Title

Investigating Euthymia in Bipolar Disorder and Neurochemical Mechanisms of Ketamine Infusions in Treatment-Resistant Depression

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Publication Date

2024

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Investigating Euthymia in Bipolar Disorder and Neurochemical Mechanisms of Ketamine Infusions in Treatment-Resistant Depression

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Neuroscience

by

Stephanie Njau

2024

ABSTRACT OF THE DISSERTATION

Investigating Euthymia in Bipolar Disorder and Neurochemical Mechanisms of Ketamine Infusions in Treatment-Resistant Depression

by

Stephanie Njau

Doctor of Philosophy in Neuroscience

University of California, Los Angeles, 2024

Professor Katherine L. Narr, Chair

Bipolar disorder (BD) and major depressive disorder (MDD) are debilitating neuropsychiatric disorders affecting millions of people worldwide. Despite the success of medications and psychotherapy in alleviating symptoms, challenges remain in achieving and maintaining remission. In BD, a key therapeutic goal is to reach a state of euthymia, characterized by reduced

intensity and frequency of symptoms or no symptoms at all. However, even in euthymia, subtle emotion regulation difficulties often persist, suggesting the illness remains active. Emotion dysregulation in BD is associated with abnormalities in brain regions involved in emotion processing and regulation, including the prefrontal cortex, amygdala, anterior cingulate cortex, hippocampus, insula and striatum (M. L. Phillips & Swartz, 2014; Strakowski et al., 2005). In Chapter 2, I explore functional abnormalities within the emotion regulation network in euthymic BD and their relationship to subclinical symptoms and clinical characteristics of the disorder. Specifically, this study investigates cognitive-emotion regulation processes in the context of negative stimuli. In Chapter 3 and 4, I address the effects of subanesthetic ketamine on neurochemical disturbances in MDD. In this disorder, a subset of patients, approximately 30%, do not achieve remission despite multiple pharmacotherapy trials, a condition known as treatment-resistant depression (TRD). Subanesthetic ketamine has shown promising rapid antidepressant effects in TRD, yet the underlying neurochemical mechanisms remain unclear. I investigated these mechanisms by utilizing proton magnetic resonance spectroscopy (^{1}HMRS) to measure in vivo neurochemistry of the dorsal anterior cingulate cortex (dACC), a region implicated in MDD pathophysiology. Chapter 3 describes a study where sixty volunteers with TRD received a single intravenous infusion of subanesthetic ketamine. ^{1}HMRS was used to measure levels of glutamate and GABA, along with additional biochemical compounds associated with MDD pathophysiology or treatment, including N-acetylaspartate, N-acetylaspartylglutamate, Creatine, Phosphocreatine and Choline-containing compounds. In

Chapter 4, I extend the experiment to explore the effects of repeated ketamine infusions in fifty volunteers with TRD. A summary of literature pertinent to each study presented in this thesis is provided in Chapter 1. Collectively, the findings from this dissertation could lead to improved therapeutic strategies and enhanced patient outcomes in both BD and MDD.

The dissertation of Stephanie Njau is approved.

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2024

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ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to the study participants who volunteered their time and energy. Your involvement was essential to the research conducted here.

A special thank you to Dr. Lori Altshuler who led the UCLA Mood Disorders Research Program and contributed the neuroimaging data on bipolar disorder utilized for the analysis in Chapter 2.

I am deeply appreciative of my friends and family, especially Rebecca, Leah and Aunt Josie, for their unwavering love, patience, and encouragement.

I am also profoundly grateful to my advisor, Dr. Katherine Narr, for a decade of mentorship and my committee members, whose constructive feedback and thoughtful suggestions greatly contributed to the quality of this work. Their expertise and insights were instrumental in shaping this dissertation.

Lastly, I wish to acknowledge the support of NIMH R03MH110877 awarded to Dr. John O. Brooks III (Chapter 2), and NIMH K24-274305 (Chapter 3) and NIMH U01-110008 awarded to Dr. Katherine Narr, which provided the financial resources necessary for my research.

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CHAPTER 1

Introduction to the Dissertation

Bipolar disorder (BD) is a prevalent psychiatric disorder (Merikangas et al., 2011) associated with considerable societal burdens such as an incidence of death by suicide that is 20 times higher than the general population (Miller & Black, 2020). BD is characterized by abnormal variations in mood and/or energy that manifest in episodes of mania or depression, and mixed states where both episodes are present in the same symptomatic period (American Psychiatric Association, 2013). In the course of BD, there are phases where BD patients experience a reduction in symptoms and enter a period of relative psychological well-being and stability termed euthymia. Fava & Bech (2016) attribute to the Greek philosopher Democritus the formal definition of euthymia as a state where 'one is satisfied with what is present and available, taking little heed of people who are envied and admired and observing the lives of those who suffer yet endure.' Further, the word euthymia is the combination of 'eu' meaning well and 'thymos', which refers to the emotional element of the soul. In psychiatry, euthymia refers to a period for BD patients when the intensity of their symptoms do not meet the threshold for an episode or there are little to no symptoms experienced (Fava & Bech, 2016) which is thought to reflect recovery. However, there is a wealth of evidence that indicates euthymic BD patients continue to experience impairments in cognition and affect with specific evidence of difficulties with emotion regulation (Mercer & Becerra, 2013; Wolkenstein et al., 2014).

Emotion regulation (ER) is defined as a process by which one tries to influence the type of emotions they experience, how they are experienced and how they are expressed (Gross, 2008). ER strategies can be differentiated based on the way an emotion has been experienced or will be expressed, and include antecedent-focused strategies before an emotion has been

experienced and response-focused strategies once an emotion once has occurred (Gross, 1998). These processes can also be further differentiated by whether they are automatic or voluntary (M. Phillips et al., 2008). ER difficulties in BD can manifest at multiple levels. In one study that investigated cognitive emotion regulation processes in euthymic BD while watching three emotionally evocative films (neutral, happy, sad), BD individuals reporteduse of spontaneous suppression and reappraisal strategies across all films compared to healthy controls, but also less success with these strategies (J. Gruber et al., 2012). At the neural level, neuroimaging studies in euthymic BD also indicate impaired function within a network of brain regions involved in emotion processing and regulation, including the prefrontal cortex (PFC), amygdala, anterior cingulate cortex (ACC), hippocampus, insula and striatum (M. L. Phillips & Swartz, 2014; Strakowski et al., 2005).

Major depressive disorder (MDD) is a prevalent mental health disorder that affects approximately 280 million people worldwide (GBD 2019 Mental Disorders Collaborators, 2022). First-line treatment for MDD involves either medication with an antidepressant (e.g., selective serotonin reuptake inhibitor (SSRI)), psychotherapy or both termed pharmacotherapy (American Psychological Association, 2019). Unfortunately, a significant portion of patients with MDD who pursue treatment may not experience a clinical response after sequential treatment strategies and are considered to have treatment-resistant depression (Gaynes et al., 2009). Treatment-resistant depression (TRD) is estimated to affect up to 1 in 3 individuals with MDD (Al-Harbi, 2012; Gaynes et al., 2009).

A long-standing theory of MDD etiology describes a deficit in the monoamine neurotransmitters, primarily the serotonin system as it is often targeted by standard antidepressant medications (Cowen & Browning, 2015). However, a comprehensive review of

studies examining serotoninergic deficits in depression concluded there is no consistent evidence that MDD is caused by lowered serotonin concentrations (Moncrieff et al., 2022). Over the last two decades, a growing number of studies have demonstrated neurochemical differences between patients with MDD and healthy controls that highlight reductions in the levels of glutamate and GABA neurotransmitters in depressed patients (Luykx et al., 2012; Moriguchi et al., 2019; Yüksel & Öngür, 2010). Moreover, MRS studies also provide evidence that antidepressant medications induce alterations in glutamate, GABA and other MRS-detectable metabolites (Boucherie et al., 2023).

A low, subanesthetic dose of ketamine has been shown to function as an effective antidepressant, especially in TRD (Anand et al., 2023; Serafini et al., 2014). The efficacy of ketamine as an antidepressant has been established in numerous studies, including those employing placebo-controlled and randomized-control designs (Bobo et al., 2016). The mechanism of action underlying ketamine's antidepressant effects are still unclear. Previous studies have shown that ketamine alters glutamate and GABA levels (Abdallah et al., 2018; Danyeli et al., 2023; Deakin et al., 2008; Li et al., 2015; Milak et al., 2016, 2020) with effects focused on normalized dysfunctional activity in the anterior cingulate cortex (Alexander et al., 2021).

This introductory chapter is structured as follows: section I provides an overview of the neural circuitry that supports ER. Section II presents an overview of magnetic resonance spectroscopy and reviews studies that have shown deficits in MRS-detectable metabolites measured in this dissertation. Section III describes the neurochemical mechanisms of action of ketamine as an antidepressant. Section IV provides an overview of the studies included in this dissertation.

Section I: Neural Circuitry of Emotion Regulation

Emotion regulation is mainly accomplished through the interaction of two brain systems. The first is a ventral system that consists of predominantly limbic-subcortical structures including the amygdala, insula, ventral striatum, ventral anterior cingulate gyrus, ventromedial prefrontal cortex, and medial orbitofrontal cortex. The ventral system is engaged in recognizing emotionally salient stimuli and generating an emotional state. The second system is a dorsal system that is involved in the voluntary regulation of emotional states. The dorsal system consists of the hippocampus, dorsal anterior cingulate cortex (ACC), areas of the prefrontal cortex (PFC) such as the dorsal PFC (M. L. Phillips et al., 2003). Emotion regulation can be further differentiated into automatic and voluntary processes. Automatic processes are shown to involve the medial prefrontal cortical structures such as the anterior cingulate cortex, orbitofrontal cortex (OFC) and the dorsomedial PFC. In contrast, voluntary cognitive regulation processes recruit the lateral prefrontal cortical structures such as the ventrolateral PFC and dorsolateral PFC in addition to medial prefrontal cortical structures (M. Phillips et al., 2008).Though emotion regulation can be subdivided into voluntary versus automatic processes, these emotion regulation processes occur in parallel and possibly simultaneously with emotion appraisal and emotion generation. In Chapter 2, we will use this neural model of emotion regulation as a framework to examine the neural basis of emotion dysregulation in euthymic BD.

Section II: Magnetic Resonance Spectroscopy Studies of Major Depressive Disorders

Basic Principles of Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a neuroimaging technique that acquires proton nuclear magnetic resonance spectra from brain tissue and can be used to estimate metabolite concentrations. These metabolites indirectly convey information about neuronal

integrity, mitochondrial function, and membrane turnover (Hajek & Dezortova, 2008). ^{1}H-MRS is a widely applied method due to the abundance of hydrogen protons in numerous metabolites, and scanners are typically optimized for the detection of hydrogen. There are various pulse sequences that can be used to assess neuronal metabolites from a region of interest in single-voxel spectroscopy. For example, the widely used Point RESolved Spectroscopy (PRESS) sequence involves a 90-degree frequency selective pulse and two 180-degree refocusing pulses. Volume selection is obtained through three orthogonal pulses that form a three-dimensional volume at their intersection (Buonocore & Maddock, 2015).

For our experiments, we utilized the MEscher-GArwood-Point RESolved (MEGA-PRESS) sequence. This technique is applied in much the same way as PRESS; however, it is optimized for the detection of GABA signals as the concentration of GABA is relatively low in the brain and its peaks overlap with other spectra. MEGA-PRESS is a difference-editing technique where an inversion pulse is applied to GABA spins at 1.9 parts per million (ppm), and the remaining refocusing pulses are applied elsewhere. The J-coupling between GABA spins at 1.9 and 3 ppm is allowed to evolve freely during the echo time. Though other metabolites and macromolecules do have peaks at these locations, by computing the difference between editing on and off, only the peaks affected by the editing pulse are retained, which are predominantly from GABA (Mullins et al., 2014). Estimates of glutamate and other prominent metabolites in the spectra such as N-acetyl-aspartate (NAA), creatine and choline, are estimated from the PRESS (editing off) part of the MEGA-PRESS sequence.

To quantify metabolites, the amplitude of the signal detected can be compared to that of a known internal intensity reference signal. The reference signal can be unsuppressed tissue water, which is acquired within the same scan session by turning off the water suppression pulses (Ogg,

Kingsley, and Taylor 1994). The spectroscopy volume contains four types of compounds: water, macromolecules, lipids, and metabolites. The presence of the first three classes of compounds can limit, but in practice usually does not prevent, the detection of metabolites within a spectroscopy voxel. In particular, as the concentration of water is four orders greater than that of metabolites of interest, the water signal usually needs to be suppressed in order to measure metabolites at all. Also note, with in vivo MR spectroscopy, we can reliably observe approximately 20 metabolites, a very low number compared to the thousands of biochemical molecules in our bodies (Hajek & Dezortova, 2008). Nonetheless, those observable metabolites happen to be key to physiology and MR spectroscopy provides much noninvasive information about metabolism inside living tissue.

Glutamate

Glutamate (Glu) is an amino-acid that functions as the primary excitatory neurotransmitter in the brain (Zhou & Danbolt, 2014). Glutamate also plays an important dual role in cellular metabolism as a byproduct of the tricarboxylic acid (TCA) cycle intermediate alpha-ketoglutarate. Glu is also synthesized from the structurally similar amino acid glutamine (Gln) through deamination. In addition to its role as a neurotransmitter, glutamate is also a building block for other amino acids, such as the antioxidant glutathione (Koga et al., 2011) and the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Reubi et al., 1978). At conventional field strengths and acquisition parameters, the resonance of ^{1}H-MRS Gln protons at 2.45 ppm overlaps with those of Glu occurring around 2.35 ppm which complicates detection of Gln (Ramadan et al., 2013).

At least three recent reviews of the MRS literature have been conducted on glutamate abnormalities in mood disorders or MDD patients (Luykx et al., 2012b; Moriguchi et al., 2019;

Yüksel & Öngür, 2010). The first review (Yüksel & Öngür, 2010) included 11 MRS studies in patients with current MDD and those with a history of MDD. In this review, reductions in glutamate and glutamine levels were reported in several brain regions. Two studies were conducted in the ACC (Auer et al., 2000; Michael et al., 2003a), and one study each was conducted in the left dorsolateral PFC (Michael et al., 2003a), dorsomedial PFC and ventromedial PFC (Hasler et al., 2007), hippocampus (Block et al., 2009) and amygdala (Michael et al., 2003b). Collectively, these studies concluded that there are reductions in glutamate and glutamine levels in depressed patients predominantly within prefrontal and cingulate regions. The second review included 16 studies with 281 depressed patients and 301 controls. This analysis revealed consistent reductions in glutamine and glutamate in the ACC (Luykx et al., 2012a) and reduced levels of glutamine plus glutamate (Glx) in other brain regions investigated. The largest effect sizes were observed for the composite measure of Glx. Furthermore, Glx levels were decreased across all brain regions assessed during depressive episodes. In the third review, encompassing over 1000 MDD patients, a significant reduction in glutamate and glutamine levels was reported in the medial PFC and ACC (Moriguchi et al., 2019). Glutamate abnormalities are argued to become more pronounced with symptom severity or with chronic/recurrent episodes (Colic et al., 2019; Portella et al., 2011). However, studies have also reported elevated Glu and Glx in depressed patients. For instance, one experiment involving measures of Glu and Glx as a ratio to NAA (Glu/NAA, Glx/NAA) reported increases in the ventromedial prefrontal cortex in depressed patients (Kantrowitz et al., 2021). Of note, these participants were not medicated. There are also studies that have reported no difference in glutamate or Glx in MDD within the dorsolateral PFC (Grimm et al., 2012) or the anterior cingulate cortex (Godlewska et al., 2018).

γ-aminobutyric acid (GABA)

γ-aminobutyric acid is an amino-acid that serves as an inhibitory neurotransmitter. GABA is synthesized in neurons from glutamate via glutamic acid decarboxylase and is taken up by glial cells where it is metabolized and enters the TCA cycle. The concentration of GABA is substantially lower than other metabolites at < 1mM (Rothman et al., 1993). In proton magnetic resonance spectroscopy, GABA signals are observed at 1.9, 3.0 and 2.2 parts per million (Puts & Edden, 2012). Similar to glutamate, abnormalities in GABA in depressed individuals have been reported in the literature. Studies using proton magnetic resonance spectroscopy have reported significant reductions in GABA levels within the anterior cingulate (Bhagwagar et al., 2008), PFC (Hasler et al., 2007) and occipital cortex (Sanacora et al., 1999, 2004a). Reductions in GABA levels are reportedly more pronounced in melancholic and treatment-resistant subtypes of depression (Price et al., 2009; Sanacora et al., 2004b).

N-acetylaspartate and N-acetylaspartylglutamate

N-acetyl-aspartate (NAA) is also an amino acid that is highly abundant in the brain, second to glutamate, that is used as a marker of neuronal integrity. NAA is formed by acetylation of acetyl-CoA, a byproduct of glucose metabolism (Baslow, 2003); thus, NAA levels may also reflect mitochondrial function (Sarawagi et al., 2021). NAA is visualized as a prominent peak at ~2.0 ppm along with N-acetyl-aspartyl-glutamate (NAAG) a related metabolite that cannot be resolved from NAAG unless visualized at high scanner field strength (Edden et al., 2007). There is evidence that NAA and NAAG play important roles in myelin synthesis during development, as NAA levels increase rapidly during the first two years of life and become relatively stable in adults. Reductions in NAA have been reported in MDD in multiple brain regions (Saccaro et al., 2024) including the PFC (Jollant et al., 2017; Mishra et al., 2018; Wang et al., 2012), anterior

cingulate cortex (S. Gruber et al., 2003; Lewis et al., 2020; Merkl et al., 2011; Venkatraman et al., 2009; Wang et al., 2012) and hippocampus (Block et al., 2009). Treatment with antidepressants (Gonul et al., 2006; Paslakis et al., 2014; Taylor et al., 2012) and electroconvulsive therapy (Merkl et al., 2011; Njau et al., 2017) have been shown to alter NAA levels.

Creatine (Cr) and phosphocreatine (PCr)

Creatine phosphate/phosphocreatine (PCr) and creatine (Cr) are metabolites that are detectable in the MRS of brain tissue at ~3.029 ppm and 3.037 ppm. These two metabolites play a role in metabolism and spatial and temporal regulation of energy. Decreased levels of creatine have been reported in depressed patients in the medial prefrontal cortex (Venkatraman et al., 2009). In another study, an interaction was detected between gender and levels of creatine where male depressed patients demonstrated lower levels than male controls and female patients exhibited higher levels relative to female controls (Nery et al., 2009). Decreases in brain phosphocreatine were more pronounced in severely depressed individuals than in mildly depressed individuals (Kato et al., 1992). A strong correlation between creatine levels and the severity of depression symptoms has also been reported in the dorsolateral PFC (Michael et al., 2003). At conventional scanner field strengths (up to 3 Tesla), the differences in frequency between Cr and PCr are too small to be differentiated and thus these metabolites are typically summed together as total tCr (tCr=Cr+PCr).

Glycerophosphocholine (GPC) and Phosphorycholine (PCh)

Choline-containing compounds are important for membrane lipid synthesis, and thus, changes in choline may reflect membrane turnover. At conventional field strengths, these

compounds are difficult to dissociate on the MR spectrum, and as such, most studies have reported the combined concentration of their primary metabolites, phosphocholine (3.21 ppm) and glycerophosphocholine (3.23 ppm), as an aggregate measure of choline. In a meta-analysis of 86 peer-reviewed studies, higher choline levels were found in the frontal lobe of MDD patients (Riley & Renshaw, 2018).

Section III: Neurochemical Mechanisms of Ketamine as an Antidepressant

Ketamine is a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor that is primarily used as an anesthetic or analgesic in clinical settings (Kohtala, 2021). The demonstration (Berman et al., 2000) that an infusion with low-dose subanesthetic ketamine could alleviate depressive symptoms within hours was a seminal study for depression research. Notably, the antidepressant potential of NMDA receptor antagonists dates back to early research on cycloserine, a partial NMDA agonist, in tuberculosis patients (Crane, 1959) and experiments with rodents (Skolnick et al., 1996; Trullas & Skolnick, 1990). Although ketamine's exact mechanism of action is still under investigation, various lines of evidence suggest the modulation of glutamatergic neurotransmission in specific cortical regions contributes to its therapeutic effects (Alexander et al., 2021b; Matveychuk et al., 2020; Zanos & Gould, 2018). Apart from its antagonism of NMDA receptors, the first indication that ketamine modulates glutamate transmission was the finding that while acute administration of low dose ketamine increased GABA signals, at a delayed rate there was an increase in glutamate spiking within the same cluster of neurons (Homayoun & Moghaddam, 2007). Using microdialysis in the rat PFC, an earlier study demonstrated significant increases in extracellular glutamate levels follows infusions with subanesthetic doses of ketamine, while at anesthetic doses, there is a reduction in glutamate outflow (Moghaddam et al., 1997). At the cellular/molecular level, the antidepressant

mechanism of ketamine is thought to involve inhibition of NMDA receptors on GABAergic interneurons, resulting in the disinhibition of excitatory pyramidal cells (Zanos & Gould, 2018) (**Figure 1.1)**. By enhancing excitatory neurotransmission, ketamine triggers synaptic potentiation and ultimately synaptic plasticity in cortical and subcortical regions associated with MDD pathophysiology (Niciu et al., 2014) such as the ACC (Alexander et al., 2021b).

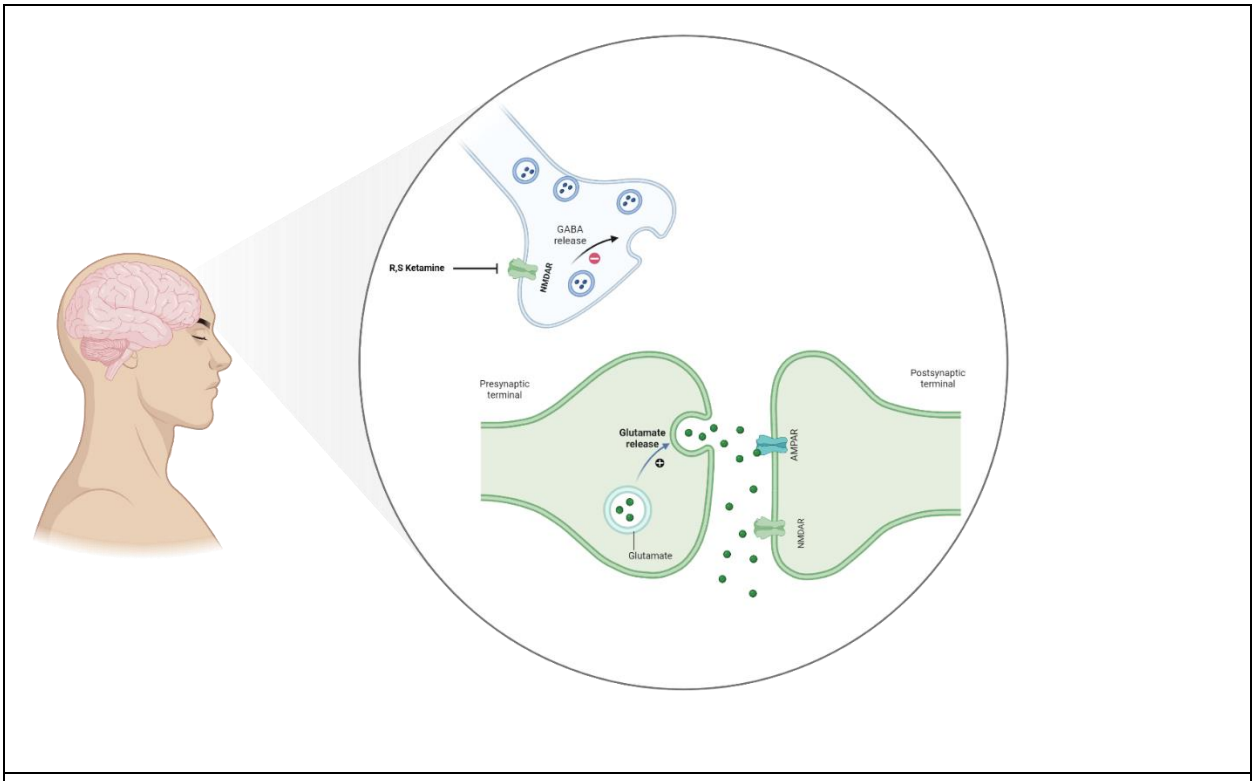

Figure 1.1 | **Ketamine Mechanism of Action.** According to the disinhibition hypothesis, ketamine blocks NMDARs expressed on GABAergic interneurons which leads to a disinhibition of pyramidal neurons and thus glutamatergic firing. Enhanced glutamatergic firing induces acute changes in synaptic plasticity, which are suggested as key to its antidepressant effects. This figure was made in Biorender.

Section IV: Overview of Studies

Neural Circuitry of Emotion Dysregulation in Euthymic Bipolar Disorder

Neuroimaging studies usually compare individuals diagnosed with BD to healthy controls to understand the underlying neural differences that drive disease states. Unsupervised machine learning techniques, such as clustering and dimensionality reduction, are of potential value in neuroimaging data in BD. These techniques aim to uncover hidden structures in data without relying on predefined outcomes. These clustering algorithms work by minimizing the distance between members of a cluster or maximizing the likelihood that data points belong to a specific cluster. Importantly, unsupervised algorithms cannot attribute meaning to the clusters they produce; rather, they report the most probable groupings based on the available data.

Based on the neural model of emotion regulation described in section I, the study described in Chapter 2 will utilize hierarchical clustering to explore variations in emotion regulation neural circuitry in euthymic BD. In this study, functional magnetic resonance imaging data was acquired from 86 volunteers with euthymic BD and 80 matched healthy controls. Participants were shown or instructed to expect and then shown emotionally negative, positive or neutral stimuli selected from the International Affective Picture System. The objective was to investigate patterns of activation during reappraisal within a priori-selected regions of interests engaged during emotion regulation and emotion processing. Our ROIs included the ventrolateral prefrontal cortex, medial prefrontal cortex and dorsolateral prefrontal cortex, and cingulate regions, which are recruited during voluntary control of emotion (Ochsner et al., 2002; M. Phillips et al., 2008), as well as subcortical structures including the amygdala, insula and hippocampus which are engaged during emotion processing (M. L. Phillips et al., 2003). We also included regions of the anterior cingulate cortex including the subgenual ACC, ventral ACC and

dorsal ACC as these regions are implicated in mood disorders and play a vital role in emotion regulation.

Neurochemical Mechanisms of Single Ketamine Infusion in TRD

In Chapter 3, we explored the effects of ketamine on glutamate and GABA levels in sixty volunteers with treatment-resistant depression. Glutamate and GABA levels were estimated via proton magnetic resonance spectroscopy from the dorsal anterior cingulate cortex. Magnetic resonance spectroscopy was employed to measure the levels of glutamate, GABA, NAA, Cr and Cho. MR spectra were acquired before and 24h after the participants received a slow infusion with 0.5 mg/kg of ketamine. It was hypothesized that the antidepressant effects of ketamine would lead to increases in these metabolites, considering previous studies indicating reduced levels of glutamate, GABA, NAA, Cr and Cho in depressed patients. Additionally, it was expected that glutamate or GABA levels would increase alongside clinical improvements after ketamine treatment.

Neurochemical Mechanisms of Serial Ketamine Infusion in TRD

In Chapter 4, we performed a follow-up experiment where fifty volunteers with treatment-resistant depression received four ketamine infusions. MR spectroscopy data were collected from the dACC on three occasions: before treatment, 24 h after the first infusion, and 48-72 h after the fourth infusion. Similar to the second experiment, the levels of glutamate, GABA, NAA plus NAAG, Cr plus PCr and Cho-compounds were measured. We hypothesized that ketamine infusions would result in steady increases in all five metabolites.

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CHAPTER 2

Neural Subtypes of Euthymic Bipolar I Disorder within Emotion-Regulation Circuitry

The content of this work is published as an original research paper to Biological Psychiatry: Cognitive Neuroscience and Neuroimaging Division: *Neural subtypes of euthymic bipolar I disorder characterized by emotion-regulation circuitry*

ABSTRACT

Current diagnostic strategy for bipolar disorders relies on symptomatologic classification. Yet, responses to both pharmacological and psychotherapeutic treatment vary widely, suggesting that neuropathological differences are not well defined by current nosology. Classifying patients with bipolar disorder based on emotion regulation network activation may account for some of the heterogeneity within the disorder. Eighty-six participants with euthymic bipolar I disorder, and eighty healthy subjects underwent functional magnetic resonance imaging scans while engaged in emotional reappraisal of negative stimuli. After determining the average regional activations in key network regions, we applied agglomerative hierarchical clustering to identify subtypes of bipolar disorder. Next, we examined relations among neural subtypes, demographic variables and mood symptoms. Analyses revealed two primary neural subtypes of euthymic bipolar I disorder participants. The first subtype, ERN cluster 1, was characterized by decreased amygdala activation and slightly increased ventrolateral prefrontal and subgenual cingulate activation. Conversely, ERN cluster 2 was defined by lower amygdala activation and increased activation in prefrontal regions, especially the dorsolateral prefrontal cortex (DLPFC). These clusters also displayed differences in clinical presentation. Cluster 1 was associated with a higher number of hospitalizations for depression and later onset of manic episodes. We also performed a parallel analysis examining ERN clustering in healthy subjects who performed the same cognitive

reappraisal tasks. These clusters were defined by differential activation of the prefrontal cortex. In conclusion, this study suggested the emotion regulation circuitry can distinguish neurobiological subtypes of bipolar disorder in the euthymic state. These subtypes, which are differentially associated with indices of illness severity and subsyndromal affective symptoms, may help to inform relapse risk and more personalized treatment approaches.

INTRODUCTION

Bipolar I Disorder (BD I) is a serious mental illness that affects an estimated 8 million adults in the United States (Merikangas et al., 2011). Though less prevalent than other psychiatric disorders, BD I exerts disproportionately greater economic costs than major depressive disorder (Dilsaver, 2011) and is associated with significant reductions in life expectancy (Chan et al., 2022) and high suicide rates (Miller & Black, 2020). Bipolar disorder is characterized by episodes of depression. Manic episodes are characterized by discrete periods of elevated or irritable mood, increased energy and heightened activity; these manic symptoms are severe enough to require inpatient treatment. BDI is characterized by episodes of depression and mania or hypomania. Although there are several treatments, both psychopharmacological and psychotherapeutic, that benefit people with bipolar disorder, response rates are generally suboptimal (Nierenberg et al., 2023).

Across bipolar disorder patients there is substantial variability in both the phenotypic expression of clinical symptoms (Fears et al., 2014; MacQueen et al., 2005) and underlying neural changes (J. R. C. Almeida & Phillips, 2013), which suggests current nosology does not fully capture neuropathological underpinnings. Thus far, efforts to describe neurobiological features of clinical phenotypes using genetics (Fears et al., 2014) or neuroimaging measures

(Phillips & Swartz, 2014; Savitz et al., 2013) have not yet succeeded to the level of clinical translation.

Prior research also suggests neural activation during emotion regulation may serve as a potential premorbid biomarker of disease status (Heissler et al., 2014) for several different reasons. First, the neural network underlying emotion regulation has been well-defined in healthy participants (Ochsner et al., 2004). Further, many studies have demonstrated that emotion regulation (Heissler et al., 2014) and its underlying neural network are dysregulated in bipolar disorder (Kurtz et al., 2021; J. Townsend & Altshuler, 2012; Zhang et al., 2018). Regions of the emotion regulation network could thus serve as an important target for understanding neural heterogeneity within bipolar disorder.

Consequently, in the present work, we used task-based functional magnetic resonance imaging (fMRI) and a hierarchical clustering procedure to address the hypothesis that neural subtypes within emotion regulation networks differentiate individuals with euthymic BD I. To achieve this goal, we adopted a data-driven approach that does not presume homogeneous neural activity within a population of BD I patients, but rather embraces neural heterogeneity to derive a 'bottom-up' approach to understanding subtypes. Specifically, we employed an agglomerative clustering approach guided by unsupervised machine learning to identify underlying neural clusters derived from patients performing an fMRI emotion regulation task. Follow-up analyses explored relations between the cluster solutions and subsyndromal clinical symptoms.

METHODS AND MATERIALS

Participants

Demographic and clinical measures of participants are provided in **Table 2.1**. Our sample included 86 subjects from whom we obtained fMRI scans during an emotion regulation task in prior projects (J. D. Townsend et al., 2013). Participants were recruited through the University of California Los Angeles (UCLA) Outpatient Clinic, the UCLA Mood Disorders Research Program, and local advertisements in the Los Angeles area. Only subjects with a DSM-IV diagnosis of BD I and currently euthymic were included in our analyses. Diagnosis was confirmed with the Structured Clinical Interview for DSM-IV (SCID) (First & Gibbon, 2004). Exclusion criteria included any current comorbid Axis 1 disorder, neurological illness, history of head trauma with loss of consciousness for more than five minutes, hypertension, ferromagnetic metal implants, and left-handedness. Subjects with a history of active substance use disorders in the past three months were also excluded. Current medication regimens were obtained at the time of scanning. For this, we differentiated between lithium, anticonvulsants, antipsychotics, antidepressants, and stimulants. Twenty-seven participants were unmedicated at the time of scanning. Additional measures relating to the course of bipolar disorder including age at first mood episode (mania or depression), number of previous manic or depressive episodes, and number of hospitalizations due to depression or mania were assessed through the SCID.

Emotion Regulation Task

Task practice was administered before scanning. Emotion regulation was modeled with a task that used negative and neutral images from the International Affective Picture System (IAPS) set (Lang et al., 2005). The task, which is described in more detail in previous work (J. D. Townsend et al., 2013), included two passive blocks and one emotion down-regulation block. During the passive blocks (observe neutral image or observe negative image), subjects were told to view the stimuli and experience any associated emotional state. For the down-regulation

block, subjects were instructed to re-evaluate the image and reduce their emotion using the training strategies. Instructions were presented for 3 s prior to each block. Each experimental block contained 8 images and all images were presented for 4 s. Experimental blocks were repeated twice. Control blocks were interleaved between each experimental block and comprised three neutral images presented for 15 s per block. Experimental conditions were counterbalanced across participants.

MRI Data Acquisition

All subjects were scanned on either a 3-T TRIO or Allegra scanner (Siemens Medical Systems, Erlangen, Germany) at the UCLA Brain Mapping Center. Though scan parameters differed, there were no significant differences in image quality, age ($t(84) = -1.11$, $p = .30$) or gender ($\chi^2(1) = 0.81$, $p = .37$) between the subgroups scanned on the two different platforms. For TRIO sequences, high resolution structural volumes were collected with a magnetization-prepared-rapid acquisition gradient echo (MP-RAGE) sequence (TR/TE=1900/2.26 ms, flip-angle=9°, matrix size = 256 x 256, FOV = 250 mm, partial Fourier 7/8, voxel size = 1 mm^3). For Allegra sequences, echo planar image high-resolution structural images (TR/TE=5000/33 ms, flip-angle=90°, matrix size = 128 x 128, FOV = 240 mm, 28 axial slices, 3 mm thick with a 1mm gap) were obtained co-planar to functional scans. TRIO sequences assessed blood oxygen dependent (BOLD) signals through an EPI gradient echo-pulse sequence (TR/TE=2500/25 ms, flip-angle = 90^0, matrix size = 64x64, FOV=200 mm, in-plane voxel size 3.125mm x 3.125 mm, 28 axial slices, slice thickness = 3 mm with a 1 mm gap). Similarly, for Allegra sequences, BOLD contrast was obtained with an EPI gradient echo pulse sequence (TR/TE = 2500/25 ms, FOV 24 cm, matrix size = 64 x 64, 25 axial slices, 3.75 mm x 3.75 mm in plane voxel size, slice thickness 3mm with a 1mm gap).

fMRI Data Analysis

Functional volumes with more than 1 voxel (~3 mm) of motion (in any direction) and those with severe motion or spike artifacts were excluded from analyses. Functional volumes were pre-processed with the FEAT package (FMRI Expert Analysis Tool; Version 6.0) in the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library v4.0 (www.fmrib.ox.ac.uk/fsl). Pre-processing steps included removal of non-brain elements with the Brain Extraction Tool, head motion correction with FSL's MCFLIRT, spatial smoothing with a 5 mm Gaussian kernel and high-pass temporal filtering (Gaussian-weighted least-squares fitting, sigma = 65.0s). Functional volumes were registered to high-resolution structural images and then to a standard space template MNI-152.

First-level time-series statistical analysis was performed in FMRIB's Improved Linear Model (FILM) with local autocorrelation correction. The three conditions in the experimental design (observe negative stimuli, observe neutral stimuli, and decrease response to negative stimuli) were entered into the general linear model (GLM) as explanatory variables. Thereafter, statistical parametric maps of activation were computed to evaluate differences in activation between passive viewing of negative stimuli ("Observe Negative") and during emotional down-regulation to negative stimuli ("Decrease Negative").

Clinical and Behavioral Measures

For all participants, depressive and manic symptoms were assessed through the 17-item Hamilton Depression Rating Scale (HAMD) (Hamilton, 1960) and the Young Mania Rating Scale (YMRS) (Young et al., 1978) . Participants were required to be euthymic for inclusion in our analyses (HAMD < 7 and YMRS < 7). The HAMD is the standard instrument used to define depressive symptoms in clinical research. Prior factor analysis of the HAMD suggested

dimensionality within the HAMD composite score (Gibbons et al., 1993); thus, for our analyses, we extracted HAMD items and placed them into categories, according to the five previously defined HAMD factors (Gibbons et al., 1993). For ease of interpretation, we labeled the five factors as 1) global depression severity (including these HAMD items: psychic anxiety, somatic anxiety, work and interests, guilt, depressed mood, suicide, agitation and loss of libido), 2) insomnia (including early morning and middle insomnia items), 3) loss of insight/loss of weight (including loss of insight and weight loss items), 4) slowing behavior (including retardation, general somatic symptoms, depressed mood, work and interests), and 5) gastrointestinal/weight loss (including gastrointestinal symptoms, weight loss, initial insomnia items). Factor scores were computed by averaging the item scores on each factor, accounting for direction of factor loading.

A subset of participants (n = 56) received additional symptom or behavioral scales including the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983), which is a well-validated, transdiagnostic measure of both state and trait anxiety. We used the Cognitive Emotion Regulation Questionnaire (CERQ) to assess general ability to reappraise and suppress emotion (Garnefski & Kraaij, 2007) . The CERQ has been used previously to measure emotion regulation in bipolar disorder (Dodd et al., 2019; Green et al., 2011) and in euthymic bipolar disorder (Wolkenstein et al., 2014).

Statistical Analyses

The primary regions of interest (ROIs), which were used as features in our analyses, included key regions of the emotion regulation circuit (amygdala, insula, medial prefrontal cortex (BA 10), and ventrolateral prefrontal cortex (VLPFC; BA 45/47)), as depicted in **Figure 2.1**. We included additional ROIs that have been implicated in emotion regulation in bipolar disorders:

parahippocampal gyrus, hippocampus, BA 8, dorsolateral prefrontal cortex (DLPFC; BA 9), subgenual cingulate cortex, and ventral and dorsal anterior cingulate cortices. ROIs were extracted using structural masks defined in the Talairach-Daemon atlas (Lancaster et al., 2000). The structural masks were centered on each subject's parametric z map obtained during the emotional reappraisal task to extract mean regional activation for each ROI constrained within a 10 mm sphere. Thereafter, we applied agglomerative hierarchical clustering to identify underlying clusters of neural activation. For each subject, we modeled regional functional activation values as a vector set and used an *a priori* defined distance measure to iterate through the sample and compute pairwise distances, resulting in a distance matrix. For agglomeration measures in the clustering analyses, we used uncentered Pearson-product moment correlations with complete linkage as the distance metric. The optimal clustering solution was determined via the NbClust package in R, which uses over 30 indices to compute the optimal partition based on highest concordance among clustering indices. Lastly, we validated the stability of the proposed partition based on Jaccard coefficients in the clusterboot package in R which utilizes a bootstrapping procedure to each computation comparing the original clustering solution to new cluster obtained after permutation (Hennig, 2007) .

Follow-up exploratory analyses

We examined the relations between neural cluster membership, subsyndromal symptoms, and clinical history through stepwise binomial logistic regression with backward elimination. To develop cluster profiles, we used the following predictors of cluster membership: age at first depressive and/or manic episode, age at first treatment, number of previous hospitalizations for depression or mania, history of psychotic depression or mania, prior substance abuse, YMRS score, and HAMD factor scores (the mean score of corresponding factor items). Multicollinearity

between predictors was assessed with variance inflation factors (VIF) and tolerance values. The variance inflation factor measures the variance increase of a model from relations between a predictor and all other predictors.

Second, to explore whether demographics and behavioral measures were related to cluster membership we performed Pearson chi-square tests for categorical variables and t-tests for continuous variables. Statistical procedures were performed in SPSS software version 25 (IBM Corp. Released 2016. IBM SPSS Statistics for Mac, Version 24.0. Armonk, NY: IBM Corp.).

RESULTS

Cluster Analyses

Based on an exhaustive series of clustering indexes (**Table 2.1**) the two-cluster solution performed best according to various algorithms. Cluster stability for both clusters was high, both Jaccard similarity coefficients were above 0.85, relative to clustering solutions with more than two groups (**Figure 2.3**).

The first emotion-regulation network cluster (ERN Cluster 1), which included 28 subjects, was characterized by decreased activation of medial, dorsal and ventral PFC (BA areas 8, 9, 44, 45, 46), anterior cingulate cortex, parahippocampal gyrus, amygdala, and insula. Notably, the amygdala exhibited the greatest decrease in activation. Only two regions showed some increase in activation: ventrolateral prefrontal cortex (BA 47) and subgenual anterior cingulate cortex (**Figure 2A**). The second neural cluster, ERN Cluster 2 was comprised of 58 subjects and sharply contrasts with ERN Cluster 1. Specifically, this cluster was characterized by increased activation dorsolateral (BA 9) and dorsomedial PFC (BA 8), anterior cingulate cortex, hippocampus, parahippocampal gyrus, amygdala, and insula. The medial PFC exhibited minimal

activation in comparison to other regions whereas the subgenual anterior cingulate cortex exhibited decreased activation. The VLPFC displayed heterogeneous activation with increased activity in both the BA 44 and 47, but decreased activity in BA 45 (**Figure 2B**).

To further differentiate patterns of regional activation between the two clusters, we provide a radial-graphs illustrating the differential activation patterns defining the two clusters, (**Figure 2.4, A – C**) as well as a graph of absolute activation values, (**Figure 2.4 D**). The amygdala exhibited the greatest activation difference between the two clusters, whereas the medial PFC exhibited the least.

Clinical Characteristics of Cluster Profiles

Next, we performed exploratory analyses to evaluate the differential cluster profiles in terms of clinical, demographic and behavioral measures. Logistic regression revealed significant differences between the profiles of the clusters (**Table 2.3**). Of the predictor variables, age at first manic episode (OR = 1.06: 95% confidence interval (CI): 1.00-1.33 p = .04), number of hospitalizations for depression (OR = 1.30, CI: 1.02-1.64 p = .03), a history of alcohol use disorder (OR = 5.93, CI: 1.17-30.190, p = .03), and psychosis (OR = 11.06, CI: 1.58-77.21, p = .02) all achieved statistical significance. ERN Subtype 1 was associated with a later age of onset of their first manic episode, more hospitalizations for depression, and reduced likelihood of alcohol use disorder or psychosis. There was a trend-level of significance for HAM-D factor 4, which reflected symptoms of anorexia and initial insomnia (OR = 0.32, CI:0.10 – 1.20, p = .06). Because clinical measures included in our model may be correlated, we computed variance inflation factor (VIF) and tolerance for variables selected were within accepted bounds.

Demographics: There were no statistically significant differences between the clusters with respect to age, t (84) = 0.50, p = .62, education level t(84) = 0.85, p = .40, or race category, χ^2

(4) = 3.00, p = .56. However, the proportion of females was greater in the first subgroup, χ^2 (1) = 4.85, p = .03.

Behavioral characteristics. In a subset of participants (n = 56), we performed an independent logistic model including two additional variables, STAI and ERQ, and neither differentiated between the two clusters: STAI trait anxiety F(52) = 0.81, p = .71 and ERQ total score F(52) = .70, p = .80, and ERQ average score, F(52) = 0.90, p = .58.

DISCUSSION

In this study, we provide the first evidence that BD can be better differentiated based on patterns of neural activation in response to negatively valanced stimuli through cognitive re-appraisal strategies. A significant body of work has demonstrated neural differences in emotion processing in bipolar disorders using cross-sectional analyses comparing BD to healthy controls and participants with unipolar depression (Pan et al., 2009; Soares & Mann, 1997; Strakowski et al., 2005). Data-driven approaches have shown promise in the demonstration of unique activation patterns in subsets of participants with bipolar depression and depression that track with clinically relevant phenotypes of the disease (J. R. Almeida et al., 2009; Price et al., 2017).

However, to our knowledge, the characterization of *intra-diagnostic* differences within a key behavioral phenotype of BD, emotion dysregulation, are lacking. Here, we have shown that within the unitary diagnosis of bipolar disorder, the emotion regulation network is differentially activated in a particular DSM-defined subtype of BD, which suggests distinct underlying neurobiology. Across bipolar disorder patients there is substantial variability in both the phenotypic expression of clinical symptoms (Fears et al., 2014; MacQueen et al., 2005) and underlying neural changes (J. R. C. Almeida & Phillips, 2013), which suggests current nosology does not fully capture neuropathological underpinnings. Thus far, efforts to describe

neurobiological features of clinical phenotypes using genetics (Fears et al., 2014) or neuroimaging (Phillips et al., 2003; Savitz et al., 2013) have not yet succeeded to the level of clinical translation.

Our findings add to current literature utilizing clustering approaches in fMRI data to characterize heterogeneity in brain activity in MDD and BD. For example, Drysdale and colleagues (Drysdale et al., 2017) also used a hierarchal clustering procedure, similar to the algorithm reported here and reported evidence of four biotypes of MDD based on a lower-dimensional representation of resting state connectivity and MDD symptoms. Biotype membership could predict response to repetitive transcranial direct current stimulation (rTMS) with high accuracy and they were evaluated on an external sample. However, a subsequent replication study following a similar protocol did not detect the same biotypes (Lacerra et al., 1999). As a follow-up analysis, they evaluated the robustness of the parameters used to select the best number of clusters; the Calinski-Harabasz (CH) and the silhouette index and concluded that the calculated values of these indices were not statistically significant. In our analysis, in addition to the CH and silhouette index, we utilized 28 clustering indexes, which varied in their underlying valuation of the index. Thirteen out of 28 suggested the two-cluster solution, 9 out of 28 suggested the four-cluster solution and 2 out of 28 selected a ten-cluster solution. In analyzing the stability of the two-cluster solution via the Jaccard coefficient, we also modeled the stabilities of additional solution to verify the stability with other proposed solutions (**Figure 2D**).

The first subtype was largely driven by decreased activation of the amygdala and hippocampus, as well as decreased activation in most other regions, save for slightly increased activation of subgenual cingulate and ventrolateral PFC. In contrast, the second cluster was defined by a robust relatively lower activation of the amygdala and increased activation in

prefrontal regions, especially DLPFC. Emotion regulation reflects both implicit and explicit processes that engage neural circuits involved in emotional processing (Etkin et al., 2015). Automatic (implicit) regulation processes engage medial prefrontal areas critical for self-referential processing and emotional generating areas such as the amygdala. In contrast, voluntary (explicit) regulation engages lateral portions of the PFC (prominently in the VLPFC and OFC). At the neural level, the prefrontal and cingulate regions regulate amygdala activation to dampen affective responses to emotional stimuli (Green et al., 2007; Kanske & Kotz, 2010; Koenigsberg et al., 2010) .

In bipolar disorder, across both diseased and euthymic states, there is evidence of altered functional activity of several regions of the emotion regulation network. Abnormal activation of subcortical and medial temporal structures, such as the amygdala (Green et al., 2007), hippocampus/parahippocampal gyrus (J. R. Almeida et al., 2009; Wijeratne et al., 2013), and ventral striatum appear to occur in parallel with reduced activity within prefrontal cortical areas (Strakowski et al., 2005). Considering the amygdala's vital role in encoding, processing, and expressing emotion it is notable that amygdala activation was a strong defining feature between the two subtypes. A previous analysis conducted on an overlapping sample indicated reduced activation in ventrolateral and dorsolateral prefrontal cortex, medial frontal gyrus and posterior cingulate in euthymic bipolar 1 disorder relative to healthy controls utilizing the same emotional down-regulation task administered here (J. D. Townsend et al., 2013). During emotional regulation, increased VLPFC activation is presumed to modulate amygdala activation (Foland et al., 2008; Hariri et al., 2000; Stein et al., 2007); however, differences in VLPFC activation between the two clusters were not as strong as other regions. Nonetheless, our subtypes are consistent with findings of abnormal functioning in subcortical and medial temporal structures,

such as the amygdala, hippocampus/parahippocampal gyrus (J. R. Almeida et al., 2009; Wijeratne et al., 2013), and ventral striatum (Strakowski et al., 2005).

The bipolar disorder subtypes revealed in our analyses were differentially associated with illness characteristics and subsyndromal symptoms. We observed that individuals with early features of mania including earlier age of onset and a positive history of manic psychosis had a greater likelihood of membership in the Subtype 2, which displayed higher amygdala activity during the emotion regulation task. Amygdala hyperactivity has been associated with manic symptoms, so this subgroup may be predisposed to hyperarousal and thus may benefit more from interventions targeting emotional regulation or anxiety (McRae et al., 2014). Although the distinct functional divisions of prefrontal cortex play important roles in affective regulation, activation differences between the two subgroups within pre-frontal and cingulate regions were not strong drivers of subtype membership. Thus, prefrontal activation during emotion may be a mood-state related marker, as opposed to a trait-related one.

There are several limitations to our study. First, although our sample size was fairly large, we may have been able to identify additional clusters through the stability that an even larger sample would provide. Differences between profiles of clusters may have been minimized because, as in many neuroimaging studies, the archival data we used strove for relatively uniform recruitment criteria to *minimize* variability across subjects in many areas, such as co-morbid diagnoses and mood state. These restrictions may have limited our ability to detect neural cluster profiles as defined by demographic features and subsyndromal mood symptoms. Even so, we were able to demonstrate differential profiles with respect to demographic and clinical measures. Further, though we used a validated brain activation task for probing emotion regulation, it is possible that other functional tasks or a combination of tasks may be more

sensitive for detecting neurobiological variations relating to BD subtypes. Third, the majority of subjects were medicated, which adds to the heterogeneity of effects and future studies toward future applications.

In this work we provided evidence that BD 1 is comprised of clusters defined by differential neural function during euthymia. These clusters, largely defined by differences in activation of amygdala, dorsolateral prefrontal cortex, and parahippocampal gyrus were related to other measures of disease expression. Clusters based on neural variability within bipolar disorder may be useful in the search for readily identifiable biomarkers, as well as in explaining variability in treatment response (Drysdale et al., 2017; Price et al., 2017; Tamminga et al., 2017). Additional prospective work may define how neural clusters can be incorporated not only into diagnostic strategies, but personalized treatment approaches.

TABLES

Table 2.1. Demographics and Clinical Characteristics	
Age[a]	38.92 ± 12.58
Sex: Male/Female	58/28
Education[a]	14.36 ± 2.02
Race	
American Indian/ Alaskan Native	2
Asian	7
Pacific Islander/ Native Hawaiian	1
African American	22
White	54
Unmedicated	27
Medicated	
Medication	
Lithium	1
Anticonvulsants	36
Antipsychotics	40

Antidepressants	24
Benzodiazepines	7
Stimulants	2
YMRS	1.65 ± 2.10
HAMD total score	4.05 ± 3.05
HAMD factor scores	
Factor 1 (Global depression severity)	2.64 ± 2.06
Factor 2 (Insomnia)	0.58 ± 0.88
Factor 3 (Weight loss and loss of insight)	0.07 ± 0.29
Factor 4 (Slowing behavior)	1.54 ± 1.74
Factor 5 (Anorexia and early insomnia)	0.68 ± 0.73
Age of first depressive episode (years)[b]	21.06 ± 10.40
Age of first manic episode (years)[c]	24.14 ± 10.78
Age of first treatment (years)	30.82 ± 13.20
Number of hospitalizations for mania[d]	2.61 ± 4.24
Number of hospitalizations for depression[d]	1.75 ± 2.84

YMRS: Young Mania Rating Scale; HAMD: Hamilton Depression Rating Scale.

[a]Age and education missing for one participant. [b]Age at first depressive episode and medication missing for one participant. [c]Age at first manic episode missing for three participants. [d]Number of hospitalizations for mania and depression missing for four participants.

Table 2.2. Logistic regression analyses on identified subtypes with clinical factors as predictors				
	OR (95% CI)	*p-value*	Collinearity diagnostics	
			Tolerance	VIF
HAMD-factor-3: weight loss and loss of illness insight	7.08 (0.41- 122.52)	0.18	0.90	1.11
HAMD-factor-4: slowing behavior	1.49 (0.96 - 2.31)	0.08	0.62	1.62
HAMD-factor-5: anorexia and early insomnia	0.32 (0.10 - 1.20)	0.06	0.55	1.83
Age of First Manic Episode	1.06 (1.00 - 1.13)	0.04	0.70	1.43
Age of First Treatment	0.96 (0.90 - 1.00)	0.11	0.71	1.42
Number of Hospitalizations for Depression	1.30 (1.02 - 1.64)	0.03	0.76	1.31
History of Alcohol Abuse	5.93 (1.17 - 30.20)	0.03	0.79	1.26
History of Drug Abuse	0.34 (0.10 - 1.21)	0.10	0.89	1.12
History of Psychotic Mania[a]	11.06 (1.58 - 77.21)	0.02	0.90	1.11
Model: $\chi^2(9) = 20.90$, p = .01. OR: Odds ratio; VIF: Variance inflation factor. [a]History of psychotic mania reflects psychosis during a manic episode.				

Table 2.3. Internal Clustering Indices utilized in NbClust package in R								
Maximum Values of the Index								
Number of Clusters	**KL**	**CH**	**Silhouette**	**Dunn**	**Ptbiserial**	**Ratkowsky**	**Hartigan**	**CCC**
2	21.18	36.92	0.44	0.17	0.66	0.37	6.66	-3.94
3	0.60	22.98	0.36	0.15	0.66	0.34	1.78	-6.75

4	0.38	16.05	0.31	0.15	0.66	0.30	8.30	-9.57
5	14.11	15.14	0.32	0.16	0.66	0.29	2.68	-10.32
6	0.82	12.89	0.32	0.16	0.66	0.27	1.46	-12.11
7	0.57	11.04	0.30	0.18	0.66	0.25	0.86	-14.49
8	0.79	9.57	0.27	0.18	0.66	0.24	0.47	-16.89
9	2.76	8.37	0.27	0.18	0.66	0.23	0.92	-17.70
10	0.87	7.53	0.27	0.18	0.66	0.22	0.74	-18.30
Optimal Cluster	2.00	2.00	2.00	7.00	3.00	2.00	4.00	2.00
Best index value	21.18	36.92	0.44	0.18	0.66	0.37	6.52	-3.94

Minimum Values of The Index								
	Cindex	**DB**	**McClain**	**Scott**	**SDbw**	**Sdindex**		
2	0.36	1.51	0.55	95.09	0.72	1.35		
3	0.39	1.86	0.77	129.80	0.61	1.50		
4	0.39	1.70	0.79	152.64	0.55	1.49		
5	0.35	1.73	1.09	192.83	0.52	1.49		
6	0.33	1.68	1.16	219.91	0.47	1.44		
7	0.36	1.66	1.19	242.90	0.43	1.63		
8	0.35	1.58	1.22	254.05	0.38	1.60		
9	0.35	1.51	1.23	262.04	0.34	1.57		
10	0.35	1.45	1.24	293.10	0.32	1.51		
Optimal Cluster	6.00	10.00	2.00	5.00	10.00	2.00		
Best Index Value	0.33	1.45	0.55	40.19	0.32	1.35		

Maximum Difference Values of Index								
	TrC	**TraceW**	**Ball**	**Scott**	**Marri**			

	ov W				**ot**			
2	207 6.96	701.90	350.95	95.09	0			
3	193 8.30	650.30	216.77	129.8 0	0			
4	176 4.20	636.63	159.16	152.6 4	0			
5	175 8.81	578.12	115.62	192.8 3	0			
6	169 7.80	559.58	93.26	219.9 1	0			
7	160 6.70	549.52	78.50	242.9 0	0			
8	157 8.30	543.63	67.95	254.0 5	0			
9	154 9.51	540.40	60.04	262.0 4	0			
10	150 6.22	534.05	53.40	293.1 0	0			
Optimal Cluster	4.00	5.00	3.00	5.00	0			
Best index value	174. 09	39.97	134.18	40.19	0			
Critical Values of the Index								
	Dud a	**Pseudot2**	**Beale**	**Frey**				
2	0.85	7.46	1.58	0.78				
3	0.71	3.62	3.21	0.86				
4	0.84	7.78	1.65	0.55				
5	0.92	2.56	0.73	0.33				
6	0.77	2.74	2.44	0.36				
7	0.98	0.49	0.16	0.49				
8	0.96	0.23	0.34	0.63				
9	0.52	3.69	6.55	0.73				
10	1.38	-0.28	-1.23	0.75				
Optimal Cluster	2.00	2.00	5.00	1.00				
Best Index Value	0.85	7.46	0.73	NA				

FIGURES

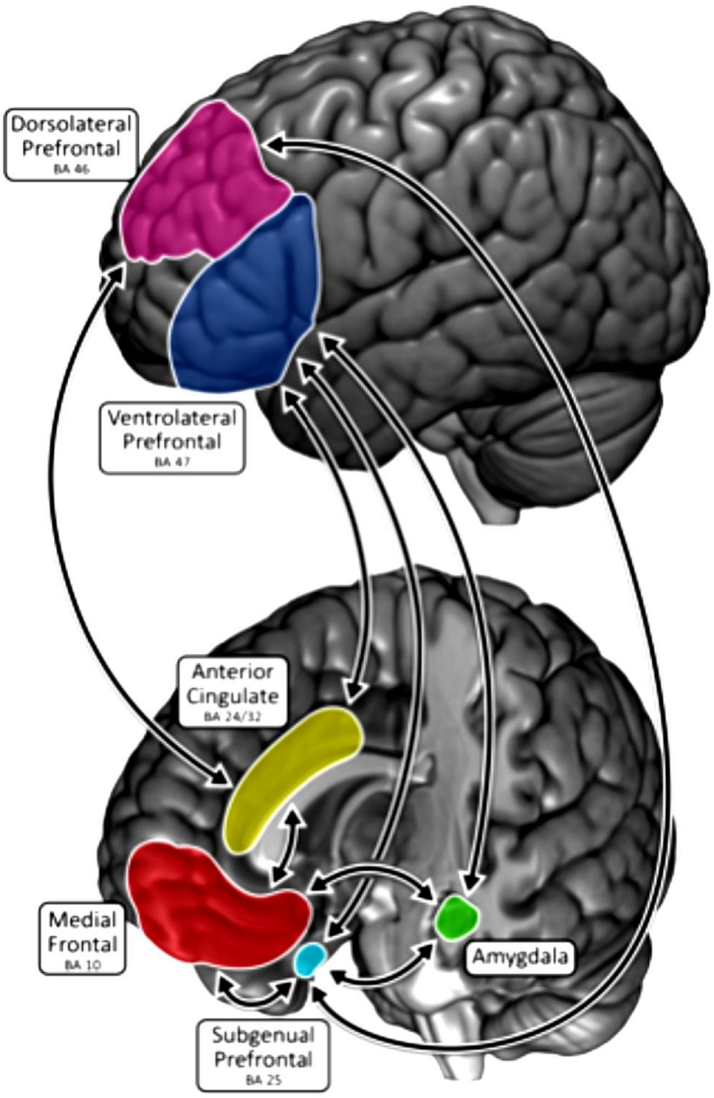

Figure 2.1. | Emotion regulation network in bipolar disorders. Lateral structures include the dorsolateral and ventrolateral PFC (top view), and medial structures include the anterior cingulate, medial frontal, subgenual prefrontal and amygdala (bottom view)

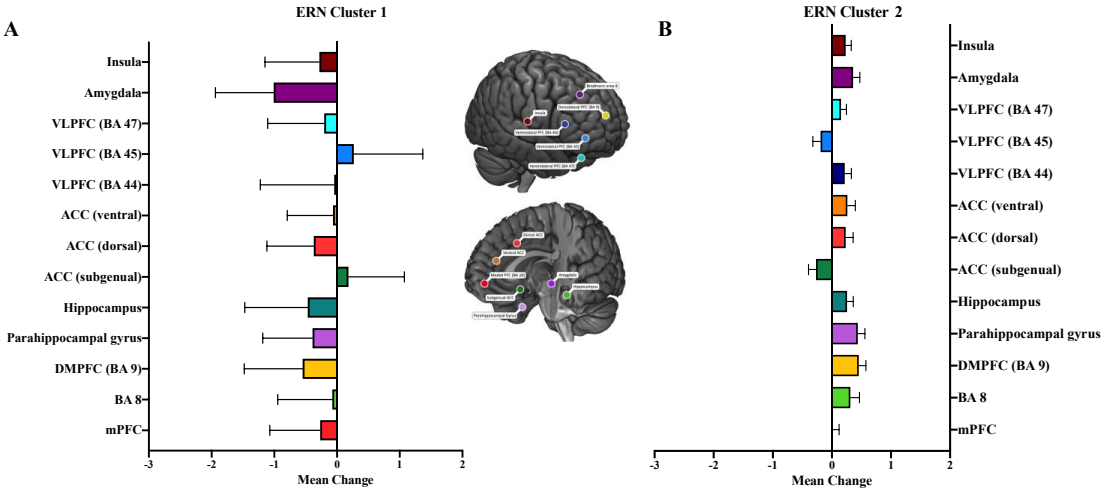

Figure 2.2. | Agglomerative hierarchical clustering on functional magnetic resonance imaging (fMRI) brain activation within the emotion-regulation network (ERN. (A and B) Bar graphs display activation in ERN regions of interest (ROI) in this order: ERN Cluster 1 (A, left) and ERN Cluster 2 (B, right). Mean change represents z-statistic from contrast (Look Negative -Decrease Negative). Error bars reflect +/- standard deviation. Each ROI is color coded on the 3D brain image (center). Lateral (top) and medial (bottom) view.

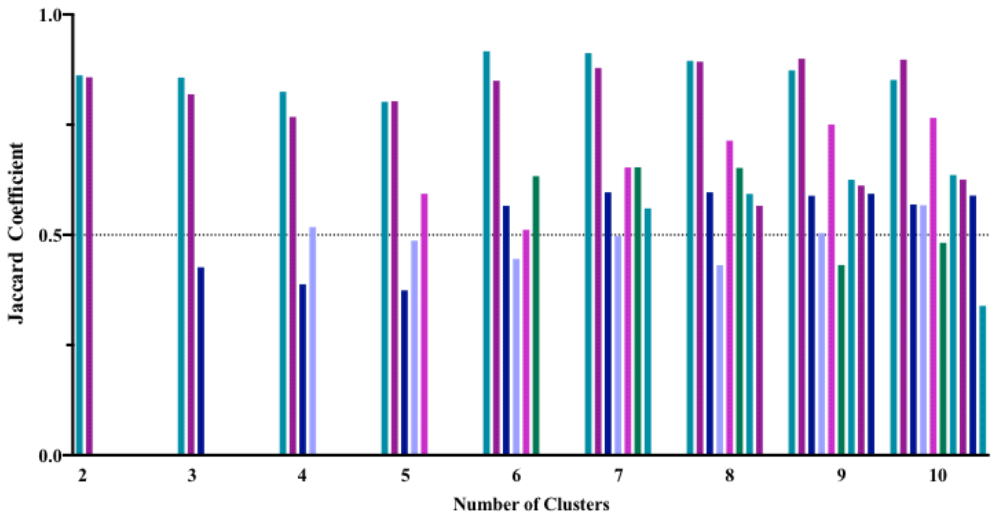

Figure 2.3. | Jaccard coefficients estimating the stability of clustering solutions (y-axis) for each potential number of clusters (x-axis).

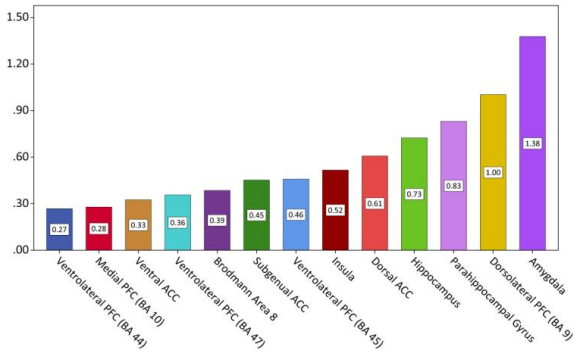

Figure 2.4 | (A) Radial plot displaying mean activation for ERN Cluster 2 (B) superimposed on ERN Cluster 1(B) for BD euthymic participants. Each cluster is displayed independently. (D) Bar graph of differences in neural activation between ERN Cluster 1 and ERN Cluster 2 as reflected in absolute mean Z-score differences. The group mean differences values are displayed within each individual bar.

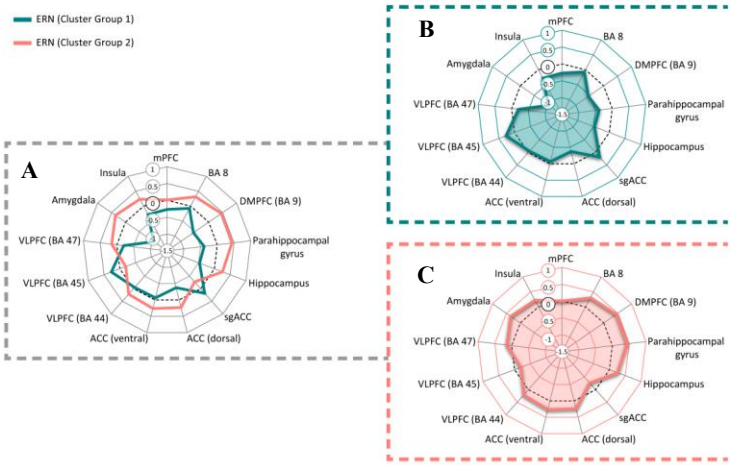

Figure 2.5 | (A) Radial plot displaying mean activation for ERN Cluster 2 (B) superimposed on ERN Cluster 1(B) for BD euthymic participants. Each cluster is displayed independently.

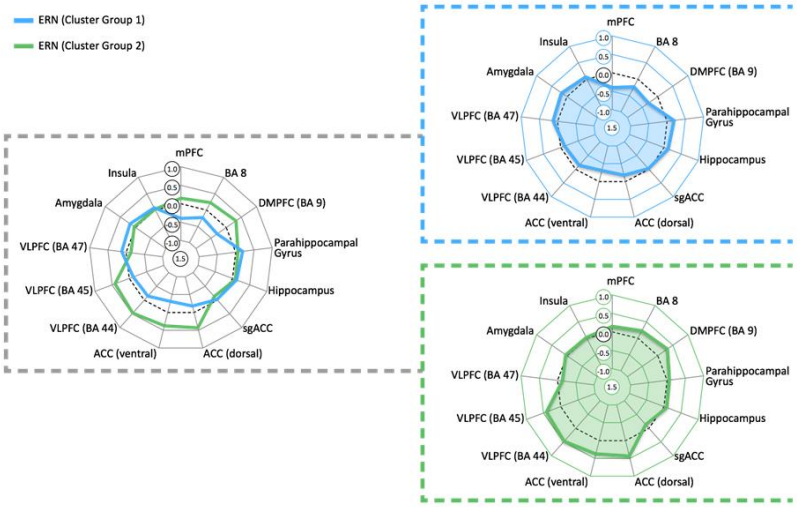

Figure 2.6 | (A) Radial plot displaying mean activation for ERN Cluster 2 (B) superimposed on ERN Cluster 1(B) for healthy controls. Each cluster is also displayed independently.

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CHAPTER 3

Effect of Single Ketamine Infusion in Treatment-Resistant Depression- a Magnetic Resonance Spectroscopy Study

ABSTRACT

Ketamine has emerged as a highly effective intervention for treatment-resistant depression (TRD). Though it acts as a non-competitive antagonist of excitatory glutamatergic N-methyl-D-aspartate receptors (NMDAR), widely expressed in the brain, including on inhibitory γ-aminobutyric acid (GABA)-ergic cells, the mechanisms of its antidepressant action are less clear. To investigate the links between glutamate and GABA neurotransmission and the clinical benefits of ketamine, we used proton magnetic resonance spectroscopy (^{1}H-MRS) to measure both glutamate and GABA levels in the dorsal anterior cingulate cortex (dACC) in sixty participants with TRD before (within 1 week), and 24 hours after a 40-minute intravenous infusion with 0.5 mg/kg of racemic (*R,S)*-ketamine. The 17-item Hamilton Depression Rating Scale ($HDRS_{17}$) was used as the primary measure of clinical improvement, and a 50% or greater improvement in $HDRS_{17}$ ratings was used to define treatment responders. Ketamine increased mean dACC glutamate levels at 24-hrs at trend-level significance ($p=.06$) across all participants. However, post-hoc comparisons revealed significant increases in glutamate in treatment responders examined separately (n=25, p=.01). Additionally, lower glutamate levels at baseline predicted greater improvements in $HDRS_{17}$ scores at 24 hrs. post treatment ($p < .0001$). GABA levels remained stable after treatment ($p=.90$). Additional metabolites associated with neuronal integrity (tNAA), metabolic function (tCr) and membrane turnover (tCho), which may serve as complementary biological evidence of ketamine-induced plasticity, also increased with treatment

(all $p<.01$). These results provide evidence of sustained enhancements of glutamate neurotransmission in treatment response and a potential role of ACC glutamate levels as a biomarker of responsivity to ketamine.

INTRODUCTION

Major Depressive Disorder (MDD) is a highly prevalent illness and is one of the most common mental health disorders worldwide ("Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019," 2022). In the US alone, in 2021, an estimated 21 million adults met diagnostic criteria for MDD (del Vecchio et al., 2012), a number that continues to grow (Goodwin et al., 2022). Clinical guidelines indicate pharmaco- and/or psychotherapy as first-line treatments for MDD (American Psychological Association, 2019). Unfortunately, there are some shortcomings associated with standard antidepressants, including a weeks-to-months delay for potential therapeutic response (Gaynes et al., 2009; Rush et al., 2006). Moreover, an estimated 30% of individuals with MDD fail to respond adequately to two or more treatment courses (e.g., serotonin-selective reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors) and are considered to have treatment-resistant depression (TRD) (Gaynes et al., 2009). Neurostimulation therapies such as transcranial magnetic stimulation (TMS) and electroconvulsive therapy (ECT) are often prescribed for TRD. However, since the discovery that a single intravenous dose of subanesthetic ketamine produced a pronounced improvement in antidepressant response in a small sample of depressed patients (Berman et al., 2000) and the confirmation of ketamine's efficacy in numerous subsequent double-blind and placebo-controlled clinical trials and open-

label studies (Serafini et al., 2014), racemic (R/S) ketamine and S-ketamine are considered viable treatment options for TRD (Anand et al., 2023).

Converging evidence suggests excitatory glutamatergic and inhibitory GABA neurotransmission is dysregulated in MDD (Duman et al., 2019; Sarawagi et al., 2021). The administration of ketamine, an N-methyl-D-aspartate receptor (NMDAR) antagonist, leads to an immediate increase of glutamate release in the brain (Abdallah et al., 2018; Moghaddam et al., 1997). This surge in glutamate occurs at low, subanesthetic doses, which is believed to initiate processes that promote synaptogenesis and synaptic plasticity (Zanos & Gould, 2018). NMDARs also regulate the activity of GABAergic interneurons (Cohen et al., 2015), and evidence from preclinical studies show that ketamine administration modulates fast-spiking GABAergic interneuron activity in the PFC (Ali et al., 2020; Gerhard et al., 2020).

Prior human studies using proton (^{1}H) MRS in human volunteers similarly report changes in glutamatergic metabolites (glutamine and/or glutamate) and GABA within the mPFC and adjacent anterior cingulate (ACC) after ketamine infusion. For example, in healthy adults receiving ketamine (0.27 mg/kg, with a maintenance dose of 0.00225 mg/kg per minute over 2-hrs) investigators reported increased glutamine levels, a metabolic precursor for the synthesis of glutamate (Lebon et al., 2002), in the bilateral ACC (Rowland et al., 2005). Another study in healthy adults receiving similar dose (0.25/mg with a maintenance rate of 0.42 mg/kg over 1-hour) also observed an increase in glutamate in the ACC in within 1-hr of infusion (Stone et al., 2012). Further, in a larger placebo-controlled trial comparing MDD patients to healthy controls, significant increases glutamate/creatine ratios were observed in the pregenual ACC (pgACC) between baseline and 24-h after participants received subanesthetic (0.5 mg/kg) ketamine (Colic et al., 2019).

In patients with MDD, Carbon-13 MRS (^{13}C MRS) data provided evidence of enhanced glutamine to glutamate cycling in the mPFC during subanesthetic ketamine administration (Abdallah et al., 2018). Further, a larger follow-up clinical trial by the same group later reported a dose-dependent change in mPFC glutamate+glutamine (Glx) levels, but not GABA levels in MDD patients, which were linked with greater antidepressant response at 24-hrs post treatment (Matthew S. Milak et al., 2020). Further, evidence from a few investigations suggest lower pretreatment Glx/glutamate ratios (Salvadore et al., 2012) and GABA levels (M. S. Milak et al., 2016; Matthew S. Milak et al., 2020; Singh et al., 2022) in the mPFC and ACC might predispose patients with MDD towards a greater therapeutic response.

Fewer ^{1}H MRS studies have investigated GABA levels following ketamine infusion. The earliest study was performed in rats using ^{13}C magnetic resonance spectroscopy (MRS) in which increased mPFC GABA levels were detected in rats administered with 30 mg/kg of ketamine (considered a subanesthetic dose in rodents) within 2h (Chowdhury et al., 2012). In a later study, using ^{1}H MRS in a chronic unpredictable stress model of depression, ^{1}H MRS increases of GABA markers were detected in the medial prefrontal cortex (mPFC) 24 h post ketamine administration (Ghosal et al., 2020). In human patients with TRD, ^{1}H MRS data acquired from the mPFC before, during and shortly after 0.5 mg/kg ketamine infusion also showed an increase in Glx/water and GABA/water within 30 minutes of infusion, though associations with antidepressant response measured ~4-hrs post treatment were absent (M. S. Milak et al., 2016). Additionally changes in GABA have been reported in regions outside the mPFC. For instance, a recent study reported an increase in GABA levels in the ACC shortly after ketamine treatment in TRD (Singh et al., 2021). Further, changes in GABA to creatine (Cr) ratios have been reported in the hippocampus 2-h post-ketamine infusion (Silberbauer et al., 2020).

However, several MRS studies have failed to observe significant changes in glutamate, Glx or GABA after ketamine administration (Boucherie et al., 2023; Lener et al., 2017). For example, significant changes in glutamate, Glx or glutamine were not detected in the occipital cortex 3h and 72h after ketamine infusion in patients with MDD (Valentine et al., 2011). Another MRS investigation targeting the ACC in healthy subjects similarly failed to detect increases in glutamine, glutamate or Glx (Taylor et al., 2012). Since all depression studies reporting null findings included small samples (n<20 receiving ketamine) impacting statistical power, ketamine's effects on glutamate and GABA remain inconclusive. Notably, ketamine has a short elimination half-life of 2-3-hrs in humans (Clements et al., 1982). Thus, early changes in glutamate or GABA may reflect the pharmacological effects of ketamine rather than neural changes associated with its antidepressant effects, which usually peak ~24 h post infusion (Murrough et al., 2013; Zarate et al., 2006).

To clarify and expand on previous findings, the current investigation sought to address whether changes in glutamate and GABA occur at the approximate peak of ketamine's antidepressant effects at ~24-hrs. Using an open-label study design, symptom ratings and MRS data were acquired prior to and 24-hours after 60 patients with MDD received a single infusion (0.5kg/mg) of racemic ketamine (**Figure 3.1**). Single-voxel MRS was acquired in the dACC, a region widely implicated in the pathophysiology of MDD and antidepressant responses to ketamine (Alexander et al., 2021). Based on a convergence of clinical and pre-clinical evidence, we hypothesized that ketamine administration would be associated with increases in the levels of glutamate and GABA 24-h post-treatment and that changes in these neurotransmitter levels would associate with ketamine's rapid antidepressant effects. Further, we investigated whether the levels of glutamate and GABA prior to treatment may influence clinical outcomes as has

been suggested in some prior investigations (M. S. Milak et al., 2016; Matthew S. Milak et al., 2020; Salvadore et al., 2012; Singh et al., 2022). Finally, due to their clinical relevance, we explored whether changes in metabolites including *N*-acetylaspartate+*N*-acetylaspartylglutamate (tNAA), and phosphocreatine+creatine (tCr) and total choline (tCho) changed in association with ketamine treatment and antidepressant response, including separate indices of apathy and anhedonia.

MATERIALS AND METHODS

Participants

Participants, aged between 20 and 65 years, were recruited from the Southern California region through advertisements (paper and digital) and from clinician referral as part of an NIH-funded project (MH102743) investigating brain-based biomarkers of ketamine response in patients with TRD. For study participation, enrolled subjects were required to be in good physical health, showing normal physiological screens for blood pressure, electrocardiogram, liver function and illicit drugs. Eligible individuals were required to be experiencing a DSM-5-defined major depressive episode (American Psychiatric Association, 2013) for at least 6-months and to have failed at least two prior antidepressant medication trials of adequate dose and duration established by clinical interview. Exclusion criteria included (1) any neuromodulation therapy (e.g., electroconvulsive therapy, transcranial magnetic stimulation) within three months of study entry, (2) depression related to a medical condition, (3) depression onset after 50 years of age, (4) dementia, (5) neurodevelopmental disorders, (6) schizophrenia, schizoaffective or psychotic disorders, (7) a history of convulsions or withdrawal seizures and (8) pregnancy or suspicion of pregnancy in females. Antidepressant use (excluding drugs with effects on NMDAR function) were permitted to continue during study participation if stable for at least six weeks

prior to treatment. Signed and informed consent was obtained according to guidelines set by the Institutional Review Board at the University of Los Angeles, California (UCLA). **Table 3.1** describes the demographic and clinical characteristics of all participants.

Study Design

Ketamine administration

R,S-ketamine hydrochloride diluted in 60 cc of normal saline was administered intravenously though a pump at a rate of 0.5 mg/kg over 40 minutes in a private room at the Clinical Translation Research Center or Ronald-Reagan Hospital at UCLA and included continuous clinical and hemodynamic monitoring. Psychotomimetic effects, blood pressure, blood oxygen saturation, heart rate, and respiratory rate were monitored by a psychiatrist during the infusion and for 3-hrs after by a trained nurse.

Clinical assessments

The 17-item version of the Hamilton Depression Rating Scale ($HDRS_{17}$) (Hamilton, 1960) was used as the primary measure of clinical response. A 50% or greater reduction in $HDRS_{17}$ score from baseline to 24h post-treatment was used to define treatment responders (Coyle & Laws, 2015). Using specific items from the $HDRS_{17}$ scale, we further explored whether specific depressive symptoms, including apathy and anhedonia, may be preferentially targeted by ketamine. Apathy measures included items that described reduced interest in activities (item 7), psychomotor retardation (item 8), and general somatic symptoms (item 13) (Marin et al., 1991). Anhedonia measures captured one overlapping feature within the apathy measure (item 7), and included measures of guilt (item 2), gastrointestinal somatic symptoms (item 12) and impaired libido (item 14) (Treadway & Zald, 2011).

MRI data acquisition

All imaging data was collected at the Ahmanson-Lovelace Brain Mapping Center (UCLA) on a Siemens 3T Trio or PRISMA-FIT scanner (Siemens, Enlargen, Germany) using a 32-channel receiver head coil. First, high-resolution T1-weighted images were collected using a multi-echo magnetized-prepared rapid gradient-echo (MPRAGE) sequence (TR = 2500 ms, TEs = 1.81, 3.6, 5.39, 7.18 ms, flip angle = 8^0, field of view = 256 mm, 208 slices, voxel resolution = 0.8 mm isotropic). After reslicing the T1 into sagittal, axial, and coronal planes, a 20 x 30 x 40 mm^3 MRS voxel (x, y, z) was then positioned in the midsagittal dACC to surround the upper and lower banks of the cingulate sulcus maximizing the coverage of gray matter. The long axis of the voxel was aligned parallel to and extended posteriorly from the genu of the corpus callosum (**Figure 3.2**). For both scanners, single voxel proton MRS (^{1}H-MRS) data was collected from the dACC using the same Siemens WIP (#859G) MEscher-GArwood Point RESolved Spectroscopy (MEGA-PRESS) sequence (TR/TE = 2000/68, flip angle = 90^o, edit-on pulse bandwidth = 45 Hz, 128 transients) with editing pulses at 1.9 ppm and 4.7 ppm. This MRS sequence is a spin-echo version (Ogg et al., 1994) of the J-coupled difference editing sequence (Mescher et al., 1996) with one acquisition including the inversion of GABA at 1.9 ppm and one acquisition without inversion to allow J evolution of GABA (Henry et al., 2001; Mescher et al., 1998, 1996). Notably, baseline and follow-up scans were acquired on the same scanner system within subjects (Trio or Prisma).

MRS data processing

Acquired MRS spectra were processed with LCModel (Version 6.3-1L, Stephen Provencher, Oakville, ON, Canada), using a basis set optimized for GABA and glutamate (Provencher, 2001). LCModel outputs were inspected for visible artifacts (e.g., baseline

distortion, motion artifacts, lipid contamination) before using quantitative measures of signal quality, including signal to noise (SNR), linewidth (full-width at half maximum) and Cramer-Rao lower bound values (CRLB) for quality control. Based on thresholds commonly used in the literature, any spectra with an SNR less than 20 and/or full width at half maximum value > 24 Hz were excluded from analyses. Five metabolites were extracted: namely, glutamate, GABA, tNAA, tCr, and tCho. A CRLB value of 10% or greater was established as the threshold to exclude metabolites with high variance. Metabolites were corrected for cerebrospinal fluid (CSF) obtained by performing tissue classification from the T1 image from each subject on which the MRS voxel was prescribed (Njau et al., 2017).

Statistical Methods

First, the general linear mixed model (GLMM) using a compound symmetry covariance structure for repeated measures and restricted maximum likelihood for model estimation tested for pre-to-post treatment changes in glutamate and GABA levels. This model included effects of time (baseline vs. 24h post-treatment) and response group (responder vs. non-responder) as fixed factors and a term for time by response group interactions with a random intercept included for subject. Thereafter, pairwise comparisons (paired t-tests) were performed within treatment responders and non-responders separately. Effect sizes were calculated using partial eta squared. Given that changes in glutamate and GABA levels were the primary measures of interest, these analyses were performed without correction for multiple comparisons.

To determine if pretreatment levels of glutamate or GABA relate to clinical response at 24-h post-treatment, baseline MRS measures were included as predictors in separate simple linear regression models with clinical improvement based on percent change in $HDRS_{17}$ score as the outcome.

In exploratory analyses, ketamine-related effects for additional metabolites that may serve as markers of neuroplasticity, including tNAA, tCr and tCho were included as dependent measures using similar GLMMs. Here, post-hoc pairwise comparisons were only performed for metabolites showing main effects of time or a significant time-by-response-group interaction. For these analyses, a Bonferroni correction was used to address multiple comparisons where p-values of less than 0.016 (0.05/3 = 0.016) were considered as the threshold of significance. Finally, we also computed Pearson product-moment correlations between changes in glutamate or GABA and subcomponents of the $HDRS_{17}$ which capture specific symptom domains of MDD, namely apathy and anhedonia (**Table 3.6**). All statistical tests were two-tailed. Statistical analyses were performed in SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Armonk, NY: IBM Corp).

RESULTS

Clinical measures

Clinical and demographic information is provided in **Table 3.1**. All subjects were currently experiencing a major depressive episode; 56 subjects had a primary clinical diagnosis of unipolar MDD, and 4 participants had a diagnosis of bipolar depression. The mean duration of current depressive episode was 22.85 years (15.12 SD). Forty-six subjects were receiving standard antidepressant medications that had remained stable for at least 6-weeks prior to enrollment. Twelve patients were not receiving antidepressant treatment. Change in $HDRS_{17}$ scores at 24-h post-infusion compared to baseline were highly significant ($t_{59} = 9.64$, $p < .0001$). Symptoms of apathy ($t_{59} = 7.54$, $p < .0001$) and anhedonia ($t_{59} = 6.00$, $p < .0001$) were also improved significantly from pre-to-post treatment (means provided in **Table 3.2**). Based on a ≥

50 % reduction in $HDRS_{17}$ scores at 24 hours, 25 participants achieved antidepressant response and were defined as treatment responders.

Quality of ^{1}H- MRS spectra

MRS data was not collected for one participant at the 24h follow-up visit, and MRS data from two other timepoints were incomplete and could not be analyzed. Based on our quality control thresholds, five additional MRS data points were excluded. Mean and standard deviation SNR values for included non-edited spectra were 32.39±3.91 at baseline and 32.09±3.49 at the 24-hour follow-up. The mean and standard deviation values of CRLB bounds were below our 10% threshold as follows at (a) baseline (Glutamate = 7.04±1.52, tNAA = 1.44±0.50, tCr = 1.79±0.45, tCho = 2.04±0.38) (b) at the 24 h follow-up (glutamate = 6.96±1.39, tNAA = 1.45±0.50, tCr = 1.80±0.40, tCho = 2.02±0.36). For the difference spectrum (i.e., GABA), CRLB values were 6.52±2.49 at baseline and 6.25±1.13 at the 24-hour follow-up visit. Quality control metrics are provided in **Table 3.5**.

Effects of ketamine on glutamate and GABA levels

The GLMM testing for main effects of time, response status and time-by-response interactions revealed trend level increases in glutamate levels in the dACC pre-to-post ketamine ($F_{1,51.23} = 3.27$, $p = .08$) and a trend for response status across time ($F_{1,56.18} = 3.86$, $p = .06$); time-by-response interactions were below the threshold of significance ($F_{1,51.23} = 2.55$, $p = .12$). However, planned post-hoc comparisons within treatment-responders examined separately revealed significant treatment effects ($t_{19} = 3.07$, $p < .01$, partial eta-squared (ηp^2) = 0.32) that were not observed in non-responders ($t_{39} = 0.164$, $p = .87$, $\eta p^2 = 0.00$). Conversely, GABA levels appeared relatively stable over time ($F_{1,53.93} = 0.02$, $p = .90$), did not vary response ($F_{1,53.94} = 0.70$, $p = .41$), or show time-by-response interactions ($F_{1,53.93} = 0.31$, $p = .58$). Planned post-hoc

analyses showed that GABA levels were not significant in treatment-responders (t_{19} = 0.38, p = .71, ηp^2= 0.00) or non-responders (t_{30} = 0.76, p = .45, ηp^2=0.02). **Table 3.3** provided means and standard deviations for GABA and glutamate across all participants and **Figure 3.3** illustrates the means graphically. **Table 3.4** includes means for treatment responders and non-responders separately which are displayed as spaghetti plots across time within subjects in **Figure 3.4.**

Relationships between baseline levels of glutamate and GABA and clinical response

Lower baseline measures of glutamate predicted greater improvements in $HDRS_{17}$ scores at 24=h, r = .43, p = .008, but these relationships were absent for GABA(r = -.23, p = .10 (**Figure 3.6)**.

Effects of single ketamine on tNAA, tCr and tCho

GLMM models exploring effects of other ^{1}H-MRS derived compounds: tNAA, tCr and tCho indicated significant effects of time for tNAA ($F_{1,50.46}$ = 6.06, p = .005) and tCr ($F_{1,50.53}$ = 9.25, p = .004), but not tCho ($F_{1,51.30}$ = 4.369, p = 0.04) after Bonferroni correction (**Table 3.3** and **Figure 3.6**) . GLMMs did not find a significant treatment by response interactions for NAA, $F_{1,50.11}$ = 6.08, p = .017 and Cho, $F_{1,51.30}$ = 5.91, p = .019, or not tCr ($F_{1,.50.50}$ = 6.91, p = .011), after using the Bonferroni adjusted p-value of 0.016.

Relationships changes in ^{1}H-MRS measures and apathy and anhedonia

Exploratory analyses did not show any significant relationships between change glutamate, GABA, tNAA, tCr and tCho in association with improvements in symptoms of apathy and anhedonia as derived from the $HDRS_{17}$ after correcting for multiple comparisons (**Table 3.6**).

DISCUSSION

There is growing evidence that the ACC serves as a key locus of ketamine's antidepressant effects (Alexander et al., 2021). Here, we measured levels of glutamate and GABA within the dACC in 60 patients with TRD before and 24-h after the administration of intravenous subanesthetic ketamine. Forty-two percent of patients were defined as treatment responders (i.e., they exhibited ≥50% reduction in HDRS scores post infusion). In responders, but not non-responders, significant increases in glutamate levels were observed from baseline to 24-h post-treatment. Across all participants, we also found that lower pretreatment glutamate concentrations in the dACC were associated with greater antidepressant effects. These results suggest that ketamine modulates glutamate transmission well after it has been metabolized in the body, and these more lasting changes contribute to its antidepressant effects. Lower glutamate levels prior to treatment could also be an indicator of future therapeutic response. In contrast, GABA levels appeared stable pre-to-post treatment and did not relate to clinical outcomes when measured at baseline. Interestingly, our results also reveal that other neurometabolites, including tNAA and tCr, which reflect neural integrity and metabolism, are modulated by ketamine and could potentially contribute to its clinical effects. Lastly, though we were able to show that ketamine improved measures of apathy and anhedonia, and prior studies have shown relationships between improvement in reward-related symptoms of depression and dACC activity (Lally et al., 2015), we failed to observe significant relationships between change in any of the measured MRS markers and change in HDRS item-level indices of apathy and anhedonia after correction for multiple comparisons (**Table 3.6**).

Dysfunction in the homeostasis of glutamate and GABA has been proposed to play a central role in the pathophysiology of MDD (Duman et al., 2019; Sarawagi et al., 2021).

Numerous MRS studies also support that dysfunction in excitatory and inhibitory neurotransmission are present within mPFC circuits including the ACC in MDD. For example, a meta-analysis of cross-sectional data in patients with depression and non-depressed controls revealed significantly lower levels of GABA and Glx in the ACC and mPFC in patients with MDD compared to controls (Godfrey et al., 2018). Evidence from an independent meta-analysis further showed that lower mPFC glutamate levels occur in patients with greater treatment resistance (Moriguchi et al., 2019). Since the current study included patients who were defined as TRD, our observations showing that lower glutamate levels in the ACC might predispose greater clinical response fit with the context of these previous cross-sectional findings.

Change in glutamate and GABA levels 24-h post-ketamine

Results showing increased glutamate in treatment responders at 24-h post-infusion suggest that ketamine produces a lasting change in neurotransmission to impact clinical outcomes. Several previous studies have examined glutamate or GABA levels ≥24 hours after ketamine infusion. For example, significant increases in glutamine/glutamate ratios have been reported in the pgACC 24-h after ketamine administration in healthy subjects (M. Li et al., 2017), observations that align our findings. However, an independent clinical trial by the same group failed to replicate these results in patients with MDD (n=20) or in controls (n=17), though it is worth noting that mean increases in glutamate 24-h post-ketamine were detected (Evans et al., 2018). Another study measuring glutamate, glutamine, and GABA within the occipital cortex at 3h and 48h post-infusion in MDD (N=10), also failed to show changes post-infusion or relationships between these MRS measures and clinical response (Valentine et al., 2011). In the current study, we likewise failed to observe significant changes in GABA pre-to-post ketamine or any relationships between GABA concentration and clinical response. Other recent MRS

studies of GABA in MDD measuring ketamine response at least 24-h post infusion similarly yield null findings with regard to changes in GABAergic neurotransmission (Boucherie et al., 2023; Lener et al., 2017; Zavaliangos-Petropulu et al., 2023) suggesting GABA does not serve as a meaningful biomarker. However, several challenges are associated with the measurement of GABA in-vivo, including its low concentration requiring larger voxel sizes for sufficient SNR and its overlap with other metabolites in the spectra. Thus, it remains possible that changes in GABA also occur and persist following low-dose ketamine administration, but are less detectable with MRS. There are several other factors that could explain discrepancies in findings between previous MRS studies examining glutamate and GABA in MDD following ketamine therapy. Foremost, most all prior studies in MDD have included small sample sizes ($N \leq 20$) that decrease the likelihood of detecting changes of smaller effects. Methodological differences related to MRS sequence parameters and magnet field strength, variations in voxel placement (e.g. dACC, pgACC, occipital cortex), and variations in analysis strategies such comparing absolute metabolite concentrations or relative to creatine or other ratios, may also contribute to mixed results.

Despite the discrepancies amongst prior MRS studies, our observations of lasting changes in dACC glutamate levels in ketamine responders are complementary to findings reported with other neuroimaging modalities (Lener et al., 2017; Zavaliangos-Petropulu et al., 2023). For instance, using task fMRI, prior studies have demonstrated changes in ACC activation amongst other areas, which appear to correlate with antidepressant response to ketamine (Alexander et al., 2021; Kotoula et al., 2023; Salvadore et al., 2009; Zavaliangos-Petropulu et al., 2023). Similarly, resting state fMRI studies have shown that ketamine modulates large-scale brain networks such as the salience network (including the ACC and insula), default mode network and fronto-

parietal/central executive networks (Zavaliangos-Petropulu et al., 2023). Increased glucose uptake within the dACC and supplementary motor cortex have likewise been reported at 24-h post infusion with ^{18}FDG positron-emission-tomography (PET) (Chen et al., 2018). Interestingly, other fast-acting therapies in MDD also report changes in glutamate levels in the ACC in association with antidepressant response in TRD patients, including electroconvulsive therapy (ECT) , and repetitive transcranial magnetic stimulation (rTMS) (Gonsalves et al., 2022).

Associations between pretreatment glutamate and GABA levels and clinical response

Results from the current study also revealed that pretreatment dACC glutamate were correlated with antidepressant response to ketamine infusion (**Figure 3.6)**. This finding suggests that glutamate levels at baseline may influence the strength of antidepressant response to ketamine. These results are also in concordance with at least two previous MRS studies reporting that pretreatment glutamate in the DLPFC (Salvadore et al., 2012) and mPFC (Matthew S. Milak et al., 2020) associate with antidepressant response to ketamine. However, others have failed to find associations between pretreatment glutamate and clinical response (Evans et al., 2018).

Effects of ketamine on other metabolites

An auxiliary outcome from our analysis is that we were able to characterize the effects of ketamine on additional metabolic compounds that may capture treatment-related changes that complement our understanding of ketamine's antidepressant action. Specifically, we found increases in levels of tNAA and tCr, which are shown to reflect changes in neural integrity and increased cellular energy demands. These findings might reflect the rapidly occurring synapto- and dendro-genesis that occur via activation signaling pathways downstream to NMDAR activity (N. Li et al., 2011). Of potential relevance, we previously reported changes in tNAA and

increased tCr in the dACC in patients with TRD in association with ECT (Njau et al., 2017). Increased levels of tCho may reflect membrane turnover due to changes in the postsynaptic circuit.

Limitations

The current study includes several limitations, both methodological and clinical. For example, though our MRS sequence was specifically designed to isolate GABA by suppressing the much larger overlapping creatine signal, as well as to detect glutamate and other high concentration metabolites in the spectra, these estimates do not distinguish between intracellular and extracellular compartments in the brain. Though high SNR and narrow line widths were observed in our data, we acknowledge that imaging performed at higher field strength (e.g., 7T) may better resolve overlapping signals and thus be more sensitive to detecting changes in GABA and glutamate. Further, in this study we focused on the dACC due its role in mood and cognitive dysfunction (Etkin et al., 2011), observations of increased ketamine-related brain metabolism (Holcomb et al., 2001; Javitt et al., 2018; Långsjö et al., 2003) or activity (Alexander et al., 2021) in the dACC, as well on prior MRS results (Boucherie et al., 2023) and the suggested role of the dACC in antidepressant response (Godlewska et al., 2018). However, it remains possible that other brain areas (e.g, ventral striatum, hippocampus, etc.) may better indicate changes in neurochemistry related to the antidepressant effects of ketamine.

Though our study is the largest investigation to date (N=60) to examine the effects of ketamine treatment on MRS markers of excitatory and inhibitory neurotransmission in TRD, statistical power may still be lacking. In particular, despite finding changes in glutamate in ketamine responders of medium to large effect size ($\eta p^2 = 0.32$), we still failed to observe a significant interaction across time with response status. Our naturalistic study design also poses a

potential limitation since placebo effects cannot be excluded. However, since the objective of this investigation was to examine imaging biomarkers related to ketamine's antidepressant action, results should be less impacted by participant expectations unless inducing changes at the physiological level. Also, is it possible that antidepressant medications may have impacted our results. Notably, since medications were unchanged at least 6-weeks prior to enrollment and participants served as their own controls in longitudinal analysis, our within subject analysis did control for this potential confound.

Finally, it is important to note that the mechanisms associated with ketamine's therapeutic effects likely extend beyond glutamate and GABA neurotransmission. For example, low-dose ketamine is found to modulate dopaminergic and serotonergic neurotransmitter systems targeted by standard antidepressants (Can et al., 2016; Kraus et al., 2019) as well as the opiate system (Sanacora & Schatzberg, 2015; Williams et al., 2018).

Conclusion

The results of this study suggest that subanesthetic ketamine infusion may induce antidepressant effects through the modulation of glutamate in the dACC, which coincides with the timing of peak therapeutic response. Specifically, we demonstrate that glutamate levels are increased in individuals who exhibit more robust improvements in depressive symptoms 24-h post treatment. We also show that lower pretreatment glutamate concentration may serve as a biomarker of antidepressant response to ketamine. Though GABAergic interneurons are shown to maintain and/or normalize synaptic dysregulation in response to ketamine (Ali et al., 2020; Gerhard et al., 2020; Luscher et al., 2020), here we did not find evidence to support links between GABA and ketamine's antidepressant effects. Sufficiently powered placebo-controlled trials may further clarify the neurochemical signatures of lasting ketamine's therapeutic effects.

TABLES

Table 3.1 Demographics and clinical characteristics of study participants with some values displayed as mean±standard deviation

Patients with TRD, N	60
Age, years	39.55±10.42
Male/Female, %	35/25
Race	
Caucasian	50
Asian	2
Pacific-Islander/Native-Hawaiian	3
African American	3
Other	2
Responder/Non-responder[1], N	25/35
DSM-5 diagnosis (UD/BD), N	56/4
Age of First Depressive Episode, years	16.57±7.83
Age of First Treatment, years	21.35±7.86
Duration of Lifetime Illness, years	22.85±15.12
Duration of Current Episode, months	7.79±21.94
Medication Status	N
Unmedicated	12
Medicated[2]	46
SSRI	23
SNRI	16
NDRI/Other	29
Monoamine Oxidase Inhibitors	2
Other/Non-ADM	6
Lithium	0
Anticonvulsants	11
Antipsychotics	13
Stimulant	15

[1]Response is defined as $\geq$ 50% change on the 17-item Hamilton depression rating scale ($HDRS_{17}$) pretreatment to 24-h after treatment. [2] Medication data was incomplete for 3 participants. Though n=19 patients were taking benzodiazepines and n=6 patients were prescribed sleep medications, these medications were paused >24 hrs. prior to ketamine infusion and MRI acquisition. Abbreviations: 17-item - Hamilton Depression Rating Scale ($HDRS_{17}$); ADM: Antidepressant Medications; SSRI: Selective Serotonin Reuptake inhibitors (escitalopram, sertraline, fluoxetine); SNRI: Serotonin and Norepinephrine Reuptake inhibitors (venlafaxine, duloxetine); NDRI: Norepinephrine and Dopamine Reuptake Inhibitors; TCA: Tricyclic Antidepressants; Antipsychotics (typical and antitypical)

Table 3.2 Mood ratings pre and 24-h post ketamine infusion

	Baseline (N=60)	**24 h post-infusion (N=60)**
$HDRS_{17}$	19.42±5.14	11.15±5.46***
$HDRS_{17}$ - apathy	5.28±1.29	3.13±1.96***
$HDRS_{17}$ - anhedonia	6.63±1.50	4.28±2.60***

$HDRS_{17}$: 17-item Hamilton-Depression Rating Scale. *p <.05, **p<.005,***p<.0001

Table 3.3 Proton magnetic resonance spectroscopy (^{1}H-MRS) metabolite levels pre and 24-h post single ketamine infusion

Metabolite	**Mean±SD**	
	Baseline	24-h post
Glutamate	3.74±0.70	3.74±0.63
GABA	0.12±0.24	0.11±0.18
Creatine+phosphocreatine (tCr)	4.05±0.09	4.27±0.09**
N-acetylaspartate+N-acetylaspartylglutamate (tNAA)	6.37±1.23	6.63±1.13**
Glycerophosphocholine+Phosphocholine (tCho)	1.18±0.03	1.23±0.03*

*p <.05, **p<.005,***p<.0001

Table 3.4 Mean and standard deviation values at for glutamate and GABA at baseline and 24h post-infusion

	Responders				Non-responders			
	N	Baseline	N	24-h post	N	Baseline	N	24-h post
Glutamate	22	3.35±0.67	22	3.63±0.63	36	3.88±0.55	32	3.90±0.50
GABA	21	0.87±0.25	22	0.86±0.15	36	0.90±0.18	32	0.88±0.14

Abbreviations: N is the number of included datasets; GABA: γ-amino-butyric acid. P values are reported or indicated by *,** and *** for $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Significant difference at $P_{corr} < 0.05$.

Table 3.5 ^{1}H-MRS quality control indexes and dACC voxel tissue content

	Mean±SD	
Metabolite	**Baseline**	**24 post-treatment**
Cramer-Rao Lower Bound		
Glutamate	7.04±1.52	6.96±1.39
GABA	6.52±2.49	6.25±1.13
Creatine + phosphocreatine (tCr)	1.79±0.45	1.80±0.40
N-acetylaspartate +*N*-acetylaspartylglutamate (tNAA)	1.44±0.50	1.45±0.50
Glycerophosphocholine + Phosphocholine (tCho)	2.04±0.38	2.02±0.36

Linewidth	0.041±0.018	0.039±0.009
SNR	32.39±3.91	32.09±3.49
Voxel tissue content[1]		
Gray Matter, (%)	13031±193.04, (52.81)	12674.25±1334.52, (52.81)
White Matter, (%)	8397±60.81, (34.98)	8447.40±1399.39, (35.20)
Cerebrospinal Fluid, (%)	2929 ±253.85, (12.20)	2878.41±1734.97, (11.99)

[1] Total Voxel Tissue = 24,000 mm^3

Table 3.6 | Correlations between in ^{1}H-MRS measures, HDRS17 total score, HDRS-apathy and HDRS-anhedonia

	$HDRS_{17}$	$HDRS_{17}$: anhedonia	$HDRS_{17}$: apathy
Glutamate	r = 0.30, p = .042	r = -.24, p = .106	r = -.28, p = .058
GABA	r = .12, p = .427	r = -.25, p = .095	r = .01, p = .957
tCr	r = .30, p = .036	r = -.27, p = .067	r = -.34, p = .018
tNAA	r = .32, p = .022	r = -.27, p = .060	r = -.35, p = .018
tCho	r = .32, p =.025	r = -.27, p =.059	r = -.35, p =.015
Abbreviations: GABA = $_\gamma$-amino-butyric acid tCr = Creatine+ Phosphocreatine; tNAA =N-acetylaspartate+N-acetylaspartylglutamate; tCho=Glycerophosphocholine+Phosphocholine			

FIGURES

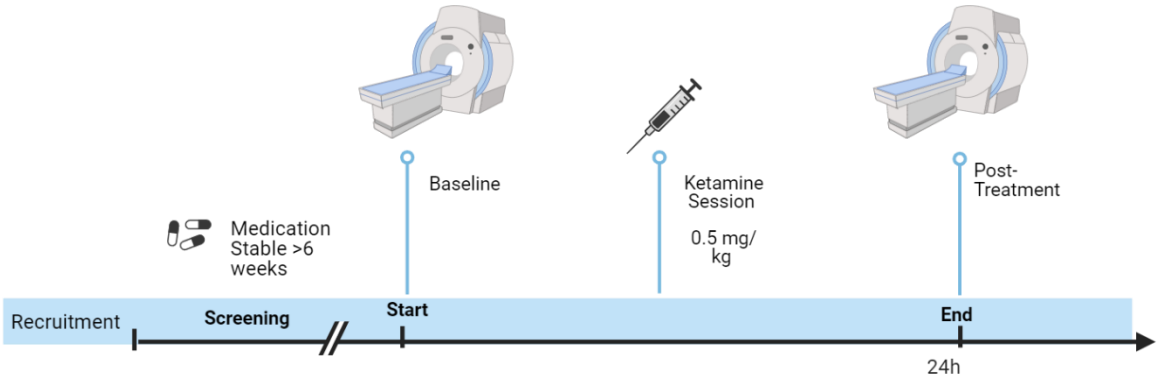

Figure 3.1 Study Design. Using a naturalistic study design, patients with TRD were scanned and received mood assessments within 1 week of receiving intravenous ketamine treatment and again 24-h following ketamine infusion.

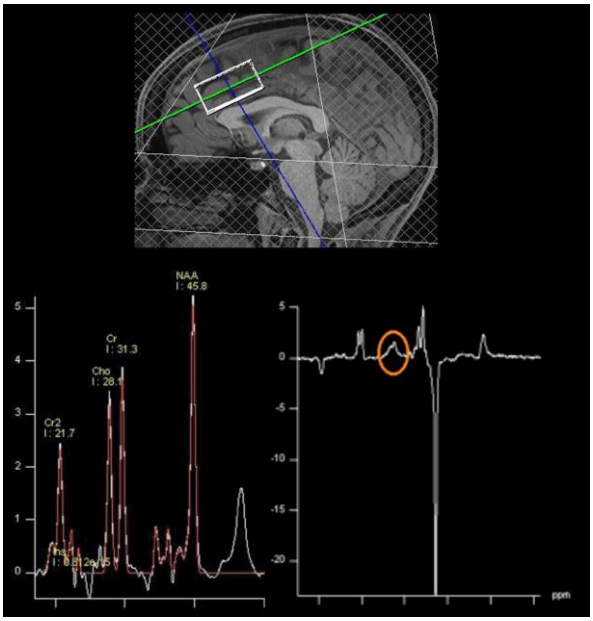

Figure 3.2. Top: Example MRS voxel positioning in the dACC. Bottom: Example of the unedited (left) and difference (right) spectra. The GABA doublet is indicated by the orange circle at 3 ppm.

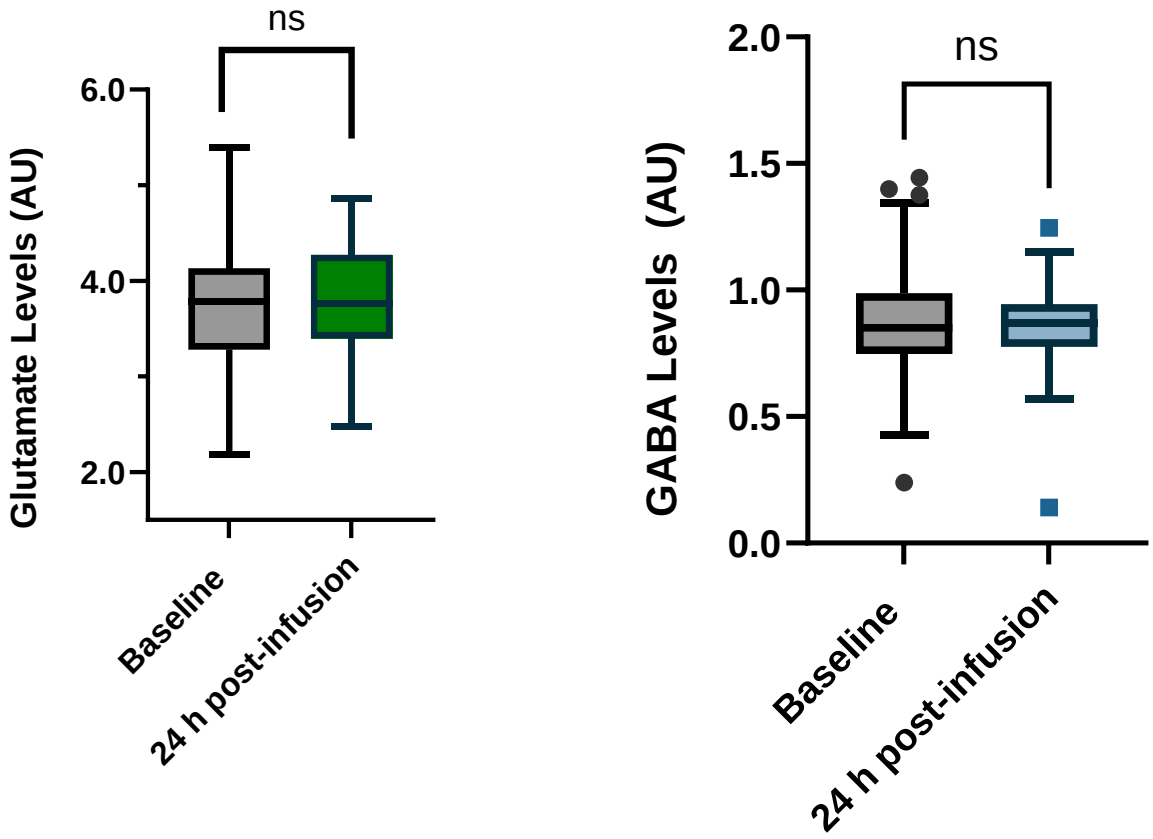

Figure 3.3 | Box plots showing mean concentration and standard errors of ^{1}H MRS glutamate and GABA levels at baseline and post-treatment across all patients. 'ns' indicates no significant difference across time points ($p>.05$), though differences in means for glutamate show trend level effects ($p=.06$). AU = arbitrary units.

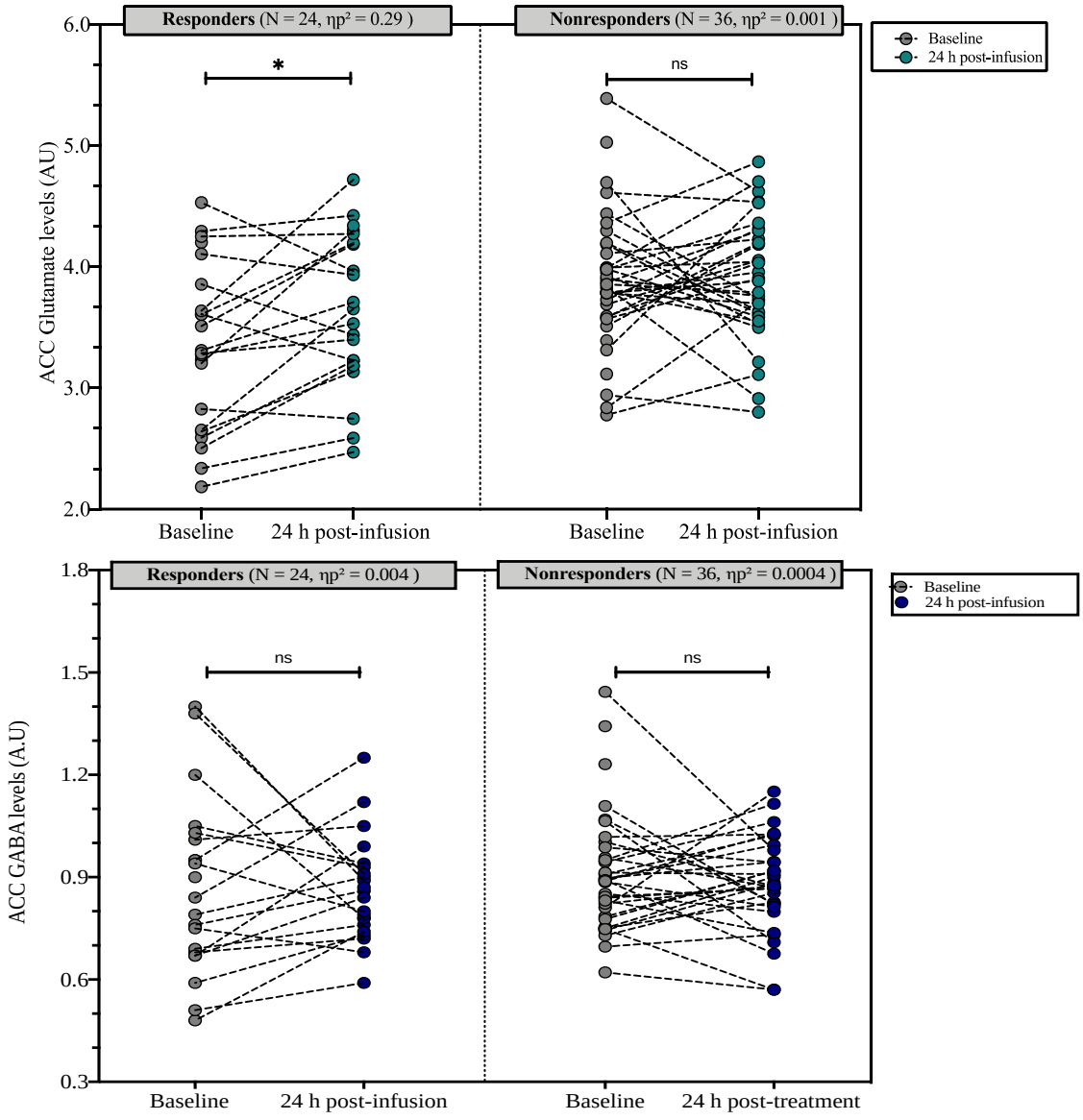

Figure 3.4 | Spaghetti plots showing effects of ketamine for [1]HMRS measured levels of glutamate (top panel) and GABA (bottom panel) in treatment-responders (R) and treatment non-responders (NR) at baseline and 24 hours after ketamine infusion. Lines connect the values from the individual subjects pre-to-post treatment. *indicates p<.01, ns indicates no significant effects (p>.05).

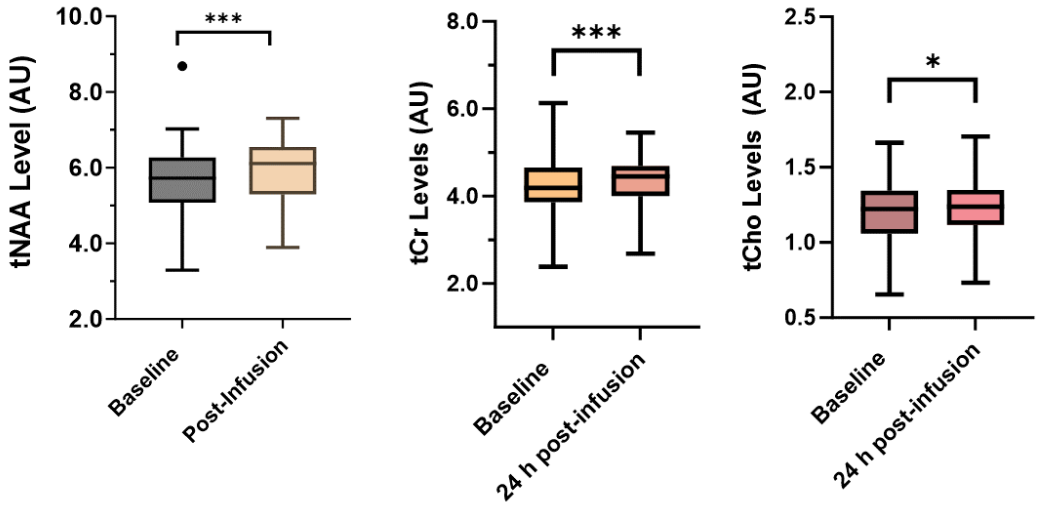

Figure 3.5 | Box plots (Tukey) for Creatine+ Phosphocreatine (tChr), N-acetylaspartate+N-acetylaspartylglutamate (tNAA) and Glycerophosphocholine+Phosphocholine (tCho) levels at baseline and post-treatment across all patients. AU = arbitrary units. P values are reported or indicated by *,** and *** for $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

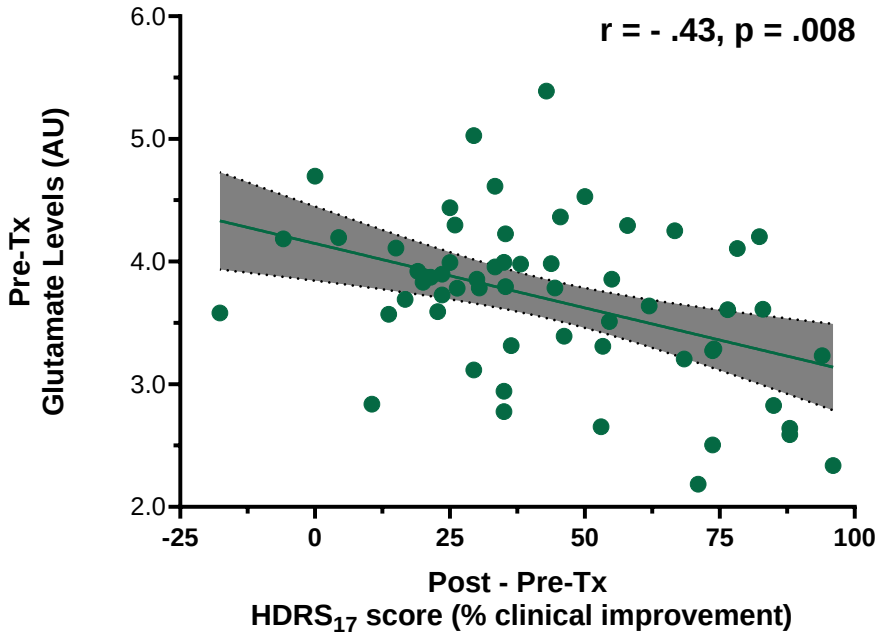

Figure 3.6 | Scatterplot of correlation between lower glutamate levels at baseline and clinical improvement 24h following single ketamine infusion. AU = arbitrary units. Clinical improvement was defined as percent change in the 17-item Hamilton Depression Rating Scale ($HDRS_{17}$) score at 24 h post-infusion relative to baseline.

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CHAPTER 4

Effects of Repeated Ketamine Infusions in Treatment-Resistant Depression: A Magnetic Resonance Spectroscopy Study

ABSTRACT

Repeated ketamine infusions enhance and prolong antidepressant response in treatment-resistant depression (TRD). Building upon the previous experiment, my second experiment explored the effects of repeated ketamine infusions on glutamate and GABA levels. Fifty volunteers with TRD were scheduled to receive four open-label infusions of subanesthetic ketamine. During each infusion, 0.5 mg/kg dose of ketamine was administered intravenously. Proton magnetic resonance spectroscopy (^{1}H MRS) scans were acquired at three time points: baseline, one to three days prior to first infusion, (TP1), 24 hours after the first infusion (TP2), and 48 – 72 hours after the fourth infusion (TP3). I measured the concentrations of several metabolites in the anterior midcingulate cortex (aMCC)/dorsal anterior cingulate cortex (dACC) and examined whether changes in neurochemistry or mood symptoms correlate with better clinical outcomes. To explore correlations between changes in neurometabolites and clinical improvement, we acquired the following behavioral measures: The 17-item Hamilton-Depression Rating Scale ($HDRS_{17}$), Snaith-Hamilton Pleasure Scale (SHAPS) and the Apathy Evaluation Scale (AES). Response was defined as a 50% or greater reduction on the $HDRS_{17}$. Results showed a mean increase in glutamate and GABA levels after the 1st and 4th ketamine infusion; however, these changes did not reach the threshold of significance. Percent changes in glutamate levels 24 hours post the first ketamine infusion were associated with improvements in $HDRS_{17}$ ($p < 0.05$). These results suggest that acute changes in glutamate relate to the antidepressant effects of ketamine in some correspondence with results from our prior experiment (Chapter 3). However, we failed to

replicate results indicating an increase of glutamate in ketamine responders, but not responders after single infusion or after serial ketamine treatment as examined here. Thus, it is possible that early changes in glutamate occurring during and shortly after ketamine infusion may trigger downstream molecular/cellular and systems-level changes to impact antidepressant response. These results indicate that glutamate as well as GABA levels following serial ketamine may not present a direct biomarker of ketamine response.

INTRODUCTION

Antidepressant medications, a first line of treatment for major depressive disorder (MDD), typically act by increasing the synaptic availability of serotonin, norepinephrine and/or dopamine (monoaminergic neurotransmitters) (Harmer et al., 2017). However, these drugs are typically slow to take effect (Gelenberg & Chesen, 2000) and a majority of patients do not experience an adequate improvement after their first treatment trial (Gaynes et al., 2009; Warden et al., 2007). Much evidence now supports that glutamate also plays an important role in antidepressant response (Duman et al., 2019; Khoodoruth et al., 2022; Sanacora et al., 2012). In particular, the noncompetitive, high-affinity NMDA receptor antagonist ketamine, has been shown effective for inducing robust and fast acting antidepressant effects (Coyle & Laws, 2015; Kishimoto et al., 2016; McGirr et al., 2015). Though the therapeutic effects of a single subanesthetic dose of ketamine usually wane within a week (Salloum et al., 2020), repeated treatment is shown to enhance and prolong antidepressant response (Kryst et al., 2020), and extend the time before relapse (Shiroma et al., 2020). For example, for the intravenous administration of racemic ketamine, typically dosed as 0.5 mg/kg infused over 40 min, 70-90% of participants were found to achieve remission following six ketamine sessions (aan het Rot et

al., 2010; Murrough et al., 2013). Several subsequent studies have since replicated these findings to show extended antidepressant response following repeated ketamine infusions (Kryst et al., 2020), where at least 3 ketamine infusions have been determined as optimal (Phillips et al., 2019).

Though ketamine alters glutamatergic signaling by antagonizing NMDA receptors on inhibitory GABA neurons (Gerhard et al., 2020), the modulation of downstream signaling pathways (Kraus et al., 2019; Zanos & Gould, 2018), other neurotransmitter systems (Kavalali & Monteggia, 2012) and circadian system regulation (Kohtala et al., 2021) appear to contribute to its antidepressant mechanisms. The dysregulation of GABA signaling is also implicated as contributing to MDD pathophysiology (Duman et al., 2019; Ghosal et al., 2017; Luscher et al., 2011; Perlman et al., 2021). Preclinical studies also show that ketamine alters GABAergic activity to benefit depressive-like behaviors (Cohen et al., 2015; Gerhard et al., 2020; Ghosal et al., 2020). Thus, the modulation of both glutamate and GABA independently or by way of interactions with other monoaminergic neurotransmitter systems may contribute to ketamine's mechanisms of antidepressant action.

Magnetic resonance spectroscopy (MRS) imaging is unique in its ability to measure neurochemical changes associated with *in vivo* drug administration. Ketamine has a short elimination half-life in adults (2-4 hrs.). Accordingly, prior MRS studies in MDD have mostly focused on examining ketamine's effects during (Abdallah et al., 2018; Stone et al., 2012) or within hours of drug administration (Milak et al., 2016; Milak et al., 2020). Since the antidepressant effects of ketamine are reported to peak at approximately 24 hrs. post administration when most of the drug is already cleared from the system, more lasting changes in glutamate, GABA or other metabolite concentrations occurring beyond 4-5 half-lives may better

represent changes in neurotransmission linked with ketamine's antidepressant effects. However, results from the few MRS investigations to address glutamate and GABA levels associate with ketamine's antidepressant effects beyond the drugs immediate effects on metabolism have been somewhat mixed (Zavaliangos-Petropulu et al., 2022), which may in part reflect differences in study designs and acquisition methods. Notably, in an independent sample, we previously found that patients who respond to single ketamine treatment show significant increases in glutamate concentrations at 24 hrs. post treatment, which are absent in non-responders (see Chapter 3). We further observed that lower levels of glutamate at baseline are associated with a greater reduction in depressive symptoms. Since repeated ketamine treatment leads to a more pronounced and lasting therapeutic response in MDD, it is thus possible that greater changes in glutamate or GABA may also be present after serial ketamine treatment.

The objective of the current investigation was to thus replicate these prior results in an independent sample and to examine how glutamate and GABA are modulated following repeated ketamine IV therapy and relate to antidepressant response in patients with TRD. Since ketamine is shown to reduce symptoms of anhedonia, which appear to outlast and be independent of overall antidepressant outcomes (Lally et al., 2015), we also examined whether change in MRS metabolites relate to ketamine's anti-anhedonic effects after ketamine treatment. Further, since improvements in depressive symptoms after single ketamine infusion may be a predictor of response to repeated ketamine infusion (Lipsitz et al., 2021), we tested whether baseline levels and changes in neurochemistry post single infusion may reflect response to repeated ketamine infusion. Finally, we explored whether changes in *N*-acetylaspartate plus *N*-acetylaspartylglutamate (tNAA), phosphocreatine (PCr) plus creatine (Cr) (tCr) and tCho levels changed pre-to-post single and repeated ketamine treatment. We hypothesized that repeated

ketamine infusions would have a more pronounced effect on glutamate and would relate with improvements in depressive symptoms. Understanding treatment-related effects in human participants could aid in the development of biomarkers of antidepressant response to ketamine and other novel antidepressant drugs.

METHODS AND MATERIALS

Participants

Participants were recruited between 2014 and 2019 for a naturalistic clinical trial investigating translational biomarkers of fast acting therapies in major depression (clinicaltrials.gov identifier no. = NCT02165449). Eligibility for this study required a DSM-5 current psychiatric diagnosis of major depressive disorder (MDD) (First et al., 2015), and 2) treatment-resistant depression (TRD), which was defined as failed response to at least two or more antidepressant medication trials of sufficient dose and duration and a depressive episode lasting at least 6-months. Exclusion criteria included: (1) depression onset after 50 years of age; (2) comorbid psychiatric disorders such as schizophrenia, schizoaffective or psychotic disorders and substance abuse; (3) dementia or neurodevelopmental disorders; (4) neurological problems including a history of convulsions or withdrawal seizures; (5) electroconvulsive therapy or other neuromodulation treatments in the past six months and (6) contraindications for ketamine treatment or magnetic resonance imaging (MRI) scanning. Participants were also screened for illicit drug toxicology, pregnancy and health conditions. Volunteers could continue treatment with any approved standard antidepressant medications so long as they remained consistent in the last 6 weeks. Benzodiazepines were not allowed within 24 hours of each infusion or brain imaging session. All volunteers provided informed consent following procedures approved by the University of California, Los Angeles (UCLA) institutional review board.

Study Design

Clinical and MRS data were acquired from TRD patients at three time points: (TP1) 1-3 days prior to treatment initiation (TP1), 24 hours after the first ketamine infusion (TP2) and 24 (or 72 in the case of an intervening weekend) hours after the last (i.e., fourth) ketamine infusion (TP3).

Ketamine Session

At each ketamine session, a solution of racemic (*R,S)*-ketamine hydrochloride was diluted in 60 cc normal saline. Ketamine was and administered intravenously at a rate of 0.5 mg/kg over 40 minutes. Vital signs were monitored by the administering physician and supporting clinical staff. Following the 1st infusion, participants received three additional infusions and the 4th infusion within 2-3 weeks. Ketamine infusions were performed as an outpatient procedure in a private room within the UCLA Clinical Translational Research Center or the Resnick Neuropsychiatric Hospital.

Clinical Measures

The primary measure of depressive symptoms in this study was the was assessed through the 17-item Hamilton Rating Scale for Depression ($HDRS_{17}$) (Hamilton, 1960). Self-reported measures of hedonic state were evaluated with the Snaith-Hamilton Pleasure Scale (SHAPS) (Snaith et al., 1995), and apathy was measured using the Apathy Evaluation Scale (AES) (Marin et al., 1991). The $HDRS_{17}$ was administered at the consultation visit, prior to study enrollment and repeated during the baseline visit, less than 2-3 days before the infusion. To adjust for small deviations in scores between the consultation and baseline visits, we averaged the two measures to obtain a composite measure of mood at baseline (T1). Follow-up ratings were obtained at 24 hrs. following single (T2) and serial infusion therapy (T3) immediately before scanning.

Response was operationalized as a percentage decrease of 50% or greater in $HDRS_{17}$ score at end of treatment relative to baseline. Remission was defined as a $HAMD_{17}$ score of 7 seven or less.

MRI data acquisition

Imaging data was collected on a Siemens 3T PRISMA-FIT system (Enlargen, Germany) with a 32-channel head coil at the Ahmanson-Lovelace Brain Mapping Center, UCLA. A high-resolution T1-weighted (T1w) volume using a multi-echo magnetized-prepared rapid gradient-echo (MPRAGE) sequence (TR = 2500 ms, TEs = 1.81, 3.6, 5.39, 7.18 ms; flip angle = 80, field of view = 256 mm, 208 slices, voxel resolution = 0.8 mm^3). This structural volume was used to position the magnetic resonance spectroscopy voxel (20 x 30 x 40 mm^3) using the mid-sagittal slice where we acquired spectra from the dorsal anterior cingulate cortex. Metabolite levels were estimated using the MEscher-GArwood Point-RESolved Spectroscopy (MEGA-PRESS) sequence (TR/TE =2000/68, flip angle = 90^o, edit-on pulse frequency or bandwidth = 1.9 ppm or 45 Hz, with water suppression (128 transients) and without water suppression (16 transients). MEGA-PRESS data was also collected without water suppression. The 'edit on' sequence at 1.9 ppm was interleaved with an 'edit-off' pulse at 4.7 ppm to acquire an additional dataset for the detection of GABA. To address this limitation of performing in vivo spectroscopy with conventional MRI scanners, we can apply techniques to suppress the water signals.

MRS data acquisition and metabolite quantification

To quantify GABA acquired with the MEGA-PRESS sequence all phase and frequency corrected 'ON' and 'OFF' spectra were averaged separately and subtracted to produce the difference-edited spectra. GABA resonates at 1.9, 2.3 and 3.0 ppm. We used the LCModel program (Provencher, 2001) (Version 6.3-1L, Stephen Provencher, Oakville, ON, Canada) to

quantify metabolite concentrations. LCModel performs quantification by fitting an observed spectrum to its corresponding predicted spectrum by applying a model based on a custom basis set which included multiple metabolites. The program was able to fit the unedited spectrum, edited spectrum and a difference spectrum using a basis set of spectra that was measured with the same acquisition sequence. These were computed as separate datasets. This program incorporated a basis set which modeled the resonance of protonated compounds within our tissue area (i.e., region of interest) restricted between 4.0 and 1.0 ppm. These included glutamine, *N*-acetylaspartate (NAA) (~2.01 ppm), *N*-acetylaspartylglutamate (NAAG) (~2.045 ppm), choline (~3 creatine (~3.03 ppm), phosphocreatine (~3.93 ppm) in addition to glutamate and GABA. The resolution of our scanner cannot differentiate between closely related compounds when their spectra exhibit high overlap. Thus, some compounds were quantified as composite peaks, in particular, NAA and NAAG as N-acetyl aspartate plus N-acetyl aspartyl glutamate (tNAA) and creatine and phosphocreatine as total creatine (tCr). (Phosphocreatine is the phosphorylation product of creatine and creatine and phosphocreatine are in fast, near equilibrium exchange readily reaching steady-state equilibrium). This is established practice for in vivo human brain proton spectra (Valentine et al., 2011). Also note that the tCr intensity in MR spectra can vary on a time scale of seconds; thus more reliable values of tCr might be measured with shorter echo-times.

We excluded data with a signal-to-noise ratio (SNR) less than 20 or a full-width at half maximum value greater than 24 Hz as these reflect measures of signal quality and spectral resolution. Individual metabolites were excluded that had a Cramér-Rao Lower Bound (CRLB) value less than or equal to 10%. (This is an estimate of the variance between the raw dat and the fit data). MRS data quality measures for included subjects are also reported in Table 4.2.

MRI data processing

To estimate metabolite concentrations, tissue volume fractions were calculated from a tissue-segmented anatomical scan. High-resolution T_1-weighted (T1w) structural volumes were processed using pipelines from the Human Connectome Project (HCP) (Glasser et al., 2013) and segmented to obtain the gray and white matter maps of brain tissue. Automated resampling of the SVS volume to the structural image volume was performed via executables in the Automated Imaging Registration Software library (Woods et al., 1998). First, we computed transformation matrices representing the positioning of the SVS volume and the MPRAGE structural volume during the acquisition. Second, we constructed a binary mask with the same dimensions as the SVS voxel (20 x 30 x 40 mm^3) using the FSL stats tool (FMRIB Software Library v4.0 (www.fmrib.ox.ac.uk/fsl) where the center represents the scanner coordinate origin. Third, the T1w volumes were pre-processed using the PreFreeSurfer minimal structural preprocessing for the HCP (Glasser et al., 2013). Thereafter, probability map of brain tissue content were estimated using BrainSuite software tools (Shattuck & Leahy, 2002) which segmented the structural volume into tissue fractions. Tissue maps (GM and WM and CSF) were calculated for each subject. The voxel mask was used to extract these values from the segmented structural volume. Thereafter, CSF correction in the voxel region was computed for each metabolite.

Statistics

To establish the cumulative effects of repeated ketamine infusions on glutamate and GABA levels, we fit a general linear mixed analysis of variance model (GLMM) to estimate treatment-related changes in glutamate and GABA. The model included participants as a random effect, time and response as fixed within-subject factors, the interaction term and the restricted maximum likelihood method was used for model estimation. We selected unstructured

covariances to estimate the effects of repeated measures as they are less constrained by assumptions regarding data structure. Based on our previous experiment (Chapter 3) demonstrating trending or significant differences in glutamate levels following the 1st ketamine session (TP1 versus TP2), we also performed post-hoc t-tests examining changes between the first (TP1) and 1st ketamine session (TP2), the 1st ketamine session and the last ketamine session (TP2 and TP3), and the 1st ketamine session (TP1 and the last ketamine session (TP3). Pearson's correlations were used to examine (1) correlations between acute change (T1-T2) and overall change (T1-T3) in glutamate and GABA and percent improvement on the $HDRS_{17}$ at the same time points and (2) correlations between baseline glutamate and GABA and percent improvement on the $HDRS_{17}$. In a secondary analysis we also analyzed the effects of ketamine on additional metabolites. Effect sizes were assessed using the Cohen d statistic. All *P* values were 2-tailed, and a significance was set at $P < .05$.

RESULTS

Quality of ^{1}H MR spectra

One study participant did not complete MRS data acquisition and was excluded from analysis. Scan data could not be collected from one participant at any point. Four datasets were excluded due to low SNR values or high CRLB. MRS data quality measures are summarized in Table 4.3. From amongst participants of this trial, forty-nine volunteers (17 females, mean age = 38.36 ± 10.71 (SD)) with TRD were included in the current study. From these participants, 45 MRS datasets could be used for the assessment of T1vT3 changes, i.e., correlates of repeated ketamine treatment.

Demographics

Table 1 documents demographic measures for participants who completed MRI/S scanning procedures. Five subjects withdrew from the study after the first infusion. See Table 1 for demographics.

Clinical effects of ketamine treatment

Repeated ketamine infusions significantly reduced depression symptoms as indexed through significant changes in HDRS scores ($F_{1.88, 87.78} = 88.03$, $p < .0001$). Twenty-six patients met response criteria as defined by >50% increase in $HDRS_{17}$ scores (mean change = 54.56%, HDRS mean change = - 8.69, 4.54 SD). Ketamine infusions also improved symptoms of anhedonia as measured through SHAPS ($F_{2, 93.27} = 11.829$, $p < .001$) and apathy via AES ($F_{2,47.01} = 32.28$, $p < .001$) (**Table 4.2; Figure 4.2**)

Effects of Repeated Ketamine on Glutamate and GABA Levels

Ketamine treatment had no significant effect on glutamate levels with time ($F_{2,36.80} = 0.148$, $p = .86$) and there was not a time-by-response interaction ($F_{2,36.80} = 0.529$, $p = .59$). Also, ketamine had no effect on GABA levels with time ($F_{2, 40.26} = 0.797$, $p = .46$). We also did not detect a time-by-response-group interaction ($F_{1,40.26} = 0.011$, $p = .99$) on GABA levels. The inclusion of age or gender as covariates did not affect these results. As a post-hoc analysis we examined effects of treatment within response groups independently. In treatment-responders, there was not a significant effect across time for glutamate levels, ($F_{2, 47} = 0.387$, $p = .68$) or GABA levels ($F_{2, 46} = 0.255$, $p = .78$). Also in non-responders, we did not find any effects for either glutamate levels ($F_{2, 30} = 0.054$, $p = .98$) or GABA levels ($F_{2, 30} = 1.108$, $p = .34$).

Associations between changes in glutamate and GABA and clinical response

Changes in glutamate showed a trend-level correlation with percent change in $HDRS_{17}$ scores after the first ketamine dose ($r = 0.28$, [95% CI -0.54, 0.01], $p = .06$), though correlations

between percent change in $HDRS_{17}$ after multiple infusions (TP1-TP3) were absent (r = 0.06, [95% CI -0.26, 0.36], p = .73) (**Figure 4.5**). Correlations between changes in GABA and percent changes in HDRS scores, and between glutamate or GABA with change in SHAPS or AES were not significant (Table 4.3).

Correlations between baseline glutamate and GABA levels and clinical response

Baseline measures of glutamate and GABA were not significantly correlated with percent changes in $HDRS_{17}$ scores after the first infusion (TP1 v TP2) or the fourth infusion (TP1 vTP3) of ketamine (all p> 0.05).

Effects of repeated ketamine treatment on other brain metabolites

Exploratory analysis did not reveal significant effects of ketamine treatment on tNAA levels ($F_{2,34.12}$ = 0.903, p = .42) and there was no time-by-response interaction ($F_{2,34.12}$ = 0.263, p = .77). Similarly, tCr and tCho levels did not show significant effects of time (tCr: $F_{2,34.74}$ = 1.268, p = .29, tCho: $F_{2,37.98}$ = 0.082, p = .92) or time-by-response interactions (tCr: $F_{2,34.75}$ = 0.535, p = .59, tCho: $F_{2,37.98}$ = 0.713, p = .50). Mean and standard deviation values at each time point are reported in Table 4.2. Statistical analyses and effect sizes are reported in Table 4.3.

DISCUSSION

This study investigated the effects of four infusions of ketamine on glutamate and GABA levels in the dACC. Consistent with previous studies, repeated ketamine infusions significantly improved depressive symptoms and symptoms of anhedonia and apathy. However, results failed to reveal significant increases in glutamate or GABA levels following 24 hrs. post single or serial ketamine infusion (**Figure 4.3**). Further, neither baseline measures of glutamate nor baseline GABA levels were associated with clinical improvement at the end of ketamine treatment. Still a

trending correlation between decreased glutamate levels 24 h after single ketamine treatment and clinical improvement was observed. Accordingly, the largest effect of ketamine on glutamate levels were observed after the first ketamine infusion (Cohen's d = 0.17), compared to between the 2nd scan and 3rd scan, observations that are generally consistent with findings reported in Chapter 3 for single ketamine infusion only.

Few studies have explored relationships between the effectiveness of ketamine in treating depressive symptoms and changes in glutamate or GABA more than 24 hrs. after serial ketamine treatment (Zavaliangos-Petropulu et al., 2023). Since ketamine is an NMDAR antagonist, increases in glutamate are found to occur during and shortly after ketamine administration (Moghaddam et al., 1997) (Stone et al., 2012). NMDARs also regulate the activity GABAergic interneurons (Cohen et al., 2015) and prior studies have also shown acute changes in GABA immediately following ketamine administration (Silberbauer et al., 2020) (Singh et al., 2021). However, our results suggest that these changes in glutamate and GABA do not persist to a significant degree after the drug has been metabolized. Still, it remains possible that changes in glutamate and GABA concentration occurring acutely during and immediately following ketamine set the stage for ketamine's subsequent and rapid therapeutic effects. Though we did not measure the metabolite changes during ketamine infusions, our results do not support that lasting changes or neuroplasticity in these neurotransmitter systems occur in the days following treatment. Our results may also indicate that glutamate or GABA measured with MRS is not a sufficiently sensitive measure of changes in neuronal activity or neurotransmitter cycling relating to ketamine treatment. We also note that ketamine did not alter the levels of NAA and Cr 24 hrs. post-infusion. These results contrast with some previous data where changes in NAA have been frequently argued to serve as a correlate of response to pharmacological treatment (Paslakis et

al., 2014) and furthermore, NAA levels in the dACC appear to normalize after successful treatment with electroconvulsive therapy (Njau et al., 2017).

These results somewhat align with prior experiments in animals. For example, in an early investigation on the effects of repeated administration of 25 mg/kg of ketamine, Lindefors et al. 1997 measured the effects of single and repeated ketamine administration on extracellular GABA levels in the mPFC using in vivo microdialysis though failed significant changes of cortical GABA levels (Lindefors et al., 1997). However, results showed increased dopamine and serotonin levels post ketamine (Lindefors et al., 1997) as partially consistent with a study showing repeated ketamine infusion induced an increase in activity in norepinephrine and dopamine neurons at 1 day post administration, though 3 days the increases in norepinephrine were no longer present (Iro et al., 2021). Interestingly, a separate investigation of repeated ketamine administration in animals showed decreased glutamate concentration in female, but not male mice (Thelen et al., 2016) though it is less clear how these results might translate to patients with TRD.

It is possible that changes in glutamate and GABA in response to ketamine may be restricted to neuronal populations or brain regions not measured in this study. For example, it has been demonstrated that GABA is reduced in parvalbumin interneurons of the PFC 2h after ketamine administration (Zhou et al., 2015). Further, repeated administration of S-ketamine (once a week for two months) in mice caused a loss of parvibillum interneuron immunoreactivity in in the medial prefrontal cortex and hippocampus (Yang et al., 2016). Regarding regional specificity of ketamine's effects, one study performed in a rat brain has investigating the long-term effects of ketamine found increased levels of purine and glycerophospholipid metabolism in

the hippocampus, striatum and prefrontal cortex (Chen et al., 2020) to indicate neurochemical changes may have occurred in regions outside of the voxel measured here.

Though we did not observe significant associations between change in glutamate or GABA with clinical response, there was a trending correlation between increases in glutamate levels and clinical improvements at 24h after the 1st ketamine infusion. These results are in accordance with our findings in an independent sample receiving single ketamine infusions reported in Chapter 3. Specifically, we showed patients with TRD that responded to single ketamine infusion demonstrated an increase in glutamate levels in the dACC that was absent in non-responders. Though the literature has shown changes in GABA levels with ketamine treatment, those findings were not replicated in our study.

Negative results were observed for other metabolites analyzed in our MRS dataset (tNAA and tCr) over time or in relation to ketamine's antidepressant effects. Thus, it is possible that neurochemical changes of interest are temporally restricted and the acute effects timing and design of MRS acquisitions may have precluded detection in any changes in glutamate or GABA. Consistent with this possibility, the Cohen's d effect size for tCr at 24h post single infusion was 0.4 compared to ~0.2 at the 3rd scan 48-72h within the fourth ketamine infusion (**Table 4.3**).

Thus far, only a few studies in small samples have shown GABA levels in MDD patients are reduced. In a meta-analysis there is a statistically significant reduction in GABA levels across varied cortical regions (Schür et al., 2016). This study is by far the largest MRS investigation with measurements of GABA level in depressed participants. Though GABA levels were largely not altered after ketamine treatment, GABA levels have been shown to increase in non-cingulate regions, namely the occipital cortex in response to treatment with selective

serotonin reuptake medications agents (Sanacora et al., 2002) and electroconvulsive therapy (Sanacora et al., 2003).

Limitations

As this was an open label study, we cannot exclude the possibility of a placebo effect. We also did not include a healthy control group for comparison of baseline differences in metabolite levels. Another reason we may not have observed a detectable change is that patients were allowed to continue antidepressant medication. It is plausible that given the interactions between monoaminergic antidepressant drugs and ketamine related glutamate/GABA systems may promote greater clinical improvement (Zhao & Sun, 2008). Methodological limitations of MRS may have impacted findings as other neuroimaging techniques may serve as better estimates of functional changes in glutamate response (Javitt et al., 2018). Changes in glutamate levels may reflect glutamate synthesis and release, as well as recycling into glia and neurons, and energy metabolism. By acquiring ^{1}H MRS data at 3T we could not resolve synaptic glutamate and glutamate release or estimate glutamine separately. Another plausible explanation for the null effects of ketamine on glutamate and GABA is that standard MRS methods can only quantify an estimate of the static levels of metabolites such that neurochemical measures reflect steady-state concentrations within a 6-10 min acquisition window, and thus we may not be able to assess changes in the dynamic of glutamate and GABA neurotransmission. Further, metabolite concentration estimates were made from a large area of the anterior cingulate cortex, which is required to obtain sufficient GABA signal from overlapping peaks in the MRS spectrum (Mullins et al., 2014). Using a smaller MRS voxel within the anatomical boundaries of the region of interest would potentially lead to different findings, though remain technical limitations common to MRS studies in general. Despite these methodological limitations, this study had

several strengths including a large sample size and high signal to noise ratio in the MRS data and consequently few MRS datasets were excluded from the analysis (Fleysher et al., 2009).

Summary and Conclusion

Our results do not support sustained changes in glutamatergic neurotransmission with repeated ketamine administration. However, in line with our previous results (Chapter 3), we did show a trend and larger effect sizes for change in glutamate after single ketamine despite glutamate levels trending back towards baseline values after the last ketamine session. Future research on repeated exposure to subanesthetic ketamine are still needed to establish a better understanding of a temporal relationship between ketamine infusions and glutamate levels. The inclusion of other imaging modalities may also help clarify the how neurochemistry relates to ketamine-induced effects in relevant neural circuits. For example, elevated neuronal activity in the anterior cingulate is one of several distributed neural regions that is shown to demonstrate maintained changes in neurotransmission at ≥ 24 hours post ketamine administration (Zavaliangos-Petropulu et al., 2022). Using arterial spin labeling, Sahib et al. 2020 reported an increase in cerebral blood flow after repeated ketamine in cingulate region (Sahib et al., 2020). Thus, future studies might combine MRS and functional imaging methods to better understand the underlying mechanisms of ketamine's antidepressant effects.

TABLES

Table 4.1 | Baseline demographics of study participants

N	47
Age, years	38.66 ± 10.71
Gender: M/F	30/17
Caucasian/Non-Caucasian, %	41/6, 86.0%
Responder/Non-Responders, %	26/19. 57.78%
Remitter/Non-Remitter, %	19/26, 42.2%
DSM-5 diagnosis: UD/BD	46/1
Age of first depressive episode, years	21.70 ± 8.42
Age of first treatment, years	17.10 ± 8.42
Duration of illness, years	23.09 ± 16.35
Duration of current episode, years	10.71 ± 23.38
$HDRS_{17}$ consult	20.16 ± 3.54
$HDRS_{17}$ baseline	19.12 ± 5.04
$HDRS_{17}$ averaged	19.64 ± 3.90

All demographics were based on self-report. Summary statistics are displayed as mean ± standard deviation. $HDRS_{17}$ = 17-item Hamilton-Depression Rating Scale; SHAPS = Snaith-Hamilton Pleasure Scale; UD = Unipolar Depression; BD = Bipolar Depression.[1]Responders demonstrated a $HDRS_{17}$ Scale score change of 50% or greater from baseline (TP1) to study-end (TP3).

Table 4.2 | Clinical and behavioral measures from study participants**.** Values are displayed as Mean ± SD.

Time Point	**T1**	**T2**	**T3**
N	**47**	**45**	**42**
HDRS	19.64 ± 3.90	12.52 ± 4.71	8.69 ± 4.54
SHAPS	7.45 ± 3.88	6.00 ± 5.48	3.16 ± 3.60
AES	48.32 ± 8.46	45.82 ± 8.78	42.71 ± 9.46

Abbreviations. SD = standard deviation; $HDRS_{17}$ = Hamilton-Depression Rating Scale (17-item); SHAPS = Snaith-Hamilton Pleasure Scale; AES = Apathy Evaluation Scale. N= included data; T1 = Baseline; T2 = 24 hours after single infusion; T3 = 24 -48 hours after the 4^{th} infusion.

Table 4.3 | ^{1}H-MRS data from the dACC after repeated ketamine infusions. Values are displayed as mean ± standard deviation.

	TP1	**TP2**	**TP3**
Glu	7.48 ± 1.00	7.57 ± 0.86	7.53 ± 0.90
GABA	1.74 ± 0. 30	1.73 ± 0.25	1.69 ± 0.21
tNAA	11.45 ± 1.49	11.67 ± 1.28	11.48 ± 1.13
tCr	8.36 ± 1.04	8.57 ± 0.87	8.44 ± 0.81
tCho	2.42 ± 0.33	2.45 ± 0.35	2.44 ± 0.32

Abbreviations: T= t-score; N= included data; T1 = Baseline; T2 = 24 hours after single infusion; T3 = 24 -48 hours after the 4^{th} infusion. Glu = glutamate; GABA = γ-amino-butyric acid; tCr = Creatine and Phosphocreatine; tNAA = *N*-acetyl-aspartate and *N*-acetylaspartate-glutamate; tCho = Glycerophosphocholine+Phosphocholine

Table 4.4 | Pairwise comparisons for ^{1}H-MRS data from the dACC after repeated ketamine infusion

	T1 v. T2			**T2 v. T3**			**T1 v. T3**		
	T	*p*	Cohen's d	T	*p*	Cohen's d	T	*p*	Cohen's d
Glutamate	0.91	.37	0.174	0.11	.91	0.020	-0.23	.81	0.050
GABA	0.73	.47	0.150	1.08	.29	0.229	1.35	.19	0.315
tNAA	1.69	.10	0.308	0.24	.24	0.209	0.39	.70	0.095
tCr	-2.24	.03	0.404	0.98	.33	0.165	-0.89	.38	0.210
tCho	0.99	.33	0.160	0.21	.84	0.300	0.42	.68	0.083

Abbreviations: T= t-score; *p* = p-value; N= included data; T1 = Baseline; T2 = 24 hours after single infusion; T3 = 24 -48 hours after the 4th infusion. GABA = γ-amino-butyric acid; tCr: Creatine and Phosphocreatine; tNAA: *N*-acetyl-aspartate and *N*-acetylaspartate-glutamate; tCho = Choline and glycophosphocholine

Table 4.4 | Mean tissue values and quality control metrics for proton magnetic resonance spectroscopy data

	T1	T2	T3
Tissue Values			
Grey Matter (%)	12990.41 ± 1026.59 (54.13 %)	12625.76 ± 1444.86 (52.61%)	12961.17 ± 949.45 (54.00%)
White Matter (%)	8358.59 ± 1108.05 (34.83%)	8438.35 ± 1458.84 (35.12 %)	8438.12 ± 1006.60 (35.15%)
Cerebrospinal Fluid (%)	2644.30 ± 1138.80 (11.02%)	2935.89 ± 1813.17 (12.23%)	2600.70 ± 1028.61 (10.84%)
Spectral Quality Measures			
PRESS Line-width (ppm)	.039 (0.01)	.038 (0.01)	.037 (0.01)
PRESS SNR (ppm)	33.49 (3.26)	33.22 (2.67)	32.88 (3.69)
MEGA-PRESS Line-width (ppm)	.024 (0.01)	.034 (0.01)	.033 (0.01)
MEGA-PRESS SNR	32.40 (4.21)	32.29 (3.68)	32.88 (3.93)
Cramer-Rao Lower Bound			
Glutamate	6.57 ± 1.08	6.57 ± 0.87	6.62 ± 0.96

GABA	5.95 ± 1.19	6.09 ± 1.16	6.00 ± 0.10
tNAA	1.32 ± 0.47	1.32 ± 0.47	1.38 ± 0.49
tCr	1.72 ± 0.45	1.76 ± 0.43	1.77 ± 0.41
tCho	6.57 ± 1.08	6.53 ± 0.89	6.00 ± 0.09

Values are displayed as mean ± standard deviation. Abbreviations: T1 = baseline; T2 = 24 hours after the first infusion and T3 = 24 – 72 hours after the fourth infusion. GABA" γ-amino-butyric acid; tCr: Creatine and Phosphocreatine; tNAA: *N*-acetyl-aspartate and *N*-acetylaspartate-glutamate; tCho: Glycerophosphocholine+Phosphocholine (tCho)

FIGURES

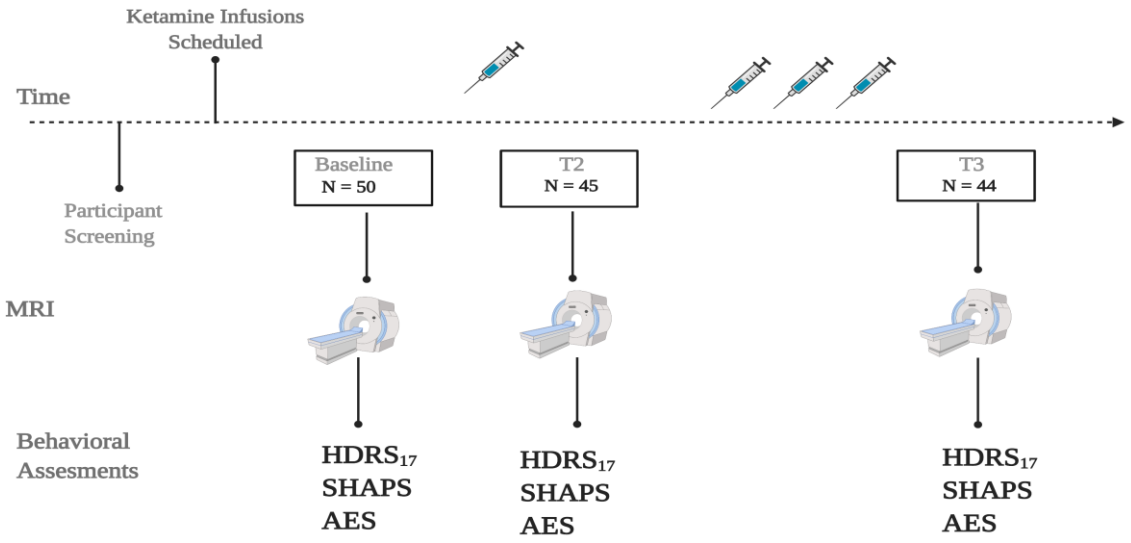


Figure 4.1 | Study Design. We collected magnetic resonance imaging data and behavioral measures at pre-treatment (baseline), 24-hours after a single dose of ketamine (T2) and 24 to 72 hours following a fourth ketamine infusion (T3). 17-item version of the Hamilton Rating Scale for Depression ($HDRS_{17}$). Snaith-Hamilton Pleasure Scale (SHAPS); Apathy Evaluation Scale (AES). Behavioral measures/ratings were obtained on the same day as scanning. Study duration ranged between 2 and 2.5 weeks depending on when the fist infusion was administered.

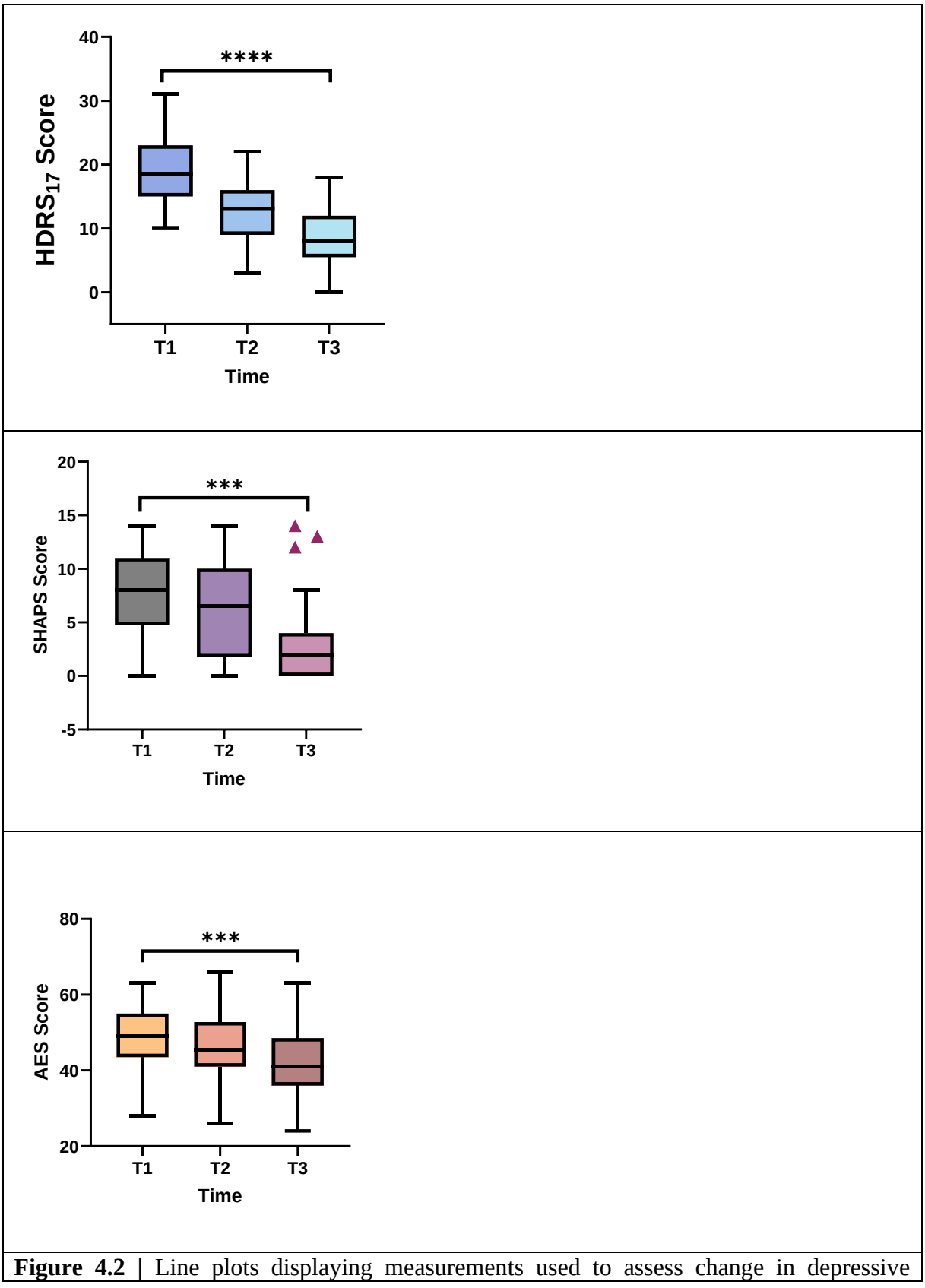
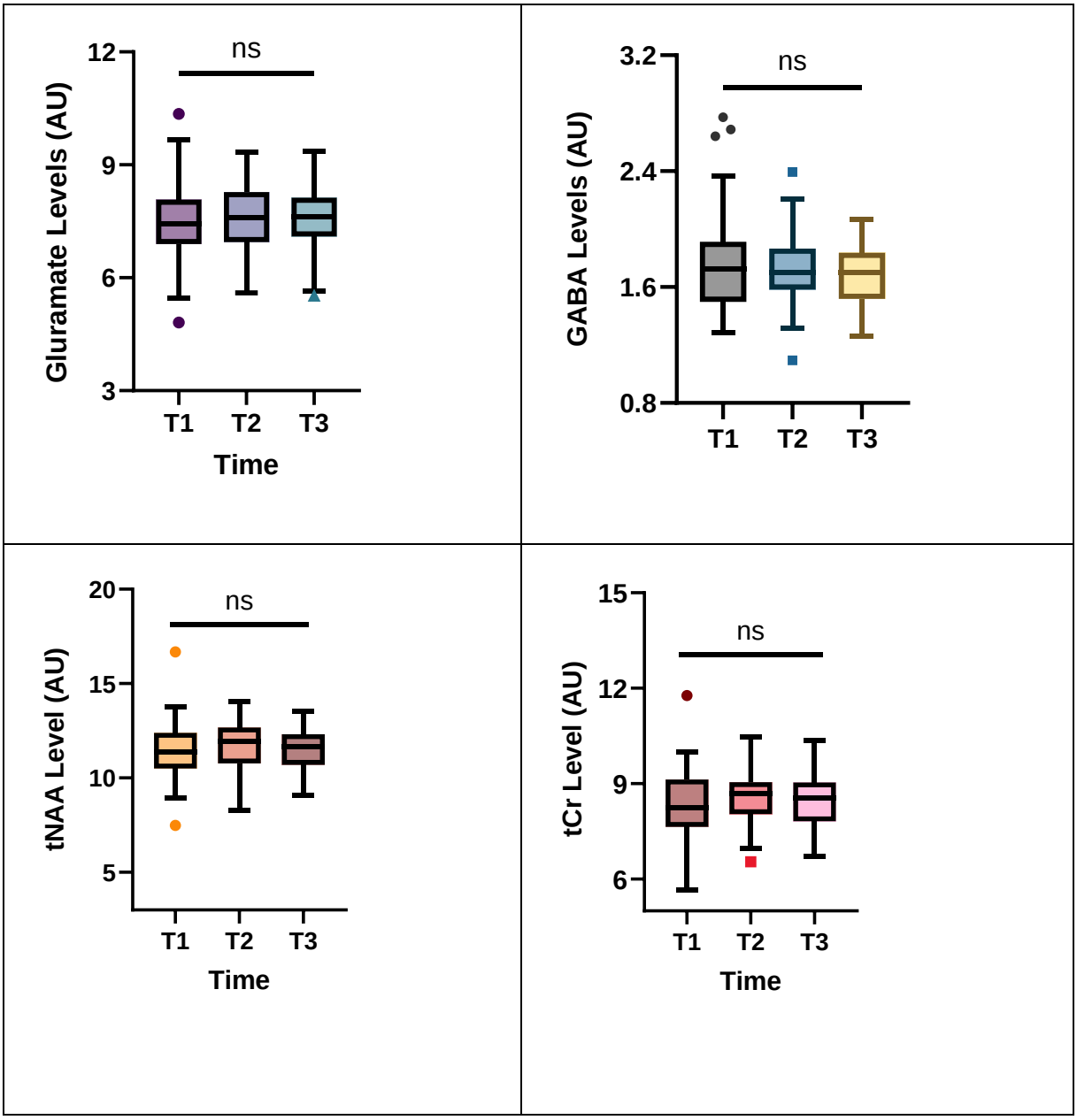


Figure 4.2 | Line plots displaying measurements used to assess change in depressive

symptoms. Top: 17-item version of the Hamilton Rating Scale for Depression ($HDRS_{17}$); Middle: Apathy Evaluation Scale (AES); Bottom: Snaith-Hamilton Pleasure Scale (SHAPS) T1 = Baseline; T2 = 24 hours after single infusion; T3 = 24 -48 hours after the 4th infusion



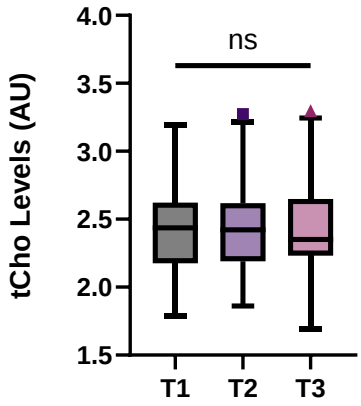
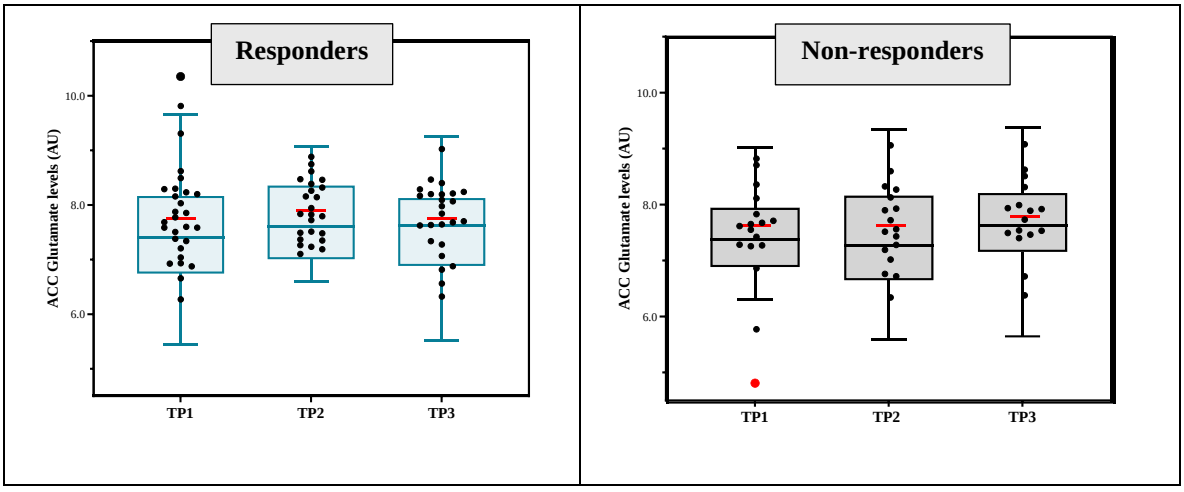


Figure 4.3 | Box plots (Tukey) of ^{1}H-MRS data from the dACC at three time points: baseline (T1); 24 hours after the first infusion (T2) and 24 hours after the fourth infusion (T3). Abbreviations: GABA = γ-amino-butyric acid; tCr: Creatine and Phosphocreatine; tNAA: *N*-acetyl-aspartate and *N*-acetylaspartate-glutamate; tCho:Glycerophosphocholine and Phosphocholine



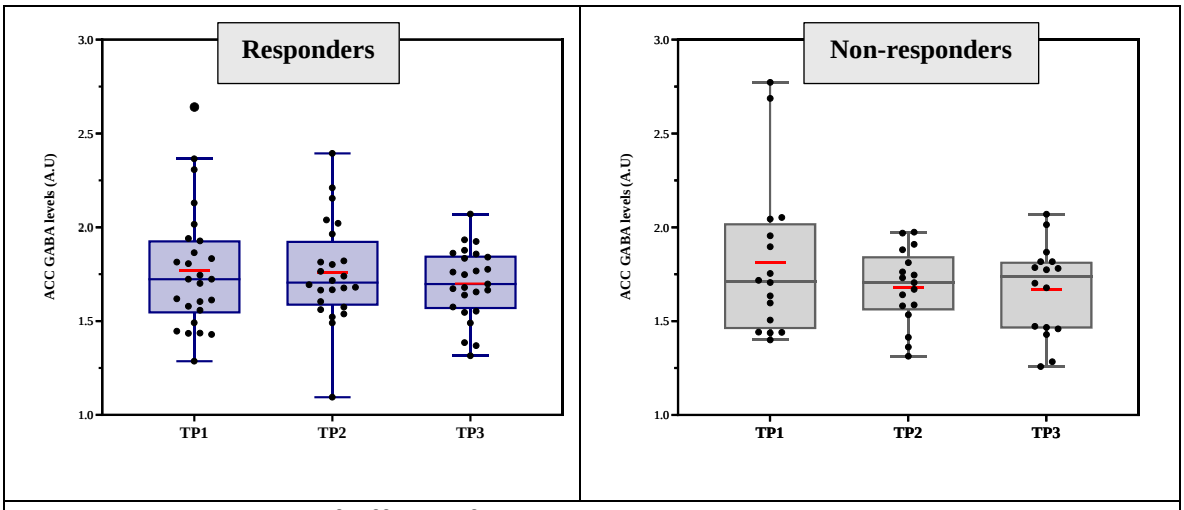
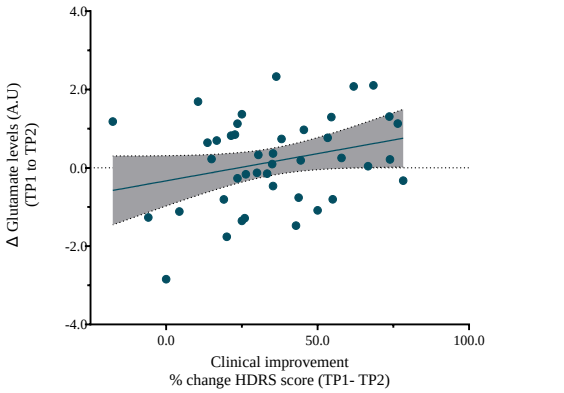


Figure 4.4 | Box plot of effects of repeated ketamine on glutamate in responders (top-left) and non-responders to treatment (top-right). GABA levels compared between responders (bottom-left) and non-responders (bottom-right) to treatment. Data was acquired from the dACC at three time points: baseline (TP1); 24 hours after the first infusion (TP2) and 24-72 hours after the fourth infusion (TP3)



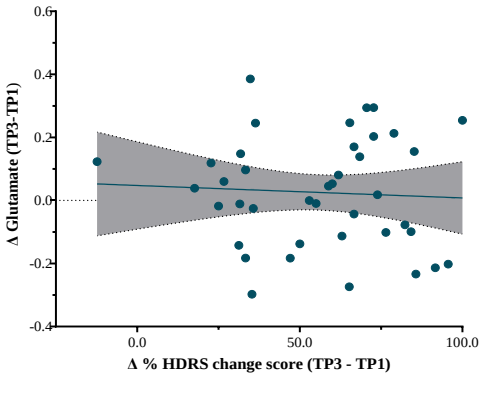


Figure 4.5 | Correlations between changes in dorsal anterior cingulate cortex (dACC) glutamate and GABA levels after repeated ketamine treatment and severity of depressive symptoms was assessed through the 17-item version of the Hamilton Rating Scale for Depression ($HDRS_{17}$). Error bars represent 85% confidence intervals.

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CHAPTER 5

Summary of Findings

The goals of this dissertation were twofold: to provide a deeper understanding of the subclinical symptoms and biological alterations in BD patients during euthymia, and to investigate the neurochemical changes induced by ketamine infusions in TRD patients. In Chapter 2, I performed a cluster analysis using patterns of neural activity during emotion regulation in a sample of BD patients with euthymia. Participants were instructed to expect and then perceive or downregulate their response to emotionally negative stimuli. The objective was to investigate patterns of activation within priori-selected regions of interest engaged during emotion regulation and emotion processing. There were two subtypes of euthymic bipolar disorder I identified based on differences in neural activation within regions of the emotion regulation network. The first subtype was characterized by decreased activation of the amygdala and hippocampus, both of which are limbic regions, and slightly increased activation of the subgenual cingulate and ventrolateral prefrontal cortex. In contrast, the second subtype was defined by lower activation of the amygdala, with the greatest increase observed in the dorsolateral prefrontal cortex. A finding highly relevant to BD is that the greatest difference in activation between the two clusters was found in the amygdala. Increased amygdala activation in response to emotional stimuli is a consistent finding in neuroimaging studies of bipolar disorder patients, even during euthymia (Chen et al., 2011). Amygdala function in the brain is largely regulated by the prefrontal cortex, in particular, the ventrolateral prefrontal cortex and the orbitofrontal cortex. It has been suggested that amygdala dysregulation coupled with ventral prefrontal under-activation may reflect a failure of healthy ventral prefrontal network modulation

of the limbic brain (Foland et al., 2008) which may drive the affective symptoms reported in BD. In contrast, the clusters found in healthy subjects were differentiated only in terms of increased activation in the prefrontal and anterior cingulate cortices.

In Chapter 3, magnetic resonance spectroscopy data were acquired from the dorsal anterior cingulate cortex of sixty volunteers with treatment-resistant depression after they received a slow intravenous infusion of ketamine for 40 minutes. Three major findings emerged. First, an increase in glutamate levels was detected 24 hours after the ketamine infusion, significant in participants who achieved a 50% or greater reduction in symptoms. Second, a lower glutamate level correlated with improvement in depressive symptoms. Third, levels of GABA were not significantly changed with ketamine treatment.

In Chapter 4, this work was extended by examining the effects of repeated ketamine infusions by obtaining longitudinal measurements of glutamate, GABA, tNAA, tCho and tCr. I hypothesized that with ketamine treatment, the levels of glutamate, GABA and other metabolites would steadily increase, and this increase would be associated with the magnitude of clinical remission, potentially suggesting the presence of neurochemical treatment response markers. MRS scans were obtained from fifty participants with treatment-resistant depression at baseline and then after 1 or 4 ketamine infusions. After the first infusion, glutamate levels increased, and effect sizes were greater after the first infusion than after the last infusion. Long-term administration of ketamine did not elicit any marked alterations (neither increases nor decreases) in levels of glutamate, GABA or other metabolites. .

Significance/Conclusion (Organized by Research Question or Hypothesis)

The findings from Chapter 2 suggest that biological subtypes of bipolar disorder and other mood disorders can be inferred based on differences in the engagement of emotion

regulation neural circuits. This analysis has important implications for neuroscience-based therapeutic approaches. Studying the function underlying emotion regulation in both normal and clinical populations provides a basis for understanding individual differences in emotional functioning.

Perhaps the most exciting findings emerged from Chapter 3. In this study, a significant increase in glutamate levels was found in treatment-responders of medium effect size. Additionally, as pretreatment dACC glutamate were correlated with antidepressant response to ketamine infusion, this finding suggests that glutamate levels at baseline may influence the strength of antidepressant response to ketamine. These results are also in concordance with at least two previous MRS studies reporting that pretreatment glutamate in the DLPFC (Salvadore et al., 2012) and mPFC (Matthew S. Milak et al., 2020) associate with antidepressant response to ketamine. In Chapter 4, we hypothesized that repeated exposure to subanesthetic ketamine would potentiate increases in glutamatergic neurotransmission which would be reflected through increases in glutamate and tNAA, tCr and tCho. Though we did not detect a significant effect of ketamine on MRS-derived measures from the dACC, studies using other imaging modalities such as arterial spin labeling have reported neural changes in several distributed regions including the ACC. It is plausible that the changes in neurochemistry occur within an acute time frame and thus we need to establish a better understanding of a temporal relationship between ketamine infusions and neurochemistry. Future studies can either integrate dynamic measurements such as functional MRS or combine MRS and functional imaging methods to better understand the multi-scale mechanisms of ketamine's antidepressant effects. Overall, the study of ketamine-induced changes in cortical neurometabolites in Chapter 3 and 4 may provide a useful marker for characterizing the treatment response to fast-acting antidepressants.

Limitations

There are many limitations to these experiments, both methodological and clinical. In Chapter 2, we utilized previously acquired data and thus were not able to incorporate additional experimental materials thus resulting in some limitations. For instance, the diagnosis of euthymia for the BD sample was based on clinician assessment rather than the administration of established measurements which would allow for us to establish a better symptom profile of emotion regulation difficulties in our euthymic BD group. Related, emotion dysregulation was not assessed directly in this study, though there are available instruments such as the Difficulties in Emotion Regulation Scale (Becerra et al., 2013). Another limitation within the design of this experiment involves the focus on reappraisal as an emotion regulation strategy. Within a clinical context, a focus on improving response tendencies may be more accessible as the emotion has already occurred and processes engaged. Contrary to this, reappraisal is ideal for testing ER within the laboratory setting as the experimenter can train the participant to engage this ER strategy. Differences in medication type in this study sample may influence the cluster subtypes; thus, we cannot exclude the contribution of medication. A similar confounding variable is also present in the experiments detailed in Chapters 3 and 4 as participants were allowed to remain on medication. While medications may have influenced the effects of ketamine treatment, perhaps augmenting response to ketamine, allowing for continued medication may have maintained the ecological validity of our findings. It is possible that the therapeutic effects of ketamine may overlap with the adaptive neural changes linked with antidepressant treatment. Alternatively, ketamine may engage neural pathways similar to those of antidepressant therapy. Another limitation involves the use of MRS. As a result of the limited temporal resolution of MRS as a technique, ketamine-induced effects on glutamatergic or GABAergic function may have been

less sensitive. Additionally, as with MRS studies performed at 3T, we were not able to distinguish glutamine from glutamate. Using other imaging modalities with the same experimental protocol, researchers have reported positive changes in neural activity within an overlapping region (Javitt et al., 2018), which suggests that it may be possible to detect changes in neurochemistry after ketamine using alternate modalities.

Future Directions/Suggestions for Future Research

There is ongoing development of new molecular entities that mimic the therapeutic benefits of ketamine without adverse effects. Future studies should consider the use of MRS in conjunction with alternate modalities with better temporal resolution to examine rapid changes such as those during ketamine treatment or immediately after ketamine infusion. Identification of these neurobiological variations could lead to valuable insight into customizing treatment approaches and reducing variability in treatment response.

Concluding Remarks

Collectively, these studies contribute a dimensional understanding of psychiatric disorders, from elucidating its neurobiological mechanisms underlying emotion regulation difficulties in euthymic BD to evaluating neurochemical variations in the clinical efficacy of ketamine treatment in TRD. Continued research in both areas holds promise for advancing our understanding of mood disorders and improving treatment outcomes for affected individuals.

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