by the Office of the Clinical Director, NIMH.

b. Quality assurance plan

The IRPAC monitors intramural research studies to ensure compliance with GCP, organizational policies and applicable federal, state and local laws and the reliability of study data. Audit frequency is determined by the IRPAC SOP based on the study level of risk. Results of IRPAC audits are provided to the PI, The Clinical Director and the CNS IRB. This study will undergo audits at least once every three years and for cause.

For this study, which is greater than minimal risk, QA will be monitored by investigator self-assessment and routine audits by the IRPAC.

17. Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the PI in consultation with the CD.

Serious unanticipated problems, serious adverse events (including deaths) that are not unanticipated problems, and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing not more than 7 days after the PI first learns of the event, unless immediate reporting is waived for specific serious adverse events as noted below. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing not more than 14 days after the PI first learns of the event. Written reports will be submitted in PTMS.

All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

18. Alternative Therapies

Currently available antidepressants (TCAs, MAOIs, SSRIs) and ECT have been reported to be effective for treating MDD. Several psychotherapeutic modalities have also been reported to be effective in patients with mood disorders and are frequently used in combination with medications. Subjects will be reminded that these alternative treatments for depression are available to them out in the community before being considered for Substudy 2 or 4. This approach will ensure that patients are clearly informed of the standard care that is available to them before being exposed to the risks of this study. Private genetic testing can be done outside of this research study at a cost to the subject.

19. Privacy

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

20. Confidentiality

a. For research data and investigator medical records

For medical records: Clinical data will be managed according on NIH's Clinical Center's policy (http://www.cc.nih.gov/participate/patientinfo/legal.shtml).

For research data: Data will be coded, no individual will be identified by name, and the data will be stored in a locked filing cabinet stored in the principal investigator's locked offices (Mark O. Hatfield CRC, Unit 7SE, Rooms 7-3445, 7-5541, 7-5561, and 7-5563) to protect subject anonymity.

Data can be sent to sites with an FWA and reported at continuing review. Prospective IRB approval will be obtained for sharing with sites that do not have an FWA.

For imaging: Imaging data will be stored in a secure server offsite.

This study collects sensitive medical information. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

b. For stored samples

Biological samples, such as DNA, blood and saliva, are kept in freezers by the NIMH/ETPB with no patient identifiers. Information on processing and storage of samples is on record with the Clinical and Scientific Director's offices at NIMH. Results will be published as group data without the use of characteristics that would identify individual subjects. Samples and date will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only Substudy 2 and 4 investigators will have access to the samples and data.

This study requires submission of genomic data under the NIH GDS policy. Genomic data will be submitted to the following NIH-designated repositories: dbGAP.

Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained in the original consent form.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations. If genomic data is submitted to an NIH-designated repository under the NIH GDS policy, information on whether the repository has open or restricted access will be provided at the time of continuing review.

c. Special precautions

Samples and data will be stored using codes that we assign. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.

There is always a possibility of data and samples being traced back to study subjects; in order to decrease the likelihood of this happening, identifiers are removed from samples and data, and samples and data are coded.

We obtained a Material Transfer Agreement (MTA # P-11-011) for plasma samples along with corresponding depression rating scores and other demographic and clinical information, collected from human participants under this protocol (04-M-0222) to be transferred to Associate Investigator Coral Barbas, Ph.D, at San Pablo-CEU University, in Madrid, Spain. No personally identifiable information will be provided to Dr. Barbas. Dr. Barbas will only use the Human Material for the Research Project, with the goal of analyzing the Ketamine metabolites in relation to the corresponding data.

De-identified results from clinical trials will be posted on cctrials.gov.

21. Conflict of Interest

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

The NIH and Dr. Zarate have submitted a patent application for intranasal administration of ketamine for the treatment of depression. Dr. Zarate has assigned his patent rights to the U.S. government, and he does not personally have any control over either the patent prosecution or the licensing of the technology. Dr. Zarate may receive payments from royalties should the patent be licensed.

c. Role of a commercial company or sponsor

N/A

22. Technology Transfer

We obtained a Material Transfer Agreement (MTA # P-11-011) for plasma samples along with corresponding depression rating scores and other demographic and clinical information, collected from human participants under this protocol (04-M-0222) to be transferred to Associate Investigator Coral Barbas, Ph.D, at San Pablo-CEU University, in Madrid, Spain. No personally identifiable information will be provided to Dr. Barbas. Dr. Barbas will only use the Human Material for the Research Project, with the goal of analyzing the Ketamine metabolites in relation to the corresponding data.

23. Research and Travel Compensation

Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

All volunteers will be compensated for time and research-related inconveniences. A travel compensation form has been attached in PTMS. All subjects will receive reimbursement based on NIH standards for inconvenience and time devoted to the research project. This compensation will also be used to defray the cost of days of missed work. Employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation.

Detailed compensation appears in the charts below. We compensate healthy controls slightly more than patients, because from past experience, adequate recruitment and retention requires higher degrees of compensation than that required for adequate recruitment and retention of patients.

Substudy 2:

Procedure	**Number**	**Duration**	**Inconvenience Units**	**Pay**
Psychiatric interview/ Physical examination/ECG	1	6 hr	1.0	$ 60
Blood tests	18	0.5 hr	1.0	$ 180
SCID	1	1-2 hr	1.0	$ 30
Hospitalization (x 14 days @ $40/day)*	14	24 hr	1.0	$ 560
2 i.v. lines	2	5 hr	2.0	$ 140
Ketamine/placebo administration	2	1hr	3.0	$ 80
Clinical Ratings	15	1 hr	1.0	$ 150

* Payment is only for the days patients need to be present for the entire 24-hour period. Note: One inconvenience point equals $10. Payment for procedures was determined by multiplying the number of inconvenience points for each procedure by the number of such procedures. Maximum total per subject: $1,200 for completing the study.

Substudy 4:Procedure	Number	Pay per procedure (control)	Pay per procedure (patient)
Infusion	2	150	0
MRI (3T, 90-120 min)			
Sleep Deprivation	1	60	50
Ketamine	5	60	50
MRI (7T, 90-120 min):			
Sleep Deprivation	2	50	30
Probabilistic Task	3	0-30	0-30

Ketamine	5	50	30
MEG:			
Sleep Deprivation	2	60	50
Ketamine	5	60	50
Polysomnography:			
Sleep Deprivation	3	100	100
Ketamine	7	150	100
Sleep Deprivation	1	70	70
Cognitive Tasks [a]:			
Money Maze Task			
Day 1	1	70	70
Days 2 & 3	2	50	50
Deterministic Reversal Task	3	25	25
Object Categorization Task	3	10	10
Effort Task	3	30	30
Ultimatum Game	3	15	15
Probabilistic Task	3	0-30	0-30
Probabilistic learning	3	0-15	0-15
Other outpatient visits for ratings/bloodwork	Up to 5	25	25
Genome sequencing	1	25	25
Total Compensation Possible		**Up to 3635**	**Up to 2715**

[a] Healthy controls and patients may participate in up to 7 cognitive tasks on three occasions (Phase I and sessions 1 and 2 of Phase II). The Money Maze task, the Effort Task, the Probabilistic Task, and the Probabilistic Reward Task include the opportunity to earn additional money, the amounts of which are included in the total compensation possible for the tasks. Additional details can be found in **Appendix E**.

Although some scanning sessions are contingent upon response to ketamine, patient non-responders will be compensated for an identical number of scans as patient responders to remove any financial incentive to respond. Although some healthy subjects will be asked to participate in interim scans as resources allow, these subjects will only be compensated for the scanning sessions they are asked to participate in. If a subject withdraws from the study for any reason, they will be compensated for the portion of the study they completed.

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25. Attachments/Appendices

a. Substudy 2 Attachments

Appendix A. Drugs allowed & drugs not allowed as concomitant medications

Drug Class	**Episodic Use *(p.r.n.)***	***Chronic Use***	***Restrictions***
Analgesics	Y	N	Non-narcotic analgesics only.
Anorexics (sibuteramine)	N	N	
Antacids	Y	Y	
Antianginal Agents	N	N	
Antiarrhythmics	N	N	
Antiasthma Agents	Y	Y	Systematic corticosteroids are not allowed
Antibiotics	Y	N	
Anticholinergics	N	N	
Anticoagulants	N	N	
Anticonvulsants	N*	N*	* Exception is lithium and valproate.
Antidepressants	N	N	
Antidiarrheal Preparations	Y	N	
Antifungal Agents			
Systemic	N	N	
Topical	Y	Y	
Antihistamines Nonsedating Sedating	 Y N	 Y N	
Antihypertensives	Y	Y	
Anti-inflammatory Drugs	Y	Y[a]	Systematic corticosteroids are not allowed.
Antinauseants	Y	Y	
Antineoplastics	N	N	
Antiobesity	N	N	
Antipsychotics	N	N	
Antivirals	N	N	Except for treatment of HSV with agents without CNS activity e.g. acyclovir, ganciclovir, famciclovir,

			valacyclovir
Anxiolytics	N	N	
Cough/Cold Preparations	Y	N	Dextromethorphan preps N/N Guanfacine Y/Y Pseudoephedrine N/N
Diuretics	Y	Y[b]	
H2-Blockers/ PPI	Y	Y[b]	
Hormones	N	Y[b]	Only thyroid hormone replacement, oral contraceptives, and estrogen replacement therapy are allowed.
Hypoglycemic Agents	N	Y[b]	Only oral hypoglycemic agents are allowed.
Antihyperlipidimics	N	Y[b]	
Insulin	N	N	
Laxatives	Y	Y	
Muscle Relaxants	N	N	
Psychotropic drugs not otherwise specified (including herbal products)	N	N	No drugs with psychomotor effects or with anxiolytics, stimulant, antipsychotic, or sedative properties are allowed. Exception is lithium and valproate.
Sedatives/Hypnotics	N	N	
Analgesics	Y	N	Non-narcotic analgesics only.
Anorexics (sibuteramine)	N	N	
Antacids	Y	Y	
Antianginal Agents	N	N	
Antiarrhythmics	N	N	
Antiasthma Agents	Y	Y	Systematic corticosteroids are not allowed
Antibiotics	Y	N	
Anticholinergics	N	N	
Anticoagulants	N	N	
Anticonvulsants	N*	N*	* Exception is lithium and valproate.
Antidepressants	N	N	
Antidiarrheal Preparations	Y	N	
Antifungal Agents			
Systemic	N	N	
Topical	Y	Y	
Antihistamines Nonsedating	Y	Y	

Sedating	N	N	
Antihypertensives	Y	Y	
Anti-inflammatory Drugs	Y	Y[a]	Systematic corticosteroids are not allowed.
Antinauseants	Y	Y	
Antineoplastics	N	N	
Antiobesity	N	N	
Antipsychotics	N	N	
Antivirals	N	N	Except for treatment of HSV with agents without CNS activity e.g. acyclovir, ganciclovir, famciclovir, valacyclovir
Anxiolytics	N	N	
Cough/Cold Preparations	Y	N	Dextromethorphan preps N/N Guanfacine Y/Y Pseudoephedrine N/N
Diuretics	Y	Y[b]	
H2-Blockers/ PPI	Y	Y[b]	
Hormones	N	Y[b]	Only thyroid hormone replacement, oral contraceptives, and estrogen replacement therapy are allowed.
Hypoglycemic Agents	N	Y[b]	Only oral hypoglycemic agents are allowed.
Antihyperlipidimics	N	Y[b]	
Insulin	N	N	
Laxatives	Y	Y	
Muscle Relaxants	N	N	
Psychotropic drugs not otherwise specified (including herbal products)	N	N	No drugs with psychomotor effects or with anxiolytics, stimulant, antipsychotic, or sedative properties are allowed. Exception is lithium and valproate.
Sedatives/Hypnotics	N	N	

[a]If being taken before admission to the study; [b]If being taken for at least 3 months before study and dose has been stabilized; PPI = Proton Pump Inhibitors.

Appendix B. Rating scales

Primary Efficacy Measure

The MADRS (Montgomery and Asberg, 1979) is a 10-item instrument used for the evaluation of depressive symptoms in adults and for the assessment of any changes to those symptoms. The estimated time to administer this scale is 20 minutes. Inter-rater reliability of the scale is high and scores correlate significantly with those of the HAMD. Each of the 10 items are rated on a scale of 0 to 6, with differing descriptors for each item. These individual item scores are added together to form a total score, which can range between 0 and 60 points.

HDRS (Hamilton 1960) is a widely used observational rating measure of depression severity. The 17-item version of this scale (HAMD) will be administered to assess the severity of depression. The estimated time to administer this scale is 30 minutes. It assesses both the presence and severity of individual signs and symptoms characterizing depression without psychotic features.

*The Visual Analog Scale (*VAS; (Aitken 1969)*)* is a self-administered visual analog scale were patients rater their mood between "very sad) (on the left) and "very happy (on the right), with a median "normal" point. Raw data will be converted to a 0 to 100 rating scale, with 0, 50, and 100 denoting extreme depression, euthymia, and euphoria, respectively. Anger, anxiety, drowsiness, high, and sadness will be assessed by using clinician-rated visual analog scales. These scales are scored in millimeters from the left-hand side of a 100-mm line to a perpendicular mark made by the clinician at a point corresponding to the apparent magnitude of the feeling state reported and exhibited by the subject (range: 0, "not at all," to 100, "most ever").

The YMRS (Young et al 1978) consists of 11 items. Items 5, 6, 8, and 9 are rated on a scale from 0 (symptom not present) to 8 (symptom extremely severe). The remaining items are rated on a scale from 0 (symptom not present) to 4 (symptom extremely severe). Items 5, 6, 8, and 9 (irritability, speech, content and disruptive-aggressive behavior) are given twice the weight of the remaining 7 in order to compensate for the poor condition of severely ill patients. The YMRS total score ranges from 0 to 60 and is the primary efficacy parameter. The time to administer this scale is 15-30 minutes. The YMRS scale is obtained should hypomanic/manic symptoms develop during the study protocol.

Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962) Symptoms and behaviors that are characteristic of schizophrenia will be assessed by using the BPRS. Four key BPRS items will be used as an index of positive symptoms of schizophrenia based on previous reports that indicated their utility and validity (Bowers et al 1980; Kane et al 1988) and inclusion within the empirically derived thought disorder factor of the BPRS. These four key positive symptoms are conceptual disorganization, hallucinatory behavior, suspiciousness, and unusual thought content. Three key BPRS items, blunted affect, emotional withdrawal, and motor retardation are selected as a measure of negative symptoms of schizophrenia based on a report of their reliability and validity and their inclusion within the empirically derived withdrawal-retardation factor of the BPRS. [COMMENT: The negative symptoms were treated differently in the UP study – more items were included.]

The Clinician-Administered Dissociative States Scale (CADSS) (Bremner et al 1998) The CADSS is a clinician-administered measure of perceptual, behavioral, and attentional alterations occurring during dissociative experiences that has been validated in healthy subjects and patients with posttraumatic stress disorder. This scale involves a 19 self-report questions and eight observer ratings scored from 0 (not at all) to 4 (extremely). To characterize dissociative responses to ketamine better, CADSS items will be sorted into five subscales with apparent face validity and based on published scales that assessed dissociative states (Steinberg et al 1990): body perception, environmental perception, feelings of unreality, memory impairment, and time perception.

b. Substudy 4 Attachments

Appendix C. Table 1. Schedule of Events for All Subjects

Time		Procedure
Study Phase I (Day –28 to -1, no TSD study)	**Study Phase I (Day -35 to -1, TSD study)**	Informed consent, demographic information, psychiatric examination, physical examination, SCID (SCID-NP for healthy controls), FIGS, vital signs (blood pressure, pulse [supine and standing], temperature, weight, ECG, labs: CBC, electrolytes, thyroid function tests, fasting blood glucose, liver function tests, urinalysis, and toxicology** screening, pregnancy test and HIV. MADRS, HDRS, FHS, SDS, PDS. Drug-free period and patients tapering off medications
	Day -21	Drug-free period; Actiwatch monitoring begins (worn through to the final EEG)
	Day -19	Adaptation sleep EEG may occur this night
	Day -18	Adaptation sleep EEG may occur this night if not already collected
	Day -17	Adaptation sleep EEG may occur this night if not already collected
	Day -16	HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SIGH-ADS, MES, CGI-I, SSS (all ratings 10am, 10pm); peripheral neurochemicals at 10 am, 2pm, 6pm and 10pm *; baseline 3T MRI may occur; baseline MEG may occur; Baseline sleep EEG may occur; baseline 7T MRI may occur; urine pregnancy w/in 24 hr of each scan
	Day -15	HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SIGH-ADS, MES, CGI-I, SSS (all ratings 7am, 10am, 1pm, 4pm, 7pm, 10pm); peripheral neurochemicals at 10 am, 2pm, 6pm and 10pm *; baseline 3T MRI may occur if not already collected; baseline 7T MRI may occur this day if not already collected; baseline MEG may occur if not already collected; Baseline sleep EEG may occur if not already collected; urine pregnancy w/in 24 hr of each scan
	Day -14	HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SIGH-ADS, MES, CGI-I, SSS (all ratings 7am, 10am, 1pm, 4pm, 7pm, 10pm); peripheral neurochemicals at 10 am, 2pm, 6pm and 10pm *; baseline 3T MRI may occur if not already collected; baseline 7T MRI may occur this day if not already collected; baseline MEG may occur if not already collected; urine pregnancy w/in 24 hr of each scan;
	Day -13	HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SIGH-ADS, MES, CGI-I, SSS, (all ratings 1am, 4am, 7am, 10am, 1pm, 4pm, 7pm, 10pm); peripheral neurochemicals at 10 am, 2pm, 6pm and 10pm*; 7T MRI; MEG; Sleep EEG; urine pregnancy

Time		Procedure
		w/in 24 hr of each scan
	Day -12	HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SIGH-ADS, MES, CGI-I, SSS, peripheral neurochemicals at 10 am, 2pm, 6pm and 10pm *
	Day -10	HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SIGH-ADS, MES, CGI-I, SSS (all ratings 10am, 10pm); peripheral neurochemicals at 10 am, 2pm, 6pm and 10pm *
	Day -8	HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SIGH-ADS, MES, CGI-I, SSS (all ratings 10am, 10pm); peripheral neurochemicals at 10 am, 2pm, 6pm and 10pm *
	Day -6	HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SIGH-ADS, MES, CGI-I, SSS (all ratings 10am, 10pm); peripheral neurochemicals at 10 am, 2pm, 6pm and 10pm *
Day –14 to -1		Drug-free period
Day -7		Actiwatch monitoring begins (worn through the final EEG)
Day -4 to -1		Baseline 3T and 7T MRI scans, MEG, and adaptation sleep EEG if not participating in TSD study; urine pregnancy w/in 24hr of each scan
Day -1		Baseline Sleep EEG for Infusion 1; SSS (8AM, 12NN, 4PM, 8PM & 10PM)
Study Phase II (Day 0-28)		
Day 0 8:00 AM –80 minutes –60 minutes		--Insert IV line in antecubital region; begin oximetry, pulse and blood pressure monitoring. Urine pregnancy test. --HDRS, MADRS, BDI, 7-item VAS, POMS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A (baseline), SSI, MES, CGI-S, SSS (8AM), Q-LES-Q, MPQ, BPI-SF; PCL-C, peripheral neurochemicals*
0 minutes		Infusion of ketamine 0.5 mg/kg or saline solution over 40 minutes
+40 minutes		End of infusion. HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SIGH-ADS, MES, CGI-I, ketamine levels (ketamine and metabolites) and peripheral neurochemicals*
+80 minutes		HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; ketamine levels (ketamine and metabolites) and peripheral neurochemicals*
+120 minutes		HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SSS, SIGH-ADS, MES, CGI-I, ketamine levels (ketamine and metabolites) and peripheral neurochemicals*
+230 minutes		HDRS, MADRS, BDI, 7-item VAS, POMS, SHAPS, TEPS, ATQ, BPRS, YMRS, CADSS, HAM-A, SSI, SIGH-ADS, MES, SSS, Q-LES-Q, CGI-I, MPQ, BPI-SF, PCL-C, ketamine levels (ketamine and metabolites) and peripheral neurochemicals*
+260 minutes		Post-infusion1 MEG, possible 7T MRI

Time	Procedure
4 – 10 PM	Sleep EEG, SSS (4PM, 8PM, & 10PM)
Day 1 or 24 hours	HDRS, MADRS, BDI, 7-item VAS, POMS, SHAPS, TEPS, ATQ, BPRS, YMRS, CADSS, HAM-A, SSI, SSS, and peripheral neurochemicals*
Day 2 or 48 hours	HDRS, MADRS, BDI, 7-item VAS, POMS, ATQ, SHAPS, TEPS, BPRS, YMRS, HAM-A, SSI, Q-LES-Q, SIGH-ADS, MES, CGI-I, PCL-C, SSS, and peripheral neurochemicals*
Day 1 to 2	3T (whole brain structural, fMRI, MRS) and 7T MRI ((if not performed on day 0); urine pregnancy w/in 24hr of scan
Day 3 or 72 hours	HDRS, MADRS, BDI, 7-item VAS, POMS, SHAPS, TEPS, BPRS, YMRS, HAM-A, SSI, Q-LES-Q, SIGH-ADS, MES, CGI-I, MPQ, BPI-SF, PCL-C, SSS, and peripheral neurochemicals*
Day 6 or 7	In all patient responders and other subjects as resources allow, , interim Sleep EEG
Day 7	HDRS, MADRS, BDI, 7-item VAS, POMS, ATQ, SHAPS, TEPS, BPRS, YMRS, HAM-A, SSI, Q-LES-Q, CGI-I, PCL-C, SSS (8AM), and peripheral neurochemicals*
Day 10 or 11	In all patient responders and other subjects as resources allow, , MEG (task battery) and 3T and 7T MRI scans (whole brain structural image, fMRI, rest, spectroscopy); urine pregnancy w/in 24hr of scan; HDRS, MADRS, BDI, 7-item VAS, POMS, ATQ, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI, SIGH-ADS, MES, Q-LES-Q, CGI-I, MPQ, BPI-SF, PCL-C, SSS (8AM, 12NN, 4PM, 8PM, & 10PM), and peripheral neurochemicals*
Day 13	Baseline sleep EEG for infusion 2 ; SSS (8AM, 12NN, 4PM, 8PM & 10PM)
Day 14 8:00 AM –80 minutes –60 minutes	--Insert IV line in antecubital region; begin oximetry, pulse and blood pressure monitoring. Urine pregnancy test. --HDRS, MADRS, BDI, 7-item VAS, POMS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A (baseline), SSI,MES, CGI-S, SSS (8AM), Q-LES-Q, MPQ, BPI-SF; PCL-C, peripheral neurochemicals*
0 minutes	Infusion of ketamine 0.5 mg/kg or saline solution over 40 minutes
+40 minutes	End of infusion. HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SIGH-ADS, MES, CGI-I, ketamine levels (ketamine and metabolites) and peripheral neurochemicals*
+80 minutes	HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; ketamine levels (ketamine and metabolites) and peripheral neurochemicals*
+120 minutes	HDRS, MADRS, BDI, 7-item VAS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI; SSS, SIGH-ADS, MES, CGI-I, ketamine levels (ketamine and metabolites) and peripheral neurochemicals*

Time	Procedure
+230 minutes	HDRS, MADRS, BDI, 7-item VAS, POMS, ATQ, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI, SIGH-ADS, MES, SSS, Q-LES-Q, CGI-I, MPQ, BPI-SF, PCL-C, ketamine levels (ketamine and metabolites) and peripheral neurochemicals*
+260 minutes	Post-infusion1 MEG, possible 7T MRI
9:00 PM	Sleep EEG, SSS (4PM, 8PM, & 10PM)
Day 15 or 24 hours, 8:00 AM	HDRS, MADRS, BDI, 7-item VAS, POMS, ATQ, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI, SSS, and peripheral neurochemicals*
Day 16 or 48 hours, 8:00 AM	HDRS, MADRS, BDI, 7-item VAS, POMS, ATQ, SHAPS, TEPS, BPRS, YMRS, HAM-A, SSI, Q-LES-Q, SIGH-ADS, MES, CGI-I, PCL-C, SSS, and peripheral neurochemicals*
Day 15 or 16	3T (whole brain structural, fMRI, MRS) and 7T MRI ((if not performed on day 0); urine pregnancy w/in 24hr of scan
Day 17 72 hours, 8:00 AM	HDRS, MADRS, BDI, 7-item VAS, POMS, SHAPS, TEPS, BPRS, YMRS, HAM-A, SSI, Q-LES-Q, SIGH-ADS, MES, CGI-I, MPQ, BPI-SF, PCL-C, SSS, and peripheral neurochemicals*
Day 20 or 21	In all patient responders and other subjects as resources allow, interim Sleep EEG
Day 21	HDRS, MADRS, BDI, 7-item VAS, POMS, ATQ, SHAPS, TEPS, BPRS, YMRS, HAM-A, SSI, Q-LES-Q, CGI-I, PCL-C, SSS (8AM), and peripheral neurochemicals*
Day 24 or 25	In all patient responders and other subjects as resources allow, , MEG (task battery) and 3T and 7T MRI scans (whole brain structural image, fMRI, rest, spectroscopy); urine pregnancy w/in 24hr of scan; HDRS, MADRS, BDI, 7-item VAS, POMS, ATQ, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI, SIGH-ADS, MES, Q-LES-Q, CGI-I, MPQ, BPI-SF, PCL-C, SSS (8AM, 12NN, 4PM, 8PM, & 10PM), and peripheral neurochemicals*
Day 28. End of Study	HDRS, MADRS, BDI, 7-item VAS, POMS, SHAPS, TEPS, BPRS, YMRS, CADSS, HAM-A, SSI, SIGH-ADS, MES, Q-LES-Q, CGI, MPQ, BPI-SF, PCL-C, SSS (8AM, 12NN, 4PM, 8PM, & 10PM), and peripheral neurochemicals* Physical examination, vital signs, weight, ECG, labs: CBC, electrolytes, liver function tests, pregnancy test.

Abbreviations: BDI = Beck Depression Inventory; BPI-SF=Brief Pain Index-Short From; BPRS = Brief Psychiatric Rating Scale; CADSS= Clinician-Administered Dissociative States Scale**;** CBC=complete blood count; CGI=Clinical Global Improvement/Severity; DTI=diffusion tension imaging. ECG=electrocardiogram; EEG=electroencephalography; FIGS = Family Interview for Genetic Studies; HAM-A = Hamilton Anxiety Scale; HDRS = Hamilton Depression Rating Scale-17 item; MADRS = Montgomery-Asberg Depression Rating Scale; MES=Bech-Rafaelsen Melancholic Scale; MPQ=McGill Pain Questionnaire; MRI= Magnetic Resonance Imaging; *Neurochemicals for transcriptional profiling and to measure brain derived neurotrophic factor

(BDNF), other neurotrophic factors, metabolomics, proteonomics, cytokines and other neuroinflammatory measures; PCL-C=PTSD Checklist Civilian Version; PDS=Posttraumatic Diagnostic Scale; POMS=Profile of Mood States; Q-LES-Q-SF=Quality of Live Enjoyment and Satisfaction Questionnaire-Short Form; SDS=Sheehan Disability Scale; SSI = Scale for Suicide Ideation; SHAPS= Snaith-Hamilton Pleasure Scale; SIGH-ADS=Structured Interview Guide for the Hamilton Depression Rating Scale with Atypical Depression SSS=Stanford Sleepiness Scale; TEPS= Temporal Experience of Pleasure Scale; VAS = Visual analogue scale; YMRS=Young mania rating scale.**Urine toxicology will be obtained on the day prior to infusions and at study exit.

Appendix D. Putative biomarkers and targets of antidepressant response to ketamine: description of background, fMRI, and structural MRI procedures

Functional Magnetic Resonance Imaging (fMRI)

During the fMRI scanning sessions, subjects will be asked to perform two emotionally-salient tasks (i.e., the Emotional Evaluation task and the Dot-Probe task), and a cognitively demanding task (i.e. the N-back task. To evaluate the integrity of the frontolimbic circuitry in response to emotional stimuli as a correlate and putative predictor of ketamine's antidepressant effects, we will use a facial information processing task that requires both implicit and explicit processing of emotion. We will evaluate 1) the integrity of the amygdalar response to perceptual processing of a set of emotional human facial expressions; 2) the ability of neocortical systems involving the anterior cingulate prefrontal cortex to modulate this response during the cognitive appraisal of these same affective faces; and 3) whether the functional integrity of these processes is relevant as a predictor and target of ketamine's antidepressant effects.

This paradigm has been used to illustrate activity in the functional circuitry underlying fear regulation in autism, Williams syndrome, and in healthy volunteers carrying the short variant of the serotonin transporter (Pezawas, Meyer-Lindenberg et al. 2005), which correlates well with the social and emotional behavioral profiles of these populations. Furthermore, there is extensive evidence of abnormal activity in the frontolimbic circuitry including the ACC, the amygdala, and the ventrolateral prefrontal cortex during the processing of negative stimuli both in patients with MDD and BD (Sheline, Barch et al. 2001; Fu, Williams et al. 2004; Blumberg, Donegan et al. 2005; Blumberg, Fredericks et al. 2005), which is normalized by treatment with SSRIs and mood stabilizers.

Subjects participating in Substudy 4 will also be administered the N-back task, another well-described functional imaging paradigm (Callicott, Mattay et al. 1999; Callicott, Bertolino et al. 2000). The N-back is a working memory task that activates several nodes involved in the pathophysiology of MDD, including the dorsolateral prefrontal cortices and the dorsal anterior cingulate cortex (Owen, McMillan et al. 2005). Walsh and colleagues (Walsh, Williams et al. 2007) used the N-back task to study functional correlates of antidepressant response to fluoxetine in patients with MDD and found that patients who displayed higher engagement of the dorsal ACC as working memory load increased showed worse treatment response. Notably, patients with MDD display abnormal activity during the N-back task not only in working memory nodes that show load-dependent activity, such as the dorsal ACC and the DLPFC (Harvey, Fossati et al. 2005; Matsuo, Glahn et al. 2007), but also in the pgACC, which is not normally activated during this task (Rose, Simonotto et al. 2006). Similarly, abnormal ACC, as well as amygdala activity during working memory processes has been reported in patients with BD (Gruber, Tost et al. 2009) (Chang, Adleman et al. 2004).

Participants in this study also will be asked to perform the dot-probe task, which is designed to evaluate attentional and emotional processing biases. A dominant feature of mood disorders is associated with attention difficulties, and the most commonly described neuropsychological finding is associated with a negative emotional processing bias (Peckham et al., 2010). This task implicates parietal attention areas as well as emotion processing areas including amygdala (Carlson et al, 2009).

Our previous research with MEG found that MDD subjects who showed the least engagement of the pgACC with increased working memory load had the greatest symptomatic improvement within four hours of ketamine administration; moreover, we found that subjects who showed the lowest source coherence between the pgACC and the left amygdala were the most likely to respond to ketamine. Therefore, Substudy 4 will be a logical continuation of our previous research, as we will use those fMRI paradigms in a longitudinal, placebo-controlled design to investigate the neural underpinnings of rapid antidepressant response to ketamine.

Magnetoencephalography (MEG)

MEG allows for measures of neural responses that compliment fMRI in that MEG measures reflect presynaptic neural activity at millisecond temporal resolution while the spatial resolution is poor. MEG is a technique that allows for the non-invasive recording and analysis of the minute magnetic fields generated by neural activity.

The participant will either sit or lie supine in the shielded recording room with her head in the helmet. Brain magnetic fields will be recorded with the 275-channel OMEGA system. The 275 SQUID sensors are uniformly distributed, in a grid, over the inner surface of the helmet that covers the entire head with provisions for the eyes and ears. Visual and two-way audio communication with the participant will be maintained throughout the session. Head position within the magnetometer will be determined before and after the MEG session by digitizing the position of three indicator coils that are attached to the preauricular and the nasion fiducial points. The positions define the coordinate system for the signals and allow for post-hoc correction of head movement artifacts. Digital photographs of the fiducial points will also be taken to localize the same points on the participant's anatomical MRI scan.

During MEG scanning sessions, participants will perform the same battery of neuropsychological tasks. Obtaining complementary data using multiple imaging methods allows for a more in-depth evaluation of the functional response to the task and drug manipulations.

Resting state fMRI

The idea that the resting brain has a specifically activated network of regions was first proposed by Raichle and colleagues in 2001 (Raichle, MacLeod et al. 2001). Network analysis techniques were first applied to study this “default mode” in 2003 (Greicius, Krasnow et al. 2003). The default mode network (DMN), which has been validated with a variety of techniques, including resting state fMRI, and encompasses structures commonly found to be deactivated in cognitive tasks, including the posterior cingulate, ventral anterior cingulate, medial prefrontal cortex, and inferior parietal cortex. Notably, several of those regions have been implicated as predictors of antidepressant response or targets of conventional antidepressants in patients with depression (Anand, Li et al. 2005; Anand, Li et al. 2009). We plan to investigate whether pretreatment resting-state connectivity between nodes of the frontolimbic circuitry is a putative predictor of antidepressant response to ketamine. We also plan to evaluate how ketamine alters connectivity, and how these changes are related to treatment response.

Resting state MEG

As with resting state fMRI, we plan to evaluate whether pretreatment resting-state connectivity among nodes of the frontolimbic circuitry, as measured with MEG, predict antidepressant response to ketamine. Moreover, we plan to evaluate how ketamine alters connectivity, and how such changes are related to treatment response.

Structural Imaging and Magnetic Resonance Spectroscopy (MRS)

To investigate putative predictors of treatment response using imaging, several other methods can be applied besides fMRI, given the recent technical developments of the field (Bandettini 2009). For example, the development of more reliable voxel-based morphometry (VBM), diffusion tensor imaging (DTI), and magnetic resonance spectroscopy (MRS) algorithms have provided important insights into the genetics and pathophysiology of mood disorders (Pezawas, Meyer-Lindenberg et al. 2005; Hasler, van der Veen et al. 2007; Versace, Almeida et al. 2008).

Voxel-based morphometry (VBM)

Recent evidence from structural imaging studies suggests that pretreatment gray matter volume in structures belonging to the mood regulating circuitry, such as the hippocampus, amygdala and the ACC is a putative biomarker of treatment response to conventional antidepressants (Frodl, Jager et al. 2008; MacQueen, Yucel et al. 2008). Although we do not expect any volumetric changes after a single infusion of ketamine, we will investigate whether pretreatment hippocampal and ACC volumes predict antidepressant response to ketamine; several studies have demonstrated that subjects who initially present with larger gray matter volumes in those regions may be more likely to remit following treatment with antidepressants (reviewed in (MacQueen, Yucel et al. 2008)). Similar predictors of treatment response may also be evident in other regions, such as the insula and the right temporoparietal cortex (Chen, Ridler et al. 2007). We plan to acquire high resolution T1 weighted whole brain MRI scans to investigate whether pretreatment gray matter volume in the ACC and the hippocampus predicts antidepressant response to ketamine in patients with MDD or BD.

Diffusion Tensor Imaging (DTI)

DTI uses MRI imaging to measure the diffusion coefficient of water in each voxel in many different directions. When adjacent voxels have similar preferred directions of diffusion, the presence of a tract can be inferred. White matter tracts measured through DTI have been shown to closely follow known white matter tracts in post-mortem brains (Basser, Pajevic et al. 2000). DTI imaging also results in a measure of diffusion anisotropy (DA), a relative measure of the degree of directionality of diffusion. Late-life depression has been studied extensively using DTI and fractional anisotropy (FA), due to the hypothesized involvement of white matter lesions in this population (reviewed in (Lim and Helpern 2002)). Younger subjects with MDD have also been studied, with these subjects exhibiting significantly reduced FA in frontal white matter as compared to healthy controls (Li, Ma et al. 2007). Given these preliminary reports, as well as the extensive literature regarding white matter abnormalities in MDD and BD (reviewed in (Fields 2008)), and the increase in interest in network analyses of psychiatric disorders, the application of DTI to MDD and BD is a potentially fruitful line of research. In fact, recent research has

established the reliability of identifying limbic and paralimbic fiber tracts, which are of particular interest in both MDD and BD research (Malykhin, Concha et al. 2008; Versace, Almeida et al. 2008). Here, we propose to use DTI to investigate whether the integrity of white matter fibers between the pgACC and the amygdala predicts antidepressant response to ketamine.

Magnetic Resonance Spectroscopy (MRS)

^{1}H-MRS allows investigators to perform a noninvasive assay of specific, biologically-relevant biochemical markers of alterations in neuronal anatomy and function. Typically, short echo-time MRS studies measure myo-inositol (mI), a marker of glial cell viability, N-acetylaspartate (NAA), a measure of neuronal viability, creatine (Perry, Cramer et al.), choline, and Glx (a combined measure of glutamate and glutamine). Interestingly, several MRS studies suggest that patients with MDD and bipolar depression show abnormalities in Glx, glutamate and GABA levels in different brain areas (Dager, Friedman et al. 2004; Frye, Watzl et al. 2007; Hasler, van der Veen et al. 2007) and that these abnormalities might be reversed by successful antidepressant treatment, at least in MDD (Michael, Erfurth et al. 2003; Pfleiderer, Michael et al. 2003; Sanacora, Gueorguieva et al. 2004). Our preliminary MRS data indicate that prefrontal cortex amino acid neurotransmitters—and in particular the ratio of Glx/Glutamate, a surrogate measure for glutaminergic capacity—might provide other putative biomarkers of antidepressant response to ketamine in patients with MDD. Interpretation of those findings is limited by the small sample size (N=13) and by the lack of a placebo group. Here we propose to replicate those findings using a larger-sample and a placebo-controlled design

Magnetization Transfer Imaging

During conventional MRI imaging, the MRI signal is produced by protons undergoing relatively unrestricted diffusion. Tissue, however, also contains abundant protons which are heavily restricted or bound to large macromolecules. These bound protons exhibit extremely short T_2* relaxation times, and are thus difficult to image directly. However, these restricted protons may transfer magnetization to free protons. If an MRI image is acquired with an off-resonant preparation pulse, the bound proton pool can be saturated, eliminating transfer of magnetization from the bound to the free pool. The acquisition of two images, one with and one without the off-resonant pulse, thus enables the calculation of a map of this magnetization transfer (MT), usually expressed as magnetization transfer ratio (MTR) or magnetization transfer coefficient (MTC). Focal decreases in MT are associated with demyelination and axonal loss in both post mortem and in vivo studies (van Buchem and Tofts 2000; Arnold 2007) , thus MT imaging is frequently used in the study demyelinating diseases such as multiple sclerosis (MS). The MTR has also been shown to correlate with N-acetyle-aspartate (NAA) in *in vivo* spectroscopy studies (Pendlebury, Blamire et al. 1999). MT has been used to study psychiatric disorders such as late-life MDD (Kumar, Gupta et al. 2004) and bipolar disorder (Bruno, Barker et al. 2004; Bruno, Papadopoulou et al. 2006). Subjects with late-life depression were found to have significantly reduced MTR in the corpus callosum, right caudate and putamen, and occipital white matter (Kumar, Gupta et al. 2004). Bipolar subjects were found to have lower MTR as compared to healthy controls in the right subgenual cingulate cortex and sub gyral white matter (Bruno, Barker et al. 2004). Also in bipolar subjects, reductions in IQ at the time of imaging as

compared to premorbid IQ were associated with decreases in MTR in the left superior temporal gyrus, uncus, and parahippocampal gyrus (Bruno, Papadopoulou et al. 2006). Furthermore, there is justification for acquiring MTR measurements in mood disorders, given that post-mortem studies have shown abnormalities of myelination in both unipolar and bipolar depressive disorders (Regenold, Phatak et al. 2007). We are not aware of any studies of response prediction with MTR in MDD. We propose to acquire whole brain maps of MTR and assess whether MTR predicts antidepressant response to ketamine.

Appendix E. Emotional and Cognitive Tasks

The dominant behavioral deficit observed in subjects with depression is a mood congruent processing bias, which has been found to alter performance on various tasks (i.e. Affective Shifting). We believe that the magnitude of impairments observed in memory and attention tasks may be modulated by the emotional content in the task stimuli. To evaluate this, inherent in the designs of the working memory and selective attention tasks described, we will utilize pictures of faces that have a happy, fearful, sad or neutral expression. This will allow us to evaluate systematically the role of emotional content on performance and on the functional brain response within the context of working memory and selective attention tasks. Further, the role of the cholinergic system in the emotional and cognitive processing deficits observed in depressed subjects can be dissected using this approach.

Evaluation of Emotion in Faces

Subjects suffering from depression exhibit emotional processing deficits, including the evaluation of emotion in faces (Gur, Erwin et al. 1992; Rubinow and Post 1992; Drevets, Gautier et al. 2001; Thomas, Drevets et al. 2001). This study is designed to identify the neural systems recruited to perform a task that requires the identification of an emotion in another person and to determine the role of the cholinergic system in this process. Subjects will be scanned as they are shown pictures of faces with happy, sad, fearful or neutral expressions that are presented in a randomized order. Pictures will be presented for 750ms second, followed by a 3 second inter-stimulus interval. The imaging studies may include several runs, with each run including two blocks of each stimulus condition, and each block lasting for 30 seconds. In separate blocks, subjects will be asked to make two different types of responses:

1. **Emotion Matching**- Subjects are instructed to evaluate the emotion expressed in the picture and press hand held response buttons to indicate if the emotion is positive: positive (happy or neutral) or negative (to include fearful or sad).

2. **Gender Matching**- In this condition, subjects are asked to determine the gender of the individual. Using the same response apparatus as described above, subjects will indicate whether the picture is of a male or female.

Immediately following the scan session, subjects will be shown a picture of each face that was used previously during the study and will be asked to evaluate (1) the emotion expressed in the face based on 4 options: happy, sad, fearful or neutral, and (2) the intensity of the emotional expression based on a four point analog scale. The timing proposed here is approximate, and may be modified as needed for the imaging studies. Upside-down faces will be used as a control stimulus to eliminate/minimize emotional processing during the gender matching task (while still utilizing recognizable faces). Under one control condition, subjects will be instructed to evaluate the emotion, and in another condition they will be instructed to determine gender.

Cued Attention Task- Dot-Probe Task

This task is designed to determine the influence of the emotional content of a spatial cue on a simple attention task. One of two types of dot-probe task designs will be used. In the first design, while looking at a fixation cross, a picture of a face will be presented either to the left or right of the fixation cross to selectively cue the subject's attention to a spatial location. Following termination of the cue, a probe stimulus (a dot) will be presented; again either to the left or right of the fixation cross, and the subject is instructed to press the left or right button to indicate where the dot-probe was presented. The trial types will include: valid cues (face and probe in same spatial location), invalid cues (face and probe in different locations) and neutral cues (pictures of faces expressing the same emotion in both locations). The emotional content of the face will be manipulated to include sad, happy, angry and neutral expressions. The probability of the cue appearing on the left or right will be the same, and the probability of each emotional expression will be equal. Phase-scrambled faces also will be used in valid, invalid and neutral cue trials. The frequency of valid cues will be 60%, the frequency of invalid cues will be 20% and the frequency of neutral cues will be 20%. The cue will be blanked, followed by a 50 ms delay, and then followed by a dot-probe stimulus. Reaction time and accuracy will be recorded for each subject. The timing proposed here is approximate, and may be modified as needed for the imaging studies. In the second design, faces will be presented to both the left and right of the initial fixation cross. Following termination of the cue, a probe stimulus (a dot) will be presented; again either to the left or right of the fixation cross, and the subject is instructed to press the left or right button to indicate where the dot-probe was presented. The pairs of faces will be presented in blocks consisting of neutral-neutral, neutral-happy, neutral-sad, neutral-angry, and neutral-fearful pairs. Blocks will be either "neutral-match" (NM), where the dot appears behind the neutral face, or "emotion match" (EM), where the dot appears behind the emotional face. The timing proposed here is approximate, and may be modified as needed for the imaging studies.

N-Back task

To further investigate the neural effects of ketamine compared to placebo, particularly as it pertains to treatment response, participants will be also administered the n-back task in each MEG session. The n-back is a working memory task which elicits activity in several brain areas which are relevant for depression pathophysiology, including the left dorsolateral prefrontal cortex and the dorsal anterior cingulate. The n-back task has been recently used by Walsh and colleagues (Walsh et al., 2007) to investigate predictors and correlate of treatment response to fluoxetine in 20 patients with major depressive disorder. Patients were administered this task three times during the course of the clinical trials. Baseline activity in the anterior cingulate cortex was a predictor of treatment response to fluoxetine.

For all of the above tasks (emotional evaluation, dot probe, and n-back) we will use analysis of variance (ANOVA) to identify brain regions that demonstrate a main effect of drug (Ketamine vs. placebo) and a drug x time interaction (attributable to the direct effects of Ketamine). This method, along with the counterbalanced order of ketamine and placebo administration will allow investigating specifically the neural changes induced by ketamine, minimizing the contribution from possible practice effects.

Somatosensory Stimulation task (MEG only)

In this task, participants are passively presented with tactile stimuli to the index finger. Participants are asked to remain attentive with their eyes open, but do not make any responses. The stimulation is controlled by a pneumatic system that uses pressurized air (2 psi) to briefly move a diaphragm, making contact with the skin of the finger for approximately 25 ms. The stimulus feels like a brief, soft touch of the finger. The total number of stimuli presented is 500, with an average interval between stimuli of 500 ms. The scan time is approximately 4 min. The scan will be repeated to stimulate both the left and right index fingers.

Cortical Excitability Task (MEG only)

The task involves the mechanical stimulation of the index finger to induce cortical excitability. Mechanical stimulation will be induced by a pneumatic stimulating device that uses a brief burst of air (30 psi) to displace a plastic membrane resting against the skin. MEG source analyses for the stimulus-evoked responses and spontaneous activity during rest will be employed using the same algorithmic steps as described previously (Rutter et al., 2009; Genovese et al., 2002). Time-frequency analyses will first be conducted on the sensor data to examine the temporal and spectral characteristics of the stimulus-evoked response. Stimulus epochs (-100 to 300 ms, locked to stimulus onset) will then be time-domain averaged before applying a Stockwell transform to the data (Stockwell et al., 1996). This task will occur during the MEG and will take approximately 10 minutes.

Non-brain imaging cognitive tasks

Background: Clinical evidence suggests that ketamine, a nonselective N-methyl-D-aspartate (NMDA) receptor antagonist, has therapeutic antidepressant effects within hours in people suffering from major depressive disorder (MDD) and bipolar disorder (BD). However, the primary method of assessing improvement thus far has been the use of questionnaires that assess mood primarily without focusing on other aspects of well-being that could be ameliorated. In a preliminary analysis, we found a dramatic decrease in levels of anhedonia, as measured by the Snaith-Hamilton Pleasure Scale (SHAPS, (Snaith, Hamilton et al. 1995)), within 40 minutes of ketamine infusion. This improvement was still significantly better than placebo to 3 days post-infusion. Moreover, we also explored specific cognitive questions within the Montgomery-Asberg Depression Rating Scale (MADRS), our primary scale of mood improvement in this study, and found that self-reported levels of concentration 40 minutes post-ketamine infusion improved significantly compared to placebo and this improvement was again maintained to 3 days post-infusion. Here we are seeking to explore these effects via cognitive tasks that assess aspects of reward guided behavior and memory performance.

We will be administering the Wechsler Abbreviated Scale of Intelligence (WASI, (Wechsler 1999)) to better match patients and healthy controls participating in certain cognitive tasks. The WASI provides a reliable measure of intelligence quotient (IQ). It is a pen-and-paper test that can be administered in less than 30 minutes, and comes in both Spanish and English. The test measures IQ by assessing vocabulary, problem solving, visual processing, and spatial perception and reasoning. It has been shown that IQ can affect cognitive task performance (Brittain, La Marche et al. 1991). We will utilize this IQ test to minimize group differences.

Money Maze

Participants will plan a sequence of button presses to move about a reinforced maze, each move will either win or lose a fixed sum of money. The goal is to assess an individual's planning ability in the context of monetary rewards and losses. Here, participants will earn a base rate for training ($20) and task completion ($10) and can win a further $40 during the test phase of the task. Training will only take place at the baseline time point and will last 1 hour. The test will last 30 minutes. Thus, on Day 1, participants can earn between $30 and $70 for 90 minutes. On days 2 and 3 participants can earn between $10 and $50 for 30 minutes.

Deterministic Reversal Task

Participants must decide whether a highlighted stimulus (picture of a neutral face or a scene) is a rewarding or a losing stimulus and must if necessary update their predictions if the associations between the picture and the outcome change. Participants will receive information as to what the association of the highlighted picture is but not whether their button press was correct or incorrect. This task assesses the ability of an individual to update their predictions in the context of rewards and losses. Participants will be trained on the task for 2 minutes and thereafter will perform the task for 25 minutes. They will be compensated $25 and will perform the task on three occasions.

Object Categorization

Participants will view real world objects and scrambled versions of these objects repeated three times to engage repetition priming, a psychological construct that probes implicit memory processes. Participants will simply classify if an object is familiar or unfamiliar. The task will last approximately 10 minutes and participants will be compensated $10. Again it will perform the task on all three occasions.

Effort Task

Participants will be required to choose between easy and hard trials to assess their willingness to expend energy to win money available. They will have to perform button or mouse presses in order to reach a desired goal within a specific time frame. The task will last 20 minutes following a brief training period. Participants will be paid a base rate of $10 and can win a further $20 on top of this due to their task performance.

Ultimatum Game

Participants will be required to choose between accepting or rejecting offers made by fictional players. It has recently been shown that individuals with depression accept more unfair offers that healthy controls. The goal of the task is to replicate this finding and see if ketamine reinstates normative behavior. The task will take 10 minutes and participants will be paid a sum of money from three randomly selected trials (out of 40), with a maximum of $15.

Probabilistic task

Participants will be required to select between sets of two random stimuli. Stimulus sets will vary with which the probability of reward is assigned to each stimulus. For example, one stimulus might result in a reward 80% of the time and no reward for the rest of the time; the other stimulus in the set will have the opposite probability distribution. The ability to select the best stimulus between two in an uncertain environment requires processes of value comparison, value tracking and memory. The task will take approximately 20 minutes and individuals can gain up to $30 for task performance.

Probabilistic reward

Participants will be required to select between two probabilistic monetary gambles. Stimulus sets will vary with which the probability of reward. For example, participants may be required to decide between a guaranteed outcome of $0.50 and a 50% gamble of receiving $1. The task will take 15 minutes and individuals can win $15.

Appendix F. Pathophysiology of depression: cross-sectional imaging findings

Functional Brain Imaging Findings

Functional neuroimaging studies have implicated multiple brain regions in the prefrontal cortex, mesiotemporal cortex, and striatum in the pathophysiology of mood disorders, and the abnormalities observed in these regions are thought to contribute to the behavioral and cognitive symptoms that characterize depression. The ACC is known to be important for attentional mechanisms and affect, and is thought to be important for the integration of attention and emotion (Devinsky, Morrell et al. 1995; Mayberg 1997; Lane, Reiman et al. 1998; Thayer and Lane 2000). In subjects with MDD, the subgenual portion of the ACC has volumetric reductions, and (partial volume corrected) increases in glucose metabolism (Drevets, Price et al. 1997; Drevets 1999; Hirayasu, Shenton et al. 1999). Reports regarding the metabolic changes associated with the pgACC are more variable, although most studies report increased metabolism in depressed subjects that normalize with treatment (Drevets 1999; Drevets 2000; Davidson, Pizzagalli et al. 2002). The pregenual and subgenual ACC are hypothesized to comprise the affective subdivision of the ACC, and are activated during the induction of sadness and anxiety in healthy subjects (for review see (Drevets and Raichle 1998)). In contrast, the dorsal ACC is more generally activated by tasks that demand attention (Davidson, Pizzagalli et al. 2002). The dorsal ACC has reduced metabolism in patients with mood disorders that also normalizes with successful treatment (Bench, Frackowiak et al. 1995; Mayberg, Liotti et al. 1999).

Other prefrontal cortical regions, including dorsolateral and dorsomedial areas, have decreased activity, while the ventrolateral and orbital frontal areas have increased activity in depressive illnesses and these changes normalize with successful therapy (Baxter, Schwartz et al. 1989; Bench, Friston et al. 1992; Dolan, Bench et al. 1993; Biver, Goldman et al. 1994; Drevets 1999). The dorsolateral prefrontal cortex, in particular, has been implicated in working memory. The dorsomedial PFC shows increased blood flow during tasks that require emotional responses or emotional evaluations (Dolan, Bench et al. 1993; Reiman, Lane et al. 1997). This same region shows increases in cerebral blood flow that correlate with anxiety ratings and heart rate during anxious anticipation of an electric shock (Drevets 2001), suggesting that this region is important in the attenuation of emotional expression. In rats, lesions of the dorsomedial PFC results in exaggerated heart rate responses to fear-conditioned stimuli, and stimulation of this region attenuates defensive behavior and cardiovascular responses evoked by amygdala stimulation (Frysztak and Neafsey 1994). Within the striatum, the nucleus accumbens has been hypothesized to play a role in mood disorders because this region is implicated in motivation and reinforcement (Fibiger, Phillips et al. 1992). In depressed humans the ventral striatal region encompassing the accumbens area shows both elevated glucose metabolism in vivo and reduced gray matter volume postmortem (reviewed in (Drevets, Gadde et al. in press)).

Two other limbic structures implicated in depressive illnesses are the hippocampus and the amygdala, which are important in memory and emotional processing tasks. Hippocampal volumes are significantly reduced in some studies of MDD (Sheline, Wang et al. 1996; Sheline, Sanghavi et al. 1999; Bremner, Narayan et al. 2000; von Gunten, Fox et al. 2000), and abnormalities in

glucose metabolism also have been reported (Saxena, Brody et al. 2001). In the amygdala, rCBF and rCMRglc are elevated in at least some clinical subtypes including depressed individuals with a family history of MDD and patients with BD (Drevets, Videen et al. 1992). Recent reports indicate that amygdala volumes also are abnormal in subjects with depressive illness, although research findings are conflicting; some studies report increases (Bremner, Narayan et al. 2000; Strakowski, DelBello et al. 2000) and others report decreases with respect to controls (reviewed in (Drevets, Price et al. 2002)). The amygdala is crucial for processing emotional information, particularly to stimuli with negative valence, and for the direction of attention to emotionally salient stimuli (LeDoux 2000).

Resting state fMRI

Despite hypotheses that abnormalities in the default mode network may be symptomatic, or even antecedents of psychiatric disorders, relatively few studies have explored resting state connectivity in depressed subjects. For example, one study found increased connectivity between the subgenual cingulate and thalamus, and a positive correlation between this measured connectivity and length of the current major depressive episode (Greicius, Flores et al. 2007). Only five minutes of resting state data were acquired for this study; however, cardiac and respiratory effects were not controlled for, and 20 of the 28 subjects were on psychotropic medications. Another recent study showed that patients with MDD displayed a failure to normally down-regulate activity in regions targeted by antidepressant treatments, such as the subgenual prefrontal cortex, the amygdala and the hippocampus (Sheline, Barch et al. 2009). Other studies of connectivity in the resting state in depression have not focused on a data-driven default mode, and have instead investigated connectivity between *a priori* ROIs during rest. For example, one study found decreased connectivity between the supragenual cingulate and subcortical regions at rest as compared to controls, and significant increases in connectivity with sertraline treatment (Anand, Li et al. 2005). In both of these studies, only five minutes of resting state data were acquired, and cardiac and respiratory effects were not controlled for. No studies have evaluated resting state functional connectivity in drug-free patients with BD; preliminary evidence with drug-free BD subjects in various mood states suggests that dysfunction of the DMN might be implicated in the pathophysiology of that disorder as well (Anand, Li et al. 2009).

Further studies using a larger number of subjects, carefully controlling for the physiological effects of respiration and cardiac activity, are necessary to fully elucidate resting state abnormalities in MDD and BD, and how these abnormalities are altered by treatment.

Structural Brain Imaging Findings

Neuromorphometric abnormalities occurring in mood disorders have been reported in the PFC, mesiotemporal lobe, and basal ganglia. Both MRI-based and post-mortem morphometric assessments have revealed reductions in dorsal anterolateral/dorsomedial PFC, orbital cortex, subgenual ACC and hippocampus in MDD and BD (Drevets, Price et al. 1997). The time course, pathogenesis, and behavioral implications of such changes remain unclear, partly because limitations in the sensitivity and specificity of such measures have hindered the ability to investigate such issues. Moreover, the literature disagrees regarding the existence of volumetric abnormalities in the mesiotemporal lobe and basal ganglia. In the basal ganglia, an imaging

(Husain, McDonald et al. 1991) and a post mortem study (Baumann, Danos et al. 1999) found smaller putamen sizes in MDD, while another study reported smaller caudate volumes in MDD (Krishnan, McDonald et al. 1992), which could not be confirmed by other studies (Krishnan, McDonald et al. 1992). In the mesiotemporal cortex, hippocampal volume was reportedly reduced in some studies (Sheline, Wang et al. 1996), but not in others (Ashtari, Greenwald et al. 1999) in MDD relative to healthy control subjects. Moreover, the amygdala volume was reported to be enlarged in MDD in one study (Tebartz van Elst, Woermann et al. 2000), but to be decreased in another (Sheline, Sanghavi et al. 1999). Regarding BD, similar conflicting results have been yielded by studies that investigated the amygdala (Pearlson, Barta et al. 1997; Altshuler, Bartzokis et al. 2000), the basal ganglia (Strakowski, Adler et al. 2002; Hwang, Lyoo et al. 2006), and the hippocampus (Swayze, Andreasen et al. 1992; Strasser, Lilyestrom et al. 2005). The contrasting findings of some volumetric abnormalities in MDD and BD may at least partly reflect heterogeneity of medication status at the time of the scan, limited sample size, and limitations in the reliability of volumetric measures in imaging studies.

Appendix G. Drugs allowed & drugs not allowed as concomitant medications

Drug Class	Episodic Use *(p.r.n.)*	*Chronic Use*	*Restrictions*
Analgesics	Y	N	Non-narcotic analgesics only.
Anorexics (sibuteramine)	N	N	
Antacids	Y	Y	
Antianginal Agents	N	N	
Antiarrhythmics	N	N	
Antiasthma Agents	Y	Y	Systematic corticosteroids are not allowed
Antibiotics	Y	N	
Anticholinergics	N	N	
Anticoagulants	N	N	
Anticonvulsants	N	N	
Antidepressants	N	N	
Antidiarrheal Preparations	Y	N	
Antifungal Agents			
Systemic	N	N	
Topical	Y	Y	
Antihistamines Nonsedating Sedating	 Y N	 Y N	
Antihyperlipidimics	N	Y[b]	
Anti-inflammatory Drugs	Y	Y[a]	Systematic corticosteroids are not allowed.
Antinauseants	Y	Y	
Antineoplastics	N	N	
Antiobesity	N	N	
Antipsychotics	N	N	
Antivirals	N	N	Except for treatment of HSV with agents without CNS activity e.g. acyclovir, ganciclovir, famciclovir, valacyclovir
Anxiolytics	N	N	
Cough/Cold Preparations	Y	N	Dextromethorphan preps N/N Guanfacine Y/Y Pseudoephedrine N/N
Diuretics	Y	Y[b]	
H2-Blockers/ PPI	Y	Y[b]	
Hormones	N	Y[b]	Only thyroid hormone replacement, oral contraceptives, and estrogen replacement

			therapy are allowed.
Insulin	N	N	
Laxatives	Y	Y	
Lithium	N	N	
Muscle Relaxants	N	N	
Psychotropic drugs not otherwise specified (including herbal products)	N	N	No drugs with psychomotor effects or with anxiolytics, stimulant, antipsychotic, or sedative properties are allowed.
Sedatives/Hypnotics	N	N	

[a] If being taken before admission to Substudy 4; bIf being taken for at least 1 month before Substudy 4 and dose has been stabilized; PPI = Proton Pump Inhibitors

Appendix H. Imaging Data analysis

Primary Data analysis

Morphometric measurements will be derived from structural MRI images using both manual and automated procedures. We anticipate using voxel-based morphometry and SPM (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK) or similar software to obtain gray matter volume estimates across the whole brain for the participants in Substudy 4. SPM or Analysis of Functional Neuroimages (AFNI) software will be used to analyze fMRI datasets. Following correction for patient movement, individual subject and group analysis will be performed. For group analysis, the data will be normalized to common Talairach coordinates; statistical analyses will be performed using SPM within the framework of a general linear model.

DTI will be reconstructed into both fractional anisotropy images and fiber tracking maps using software created by the NICHD diffusion tensor processing center, or similar packages. Fractional anisotropy images will be compared in a voxel-wise manner across groups, using an image analysis package such as SPM (Functional Imaging Laboratory, University College London, London UK). We will also explore techniques for comparing fiber tract volumes and other attributes between groups.

Resting state functional MRI scans will be analyzed using an independent components analysis to identify primary and secondary default mode networks. These will be compared between groups in a voxel-wise manner.

Magnetization transfer coefficient maps will be calculated from magnetization transfer weighted images, and will be corrected for B1 field in homogeneities before voxel-wise analysis.

MRS data will be analyzed using commercial (e.g., LC model) and in-house written software to obtain concentrations of individual metabolites (normalized to creatine levels) in our voxel of interest.

Test/Retest Reliability of MRS Measures

The ability to interpret MRS changes associated with treatment depends upon the test-retest stability of regional neurophysiology across time.

Test/Retest reliability for NAA

The stability of NAA in the cingulate gyrus, left and right dorsolateral prefrontal cortex, white matter (WM), and a large ROI containing all the good quality voxels in the slice (WHOLE) was assessed in 10 healthy volunteers who underwent two scanning sessions on a GE 3T-Signa scanner. The mean interval between the two scanning sessions was 60 days. For each ROI the scan-to-scan reproducibility was assessed using the coefficient of variation in percent (CV) and the unbiased intra-class correlation coefficient (ICC(U)). A repeated measures ANOVA was also performed with ROI (5 levels) and scanning session (2 levels) as repeated measures. If reproducibility is adequate, no significance of scanning session is expected. The CVs and ICC(U)s are for various metabolites are displayed in the table below.

Table a – Test-retest reliability data for NAA

		ROI Av raw			
		Test	Retest	CV [%]	ICC(U)
WHOLE	Cho	4.127	4.241	6.4%	0.623
	Cre	3.492	3.566	6.8%	0.158
	Naa	8.037	8.182	6.1%	0.372
	Cho/Cre	1.181	1.191	4.8%	0.645
	Naa/Cre	2.305	2.298	3.2%	0.664
	Naa/Cho	1.971	1.941	3.0%	0.924
WM	Cho	4.685	4.784	6.8%	0.680
	Cre	3.619	3.718	6.9%	0.219
	Naa	9.174	9.339	5.9%	0.115
	Cho/Cre	1.291	1.291	4.5%	0.773
	Naa/Cre	2.542	2.527	3.9%	0.737
	Naa/Cho	1.991	1.974	3.5%	0.912
CING	Cho	5.587	5.835	8.5%	0.554
	Cre	4.064	4.218	7.9%	0.248
	Naa	8.574	8.697	7.0%	0.428
	Cho/Cre	1.376	1.383	7.9%	0.267
	Naa/Cre	2.116	2.062	4.4%	0.403
	Naa/Cho	1.558	1.504	6.4%	0.732
LDLPFC	Cho	4.491	4.550	10.3%	0.932
	Cre	3.390	3.388	10.5%	0.337
	Naa	3.137	3.190	10.8%	-0.157
	Cho/Cre	1.082	1.067	7.6%	0.475
	Naa/Cre	2.229	2.225	8.8%	-0.098
	Naa/Cho	2.077	2.106	5.4%	0.757
RDLPFC	Cho	3.700	3.815	7.8%	0.369
	Cre	3.465	3.569	11.6%	-0.257
	Naa	7.872	8.013	7.3%	0.329
	Cho/Cre	1.071	1.073	7.9%	0.021
	Naa/Cre	2.281	2.256	6.4%	0.351
	Naa/Cho	2.148	2.107	4.5%	0.789

CVs were usually less than 10%. Scanning session and the interaction of scan x ROI were not significant for all of the measures tested, confirming good overall reproducibility

The CVs for the absolute metabolite values tend to be higher than for the NAA/Cre ratios. This is expected since the ratios are independent of several factors that can make determination of absolute metabolite values more prone to error (e.g. amount of CSF in the ROI). Among the ratios, the best CVs and ICCs were found for NAA/Cho (ICCs were consistently above 0.7). NAA/Cre (the main ratio that is usually reported) and Cho/Cre appeared to have low ICCs (below 0.50) for all gray matter ROIs. The ICCs reflected similar trends as already described for the CVs.

Test-retest reliability for glutamate

The long-term stability across time for glutamate in the medial prefrontal cortex was assessed scanning six healthy volunteers in two different scanning sessions with an interval of 7-14 days between the sessions. Data about short-term reproducibility for glutamate were also acquired: 6 subjects were scanned twice in the same scanning session. All the measures were obtained with a GE 3-T Signa magnet. The CVs for glutamate were less than 10%.

Table b-Long-term reproducibility data for Glutamate

Variable	Mean value scan 1	Mean value scan 2	ICC	ICC(U)	F	p	CV%
Auto Glu/Cre	1.37	1.34	0.492	0.324	2.942	0.110	6.82
Auto NAA/Cre	1.56	1.58	0.845	0.776	11.934	0.004	3.58
Manual Glu/Cre	1.36	1.43	0.576	0.425	3.718	0.070	9.02

Table c-Short-term reproducibility data for Glutamate

Variable	Mean value scan 1	Mean value scan 2	ICC	ICC(U)	F	p	CV%
Auto Glu/Cre	1.41	1.41	0.802	0.717	9.123	0.008	3.26
Auto NAA/Cre	1.56	1.59	0.961	0.943	51.459	0.00007	2.02
Manual Glu/Cre	1.40	1.47	0.473	0.302	2.799	0.121	8.13

Appendix I. Rating Scales

The MADRS (Montgomery and Asberg 1979): is a 10-item instrument used to evaluate depressive symptoms in adults and to assess any changes to those symptoms. The estimated time to administer this scale is 20 minutes. Inter-rater reliability of the scale is high and scores correlate significantly with those of the HAMD. Each of the 10 items are rated on a scale of 0 to 6, with differing descriptors for each item. These individual item scores are added together to form a total score, which can range between 0 and 60 points.

HDRS (Hamilton 1960): is a widely used observational rating measure of depression severity. The 17-item version of this scale (HAM-D) will be administered to assess the severity of depression. The estimated time to administer this scale is 30 minutes. It assesses both the presence and severity of individual signs and symptoms characterizing depression without psychotic features.

The Hamilton Psychiatric Rating Scale for Anxiety (HAM-A)(Hamilton 1959): The HAM-A is a widely used observational rating measure of anxiety severity. The scale consists of 14 items. Each item is rated on a scale of 0 to 4. This scale will be administered to assess the severity of anxiety and its improvement during the course of therapy. The HAM-A total score is the sum of the 14 items and the score ranges from 0 to 56.

The Visual Analog Scale (VAS; (Aitken 1969)): *The 7-item Visual Analog Scale* (VAS) is a self-administered visual analog scale were patients rater their mood between 0 and 10 on seven mood domains: happy/euphoric, restless, sad, anxious, irritated/angry, drowsy, and alert. A score of 0 is described as “none”; 1-3 is “mild”, 4-6 is “moderate”, 7-9 is “marked” and 10 is “extreme.”

The YMRS (Young, Biggs et al. 1978) consists of 11 items. Items 5, 6, 8, and 9 are rated on a scale from 0 (symptom not present) to 8 (symptom extremely severe). The remaining items are rated on a scale from 0 (symptom not present) to 4 (symptom extremely severe). Items 5, 6, 8, and 9 (irritability, speech, content and disruptive-aggressive behavior) are given twice the weight of the remaining 7 in order to compensate for the poor condition of severely ill patients. The YMRS total score ranges from 0 to 60 and is the primary efficacy parameter. The time to administer this scale is 15-30 minutes. The YMRS scale is obtained to monitor the development of hypomanic/manic symptoms during the study.

Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962) Symptoms and behaviors that are characteristic of schizophrenia will be assessed by using the BPRS. Four key BPRS items will be used as an index of positive symptoms of schizophrenia based on previous reports that indicated their utility and validity (Bowers, Heninger et al. 1980; Kane, Honigfeld et al. 1988) and inclusion within the empirically derived thought disorder factor of the BPRS. These four key positive symptoms are conceptual disorganization, hallucinatory behavior, suspiciousness, and unusual thought content. Three key BPRS items, blunted affect, emotional withdrawal, and motor retardation are selected as a measure of negative symptoms of schizophrenia based on a report of their reliability and validity and their inclusion within the empirically derived withdrawal-retardation factor of the BPRS.

The Clinician-Administered Dissociative States Scale (CADSS) (Bremner, Krystal et al. 1998) The CADSS is a clinician-administered measure of perceptual, behavioral, and attentional alterations occurring during dissociative experiences that has been validated in healthy subjects and patients with posttraumatic stress disorder. This scale involves 19 self-reported questions and eight observer ratings scored from 0 (not at all) to 4 (extremely). To characterize dissociative responses to ketamine better, CADSS items will be sorted into five subscales with apparent face validity and based on published scales that assessed dissociative states (Steinberg, Rounsaville et al. 1990): body perception, environmental perception, feelings of unreality, memory impairment, and time perception.

Scale for Suicidal Ideation (Beck, Kovacs et al. 1979) SSI is a 19-item observer scale designed to quantify the intensity of current conscious suicidal ideation in various dimensions of self-destructive thoughts or wishes: the extent of the wish to die, the desire to make an actual suicide attempt, and details of any plans; also, internal deterrents to an active attempt, and subjective feelings of control and/or courage regarding a proposed attempt

The Stanford Sleepiness Scale(Hoddes, Zarcone et al. 1973): is a single measure scale designed to give a introspective measure of sleepiness. Scale ratings are between 1 and 7, with a 1 indicating "active, vital, alert, or wide awake" and a measure of 7 indicating "no longer fighting sleep, sleep onset soon, having dream like thoughts."

The Profile of Mood States (POMS)(McNair, Lorr et al. 1971): is a 65 item scale where respondents are asked to characterize how much they feel a certain mood, from 0 (not at all) to 4 (extremely). Scoring of the POMS divides the 64 "moods" into 6 domains: tension, depression, anger, vigor, fatigue, and confusion.

The CGI scale is a three-item scale that assesses treatment response in psychiatric patients. The administration time is 5 minutes. This scale consists of three items: Severity of Illness (item 1); Global Improvement (item 2); and Efficacy Index (item 3). Item 1 is rated on a seven-point scale (1 = normal, 7 = among the most extremely ill patients); as in item 2 (1 = very much improved, 7 = very much worse). Each includes an additional response of "not assessed." Item 3 is rated on a four-point scale (from "none" to "outweighs therapeutic effect"). Items 1 and 3 are assessed based on the previous week's experience. Item 2 is assessed from the period since the initiation of the current treatment.

The *PTSD Checklist Civilian version (PCL-C(Blanchard, Jones-Alexander et al. 1996))* is a 17-item self-report rating scale for assessing post-traumatic stress disorder (PTSD) which corresponds to the DSM-III-R symptoms of PTSD. Examinees are instructed to indicate how much they have been bothered by each symptom using a 5-point (1-5) scale. The anchors for the severity ratings range from "Not at all" to "Extremely." In order to assess symptom severity repeatedly in the context of a treatment protocol, the time frame of one month can be changes. It has excellent test-retest reliability over a 2-3 day period. Internal consistency is very high, correlates strongly with other measures of PTSD, and has good diagnostic utility.

The *Brief Pain Index-Short Form (BPI-SF)* is a brief, simple, and easy to use tool for the assessment of pain in both clinical and research settings. The BPI uses simple numeric rating scales from 0 to10 that are easy to understand and easy to translate into other languages. On the BPI, mild pain is defined as a worst pain score of 1 - 4, moderate pain is defined as a worst pain score of 5 - 6, and severe pain is defined as a worst pain score of 7 - 10

The *McGill Pain Questionnaire* (MPQ)(Melzack 1975) consists primarily of 3 major classes of word descriptors--sensory, affective and evaluative--that are used by patients to specify subjective pain experience. It also contains an intensity scale and other items to determine the properties of pain experience.

The *Posttraumatic Diagnostic Scale (PDS(Foa and Tolin 2000))* is a 49-item self-report measure recommended for use in clinical or research settings that queries about trauma exposure and functioning, and can be used to generate a diagnosis of PTSD consistent with the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders 4th edition.) The PDS has four sections: Part 1 is a trauma checklist; Part 2 asks the respondent to describe their most upsetting traumatic event; Part 3 assesses the severity of the 17 PTSD symptoms of the DSM-IV; Part 4 assesses interference of the symptoms. The PDS has good validity and reliability.

The *Bech-Rafaelsen Melancholic Scale (MES)(Bech 2002)* was developed to assess the severity of manic and depressive symptoms. It contains 11 items which are scored according to a five-category Likert scale (0 = not present,1 = very mild or doubtful, 2 = mild, 3 = moderate,4 = severe). Five of these items are shared with the Hamilton Depression Rating Scale. Their total score range from 0to 44 points. The MES has been found a valid and reliable scale for the measurement of changes in depressive states during short-term as well as long-term treatment. The interobserver reliability of the MES has been found adequate both in unipolar and bipolar depression. External validity including both relapse, response and recurrence indicated that the MES has a high responsiveness and sensitivity.

The *Family History Screen (FHS)(Weissman, Wickramaratne et al. 2000)* is a clinician-administered structured interview designed to collect the mental health history of an informant and his/her first-degree relatives on 15 psychiatric disorders and suicidal behavior. A series of 31 questions are used to detect any evidence of psychiatric symptomatology in the participant's relatives. Its validity is best demonstrated for major depression, anxiety disorders, substance abuse, and suicide attempts.

The *Sheehan Disability Scale (SDS(Sheehan, Harnett-Sheehan et al. 1996))* is a patient-rated instrument designed to assess the impact of perceived problems on work productivity, social/leisure activities, and family life/home responsibilities. The SDS consists of 3 questions rated on a visual analog scale (0 to 10). Higher scores represent greater impairment of activity.

The *Quality of Live Enjoyment and Satisfaction Questionnaire-Short Form (Q-LES-Q-SF)(Endicott, Nee et al. 1993)* is a 16-item well-validated instrument (consisting of the general

activities items within the longer version of the Q-LES-Q) that has been used to measure quality of life in psychiatric and nonpsychiatric populations. Each item is self-rated on a 5-point scale (from 1 = "very poor" to 5 = "very good") indicating how satisfied the individual has been over the past week with 14 specific aspects of his or her life. A separate item assesses medication satisfaction, and an overall summary item measures overall life satisfaction.

The *Structured Interview Guide for the Hamilton Depression Rating Scale with Atypical Depression* (*SIGH-ADS(Williams and Terman 2003))* rates the severity of depressive symptoms in terms of Hamilton's 17 and 21-item depression scales and the NIMH/Columbia addendum of eight symptoms of atypical depression. It was designed for general use in depression research and clinical evaluation, regardless of seasonality.

The *Snaith-Hamilton Pleasure Scale* (SHAPS, (Snaith, Hamilton et al. 1995)) is a 14-item instrument that assesses pleasure loss to previously pleasurable activities, otherwise known as anhedonia, in a self-reported manner. It has been successfully employed in Major Depressions and Schizophrenia.

The *Temporal Experience of Pleasure Scale* (TEPS, (Gard, Gard et al. 2006)) is an 18-item self-report questionnaire that measures pleasure associated with the consumption and anticipation of rewards. It was designed to assess aspects of anhedonia that not directly assessed in other questionnaires.

Appendix J. Plasma/Serum (peripheral) Biomarkers of Interest in Relationship to Ketamine in Treatment-Resistant Depression

General Background

Our conceptualization of the pathophysiology of MDD has evolved significantly over the past decade from a simple chemical imbalance model to a model of impaired neural plasticity and resiliency (Carlson, Singh et al. 2006). This new conceptual approach to the pathophysiology of the disorder now affords us the opportunity to specifically examine the clinical utility of novel drugs affecting these processes (Manji, Quiroz et al. 2003; Zarate, Du et al. 2003). Such is the case for the currently proposed study with ketamine, Substudy 4. Moreover, this novel conceptual approach to MDD also provides us the opportunity to explore the relationship between several psychological and physiological factors that are believed to moderate and mediate effects on neural resiliency and plasticity to the progression of the disorder and the response to specific medications targeting these physiological processes. Here we provide a brief outline of the supplemental studies that we wish to specifically pursue in an attempt to identify biomarkers related ketamine's anticipated antidepressant response.

Serum Biomarkers: Neurotrophic Factors and S-100B

Overview:

This portion of Substudy 4 is designed to compare the effects of ketamine vs. placebo on various serum neurotrophic factors (NTs) and S-100B in patients with MDD.

Background:

Low serum brain derived neurotrophic factor (BDNF) levels in individuals with MDD are among the most consistent and replicated findings in biological psychiatry (Brunoni, Lopes et al. 2008; Sen, Duman et al. 2008). Animal studies clearly demonstrate that the neurotrophic factors (NTs) BDNF, vascular endothelial growth factor (VEGF), glial derived neurotrophic factor (GDNF) and insulin growth factor (IGF-1), act within prefrontal cortex (PFC) and hippocampus (HP), contributing to the pathophysiology of MDD, and mediating some of the beneficial effects of antidepressant drug therapy (ADT). Exciting new preliminary data now even suggest strong associations exist between the peripherally circulating NTs and the morphological, mood, and cognitive features of MDD.

1. S-100B protein is highly specific for neural tissue, where it is predominantly synthesized and secreted by glial cells. The role of protein S-100B is not yet fully understood. However, it is suggested that it has intracellular and extracellular neurotrophic as well as neurotoxic functions. Structural damage of glial cells causes leakage of S-100B protein into the extracellular compartment and into cerebrospinal fluid, further entering the bloodstream. Recently, serum S-100B protein has been proved to be an attractive surrogate marker of brain injury and secondary insults. It can be measured in the arterial and venous serum; it is not affected by hemolysis and

remains stable for several hours without the need for immediate analysis (Beaudeux, Dequen et al. 1999; Beaudeux 2009).

Several studies have demonstrated elevated levels of S-100B in the CSF (Grabe, Ahrens et al. 2001), and serum of individuals suffering from MDD, especially those with melancholic features (Rothermundt, Arolt et al. 2001). A recent meta-analysis of the data from nine studies with 198 depressed and 209 healthy subjects investigating serum S-100B levels in MDD found very strong evidence of elevated S-100B levels in the MDD subjects with an average Cohen effect size of 2.57 ± 0.70 for the published studies (Schroeter, Abdul-Khaliq et al. 2008). S-100B levels appear to be significantly positively related with the numbers of depressive episodes, family history, and cognitive disturbance scores (Yang, Xie et al. 2008) and could be normalized by treatment (Schroeter, Abdul-Khaliq et al. 2002; Schroeter, Abdul-Khaliq et al. 2008). Other preliminary evidence suggests an association between S100B levels and memory processes in patients with recurrent depression and further suggest a neuroprotective role of moderately increased S-100B serum levels in the course of affective disorders (Zhang, Rothermundt et al. 2009). In sum, there is now mounting evidence to suggest serum S-100B levels may serve as a useful biomarker for MDD that may possibly reflect an adaptive mechanism to glial cell damage. Considering its proposed function in regulating cell survival and its putative origin (from damaged glial cells in the CNS), it makes it an extremely interesting biomarker to study in relationship to ketamine treatment.

Objective:

The objective of this part of Substudy 4 is to determine whether changes in NT and S-100B levels are present in the serum of patients with MDD receiving ketamine, and whether changes in serum concentrations are correlated with antidepressant change scores following treatment with either ketamine or placebo.

Procedures:

Serum Collection: Samples will be collected via antecubital venipuncture between the hours of 10am and 2pm, to minimize potential diurnal variability (Begliuomini, Lenzi et al. 2008) at baseline prior to randomization and at the time points listed in Table 1. This will afford us the opportunity to explore the temporal relationship between the changes in the serum biomarkers and clinical improvement. For BDNF serum sampling, blood (5 mL) will be collected in anticoagulant-free tubes and kept at room temperature for one hour, followed by one hour at 4°C, and then spun to isolate serum at 2000*g* × 10 min at 4°C. This is done do be consistent with most of the previous studies measuring serum BDNF. A second sample of 10ml will be immediate spun to isolate serum at 2000*g* × 10 min at 4°C for the measure of S-100B and other NTs. Serum will be collected and kept at −20°C at NIMH.

NT Assays:

IGF-1, VEGF, S-100B will be determined by commercially available immunoassays in duplicate according to manufacturer's instructions.

Metabolomic and proteomic measures

Overview:

This portion of Substudy 4 is designed to evaluate the effects of ketamine on metabolomic and proteomic measures in treatment-resistant patients with MDD and BD.

Background:

A metabolomics platform was previously used to successfully identify a specific metabolic profile related to other glutamatergic drugs such as riluzole (Rozen, Cudkowicz et al. 2005). Another relevant metabolomic study suggests the depressed state may be associated with alterations in metabolism of lipids and neurotransmitters strongly related to GABA, and that treatment with antidepressants adjusts some of the aberrant pathways in the disease so that the patients in remission have a metabolic profile more similar to controls than to the depressed population (Paige, Mitchell et al. 2007). Considering ketamine's purported mechanisms of action related to the amino acid neurotransmitter systems, we believe that these measures will be extremely interesting vis-a-vis the anticipated inter-individual variation in clinical response to ketamine.

Objective:

The objective of this part of Substudy 4 is to determine whether there are specific metabolomic signatures in patients with MDD or BD receiving ketamine and whether these signatures or changes in these signatures with treatment are correlated with antidepressant change scores following treatment with either ketamine or placebo.

Procedures:

Samples will be collected via antecubital venipuncture between the hours of 10am and 2pm, to minimize any potential diurnal variability at baseline and at the time points listed in Table 1. Subjects will be asked to neither eat nor drink for at least eight hours before venipuncture to minimize food effects. The collections will afford us the opportunity to explore the temporal relationship between the changes in the metabolome and clinical improvement. Plasma will be obtained from blood drawn into two 10 mL green top tubes containing sodium heparin. All samples will be centrifuged at 1800 × g for 10 min within 30 minutes of collection. The supernatants will be aliquoted in multiple cryovials and frozen at −80°C.

Transcriptional profiling

Background/Objective:

Gene expression changes in neuropsychiatric and neurodegenerative disorders, and gene responses to therapeutic drugs, provide new ways to identify central nervous system (CNS) targets for drug discovery. In the absence of a deeper understanding of disease pathology and mechanisms of side effects, CNS drug discovery would remain dominated by the redesign of

drugs for familiar targets and reduced approval rates for 'me too' drugs. The ability to evaluate changes in the expression of the entire genome in brain areas affected by CNS disease and drug effects on multiple pathways is an alternative, 'bottom–up' approach to drug discovery (Altar, Vawter et al. 2009).

Procedures:

Collect 2 x 2.5 ml samples from each patient per time pt in Paxgene tubes. Should either be last draw of a series or draw a "blank" first; should ideally be collected in AM (between 9 AM - 12 PM). Samples must be inverted 8-10 times immediately after collection -- can be kept at room temp up to 3 hrs or at 4 deg up to 24 hrs, then should be transferred to -20 deg C. Alternatively can be transferred to -20 deg C immediately after the inversions. -20 storage is acceptable for up to 1 month, but -80 deg C needed for long-term storage (must go first to -20 to avoid tubes cracking).

A time course before/after treatment (to include response and relapse):

Whole blood collected in Paxgene tubes for transcriptional analysis (2 x 2.5 ml per patient per time point). For cytokines, kynurenine pathway (KP) metabolites an additional 200 ul will be collected per sample.

<u>Extraction of RNA from blood samples:</u>

i. There are two systems for blood RNA stabilization and extraction which do not need immediate RNA extraction:LeukoLOCK from Ambion. The main advantage of this system is that it enriches for leucocytes and both - total RNA and miRNA can be extracted if needed. The blood will be transferred from blood collection tube through a filter, then the filter will be washed with PBS buffer, then with RNAse inhibitor. After this, the filter will be sent for analysis at room temperature.
ii. PAXgene blood RNA tubes from BD in combination with Qiagen's PAXgene RNA ki). 2.5 ml of blood in PAXgene tube, mix 15 times by inverting tube upside down. Pack in mailer, then send. We need to receive the tubes within 3 days to preserve integrity of RNA (can be sent on Friday). The samples will be frozen at -70C for longer storage.
iii. RNA-Seq

The total amount of blood drawn during the whole Substudy 4 will be approximately 350 ml.

Appendix K. Treatment Contract

Instructions:
1. First decide whom you would like to include in the contract. You should consider including your psychiatrist, psychotherapist, spouse or significant other, or any other people that you feel are important members of your support system. Sometimes people choose to include their family doctor, a close friend, or an ally at work.

2. Write these people's relationship to you (e.g. "wife," "boss," etc.), their names, and phone numbers in the spaces provided. You do not need to fill in all the spaces if you would only like to include one or two people.

I recognize that I have a cyclical mood disorder. This is a plan to help me identify symptoms early and take steps that will be helpful. The people who can help me with this treatment plan are:
my __psychiatrist___ (name): _____ (phone number):_____
my _____ (name): _____ (phone number):_____
my _____ (name): _____ (phone number):_____
my _____ (name): _____ (phone number):_____

When I am well:
Instructions:
3. Next, check off any behaviors that describe how you behave when you are feeling your usual self, which is when you are neither depressed nor elevated.

4. Extra lines are provided so that you can add any other behaviors which reflect how you are when you are feeling well.

When I am well and my mood is stable, I can do all of the following (Check off the ones that apply. Use the blank spaces to add in symptoms that are not on the list.):
_____ Take care of my appearance and shower regularly
_____ Attend work regularly
_____ Attend school regularly
_____ Keep up with household chores
_____ Keep up with school work
_____ Keep up with paying bills
_____ Get together with friends or do social activities _____ times per week
_____ Exercise regularly
_____ Socialize with people without being unduly irritable or starting arguments
_____
_____
_____
_____

_____
_____
_____

When I am well, I can do the following things to help myself:
Instructions:
5. Use the spaces provided to add any other coping behaviors you have found helpful when you are feeling well.

1. Take my medications as prescribed.

2. Be sure to keep regular sleeping hours (go to bed and get up the same time every day).

3. Work on solving the following problems or pursuing the following life goals:

_____
_____
_____
_____
_____
_____

Instructions:
1. Below, we list many common symptoms of depression, including thoughts, feelings, and behaviors. Read carefully over these symptoms, and check off any that apply to you when you are depressed.

2. Mark an "E" next to any symptoms that are early warning signs, that is, symptoms that generally signal the beginning of your new episodes.

3. Use the blank lines to add any symptoms that you experience that do not already appear on the form.

DEPRESSION
Thoughts
____ Difficulty concentrating
____ Memory problems
____ Difficulty making decisions
____ Frequent thoughts about dying or suicide
____ Thoughts that others do not care when they really might
____ Concerns that I am worthless or evil
____ Paranoia: unreal concerns that people are plotting against me
____ Hallucinations: unreal voices or visions
_____
_____
_

_____
_____
_

Feelings

____ Feelings of worthlessness
____ Feeling guilty without cause
____ Feeling sad without cause
____ Easy irritability
____ Not feeling good even when good things happen
____ No energy
____ No appetite
____ Lower sexual interest

_____
_____
_
_____
_____
_

Behaviors

____ Restlessness and pacing
____ Trouble sleeping or too much sleep
____ Trouble starting or finishing projects
____ Keeping away from people
____ Stopping work or usual activities
____ Fighting easily without good reason
____ Stop eating or eating too much

_____
_____
_
_____
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_____
_____
_
_____
_____
_

PERSONAL TRIGGERS OF DEPRESSION
Instructions:

Check off the events that have led to your becoming depressed in the past. Understanding what kinds of events trigger depression for you can help you identify times when you might be vulnerable to becoming depressed. You can then both be alert for symptoms and work to reduce or minimize symptoms.

Sometimes depressions come out of the blue. Other times they are triggered by certain events or situations. The kinds of situations that sometimes trigger depression for me are (Check off the ones that apply. Use the blank spaces to add triggering situations that are not on the list):

_____ The break-up of relationships
_____ Losing a job
_____ Bad life events:
_____
_____ Good life events:
_____
_____ Physical illness:
_____
_____ Drug or alcohol use
_____ Changes in smoking habits
_____ Changes in seasons
_____ Changes in medications
_____
_____
_
_____
_____
_
_____
_____
_

COPING WITH DEPRESSION

Instructions:

1. You will find suggestions for ways to cope with depression. Please read over the suggestions and fill in the blanks. This page will be yours to refer to when you start to get depressed. Under #1, fill in your doctor's name and phone number so that your family will have it for easy reference.

When I get depressed, I will do the following things to help myself:

Instructions:

1. Items #2, 3, and 4 guide you in trying to identify what may have triggered the depressed symptoms. Under #4, add in any typical personal triggers of depression to remember to think about (these may include triggering events that you have checked off above).

1. Contact my doctor early: _____

Phone number_____

2. Review whether I have had any recent medication changes for medical illnesses.

3. Get early medical attention for any physical illness.

4. Identify any triggering events.
Physical: _____
Emotional: _____

5. Avoid alcohol and drugs (even increasing tobacco can undo the effects of prescribed medication).

6. Maintain my regular daily activities.

7. Minimize sleep loss.

8. Contact support persons:
Instructions:
Fill in the names of one or two people that you could call for support (or just to talk) if you were going through a hard time.
(Name)_____ (phone #) _____
(Name)_____ (phone #) _____
(Name)_____ (phone #) _____

9. Coping skills
Instructions:
Fill in any coping strategies that you have found helpful during the past times you have been depressed. For example, some people benefit from taking short daily walks. Others find it rewarding to write down their thoughts or feelings in a journal. Still others find it beneficial to distract themselves from their thoughts and feelings by listening to music or the radio. If you cannot think of any strategies that work for you, you may want to talk this item over with your therapist or psychiatrist.
(To do) _____

10. Coping skills
Instructions:
Under item #10, fill in any coping strategies that you know ARE NOT helpful during periods of depression. For example, some people desire alcohol more when they are depressed, but this can make them feel even worse once the alcohol wears off. Other people want to stay in bed all day when they start to get depressed. This can also lead to more severe depression by making their sleep schedule even more disturbed, lowering their energy further, and making them miss out on activities that could give them more enjoyment or sense of accomplishment
(Not to do)
_____

When I am depressed, other people can help me by:
Instructions:

Read the suggestions and add any of your own on the lines provided.

1. Trusting me to be the best judge of when I am getting depressed and not contradicting me when I tell them that I am depressed (e.g. not telling me to just" snap out of it.")

2. Calling my doctor and/or taking me to the hospital if my symptoms are serious enough (e.g. if I am suicidal) and I am not sufficiently aware of what is happening to take care of myself.

3._____
_____

MOOD ELEVATION
Instructions:
1. In this contract, we list many common symptoms of elevated mood, including thoughts, feelings, and behaviors. Read carefully over these symptoms, and check off any that apply to you when your mood is elevated.

2. Mark an "E" next to any symptoms that are early warning signs that is symptoms that commonly signal the beginning of your mood episodes.

3. Use the blank lines to add any symptoms that you get that do not already appear on the form.

Sometimes my mood gets elevated (too high). This mood state is called "mania" or "hypomania." I can tell my mood is getting elevated when I experience the following symptoms. Sometimes these symptoms are noticed by other people (like my family, friends, or doctor) before I notice them myself. (Check off the ones that apply, and mark an "E" next to those that are Early Warning Signs. Use blank spaces to add in symptoms not on the list.)

Thoughts
_____ Difficulty concentrating
_____ Thoughts about having special powers
_____ Racing or speeded-up thoughts, like the rest of the world is in slow motion
_____ Thoughts that jump quickly from one idea to another
_____ Paranoia: unreal concerns that people are plotting against me
_____ Hallucinations: unreal voices or visions

Feelings
_____ Feeling "high", completely optimistic, euphoric
_____ More energy
_____ Feeling impatient, irritable
_____ Unusually cheerful and happy
_____ Feeling unusually self-confident
_____ Feeling that nothing bad can possibly happen to me
_____ Increased sex drive

Behaviors

_____ More talkative
_____ Speech loud and rapid
_____ Less sleep
_____ Overly sociable
_____ Doing more projects, sometimes more than are practical
_____ Easily fighting without a good reason
_____ Spending more money impulsively, shopping sprees
_____ Driving recklessly or fast
_____ Involvement in dangerous activities

PERSONAL TRIGGERS OF MOOD ELEVATION

Instructions:

Check off the kinds of events that have led to your becoming hypomanic or manic in the past. Understanding what kinds of events trigger mania can help you identify periods of time when you might be vulnerable to developing new episodes of mania. You can then both be alert for symptoms and work to reduce or minimize symptoms. Sometimes elevated periods come out of the blue. Other times they are triggered by certain events or situations. The kinds of situations that sometimes trigger elevated moods for me are (Check off the ones that apply. Use the blank spaces to add triggering situations that are not on the list):

_____ The break-up of relationships
_____ Losing a job
_____ Bad life events:
_____
_____ Good life events:
_____
_____ Physical illness:
_____
_____ Drug or alcohol use
_____ Changes in smoking habits
_____ Changes in seasons
_____ Changes in medications

COPING WITH MOOD ELEVATION

Instructions:

You will find suggestions for way to cope with mania or hypomania. Please read over the suggestions and fill in the blanks. This page will be yours to refer to when you start to become manic or hypomanic. Under #1, fill in your doctor's name and phone number so that you and your family members will have it available for easy reference.

When my mood gets elevated, I sometimes need the help of my doctor and other support sources (friends or family) to recognize what is happening. Just as I am usually the best judge of when I am getting depressed, others are usually the best judge of when I am getting manic. When

someone in my support system points out to me that I am getting manic, I will do the following things to help myself:

Instructions:
Items #2, 3, and 4 guide you in attempting to identify what may have triggered past manic symptoms. Under #4, add in any typical personal triggers of mania to remember to think about (these may include triggering events that you checked off above).

1. Contact my doctor early: _____
Phone number_____

2. Go over medication changes for medical illnesses.

3. Get early medical attention for any physical illness.

4. Identify any triggering events.
Physical:
_____
Emotional:
_____

5. Avoid alcohol and drugs (Even increasing tobacco can undo the effects of prescribed medication).

6. Maintain my regular daily activities.

7. Minimize sleep loss.

8. Contact support persons:
Instructions:
Fill in the names of one or two people that you could call for support (or just to talk) if you were going thought a hard time.
(name)_____ (phone #) _____
(name)_____ (phone #) _____
(name)_____ (phone #) _____

9. Coping skills
Instructions:
Fill in any coping strategies that you have found helpful during past times you have been manic or hypomanic. For example, some people prefer to stay in a darkened room without much stimulation when they are feeling overwhelmed or overstimulated.
(To do)_____-
_____

10. Coping skills
Instructions:
Fill in any coping strategies that you know ARE NOT helpful during periods of mania or hypomania. For example, some people desire alcohol more when they are manic, but this can make them even more impulsive and likely to do risky things.

(Not to do)_____

When I am manic, other people can help me by:
Read the suggestions and add any of your own.

1. Letting me stay alone in a room with minimal stimulation when I am feeling agitated.

2. Preventing my driving (for instance, holding my car keys) when a consensus of two or more of my doctors or support people judge that it is unsafe for me to drive.

3. Preventing my shopping (for instance, holding my credit cards or bank card) when a consensus of two or more of my doctors or support staff judge that I am too impulsive to shop.

4. Avoiding arguing with me, especially when I am feeling irritable.

5. Calling my doctor and/or taking me to the hospital if my symptoms are serious enough, and I am not aware enough of what is happening to take care of myself.

AGREEMENT

I hope that by signing this treatment contract and going over it with my doctors and members of my support system when I am well, I can be better prepared for and exercise more control over any further depressed or manic episodes that may arise.

_____
_____
_____
_____
_____
_____
_____
_____

Signature_____
Date_____

Other signatures

Signature_____
Date_____

Signature_____
Date_____

Signature_____
Date_____

Signature_____
Date_____

Signature_____
Date_____

Appendix L. Informed Written Consent by Telephone Script

Script for Informed Written Consent by Telephone:
Oral informed consent process for genome sequencing

Caller

Hello, my name is *(your name)* and I'm a *(job title)* from the National Institutes of Mental Health, Bethesda, Maryland.

If necessary: Am I speaking with *(subject name)*?

I am calling because we would like to obtain informed consent for genome sequencing on the genetic samples you have previously donated. In this phone call, I will explain the procedure and risks involved in having this type of genetic testing. Please feel free to ask questions at any time during this phone call.

I assume you have already received a copy of the consent form, is that correct?

Have you had a chance to read through the consent form?

Do you have any specific questions before we begin?

Can you please get your copy of the consent to look at while we discuss together.

You should then review each section of the consent form, and encourage questions and discussion as needed. Once the consent is completely reviewed, and all questions have been answered, ask the subject whether they would like to participate in the genome sequencing project.

If they no longer wish to participate, thank them and encourage them to call back if they are interested in the future.

If they agree to participate, ask them to sign and date the consent form in front of a witness and have the witness sign and date as well.

"If you have no further questions, please sign the consent form, have a witness also sign the consent form and send the consent form to the NIH in the pre-paid envelope"

If you have any questions at any time, please call me at *(your phone number)*.

After Conversation

You should then sign and date your copy of the consent as the investigator, and print your name beneath it. In the box labeled "A" Adult Patient's Consent, write the name of the patient and the words "telephone consent". Keep this copy until you receive the signed consent from the patient. Then, your copy and the patient's copy can be sent to medical records together.

26. Consent Forms

The consent forms contain all required elements.

The following consent forms are submitted with this protocol:

- Substudy 2: Ketamine-BP: Consent Form
- Substudy 4: Ketamine-MOA: Healthy Volunteer Consent
- Substudy 4: Ketamine-MOA: Depressed Consent Form
- Genome Sequencing Consent Form