7T ^{1}H-MRS in Major Depressive Disorder: a Ketamine Treatment Study

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For submission to *Neuropsychopharmacology* as an Original Article, November 2017

Data previously presented at the annual meeting of the International Society for Magnetic Resonance in Medicine (April 22-27, 2017)

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Running title: 7T ^{1}H-MRS in MDD

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Abstract

The glutamatergic modulator ketamine has striking and rapid antidepressant effects in major depressive disorder (MDD), but its mechanism of action remains unknown. Proton magnetic resonance spectroscopy (1H-MRS) is the only non-invasive method able to directly measure glutamate levels in vivo; in particular, glutamate and glutamine metabolite concentrations are separable by 1H-MRS at 7T. This double-blind, placebo-controlled, crossover study that included ^{1}H-MRS scans at baseline and at 24 hours post-ketamine and post-placebo infusions sought to determine glutamate levels in the pregenual anterior cingulate (pgACC) of 20 medication-free MDD subjects and 17 healthy volunteers (HVs) 24 hours post-ketamine administration, and to evaluate any other measured metabolite changes, correlates, or predictors of antidepressant response. Metabolite levels were compared at three scan times (baseline, post-ketamine, and post-placebo) in HVs and MDD subjects at 7T using a ^{1}H-MRS sequence specifically optimized for glutamate. No significant between-group differences in ^{1}H-MRS-measured metabolites were observed at baseline. Antidepressant response was not predicted by baseline glutamate levels. Our results suggest that any infusion-induced increases in glutamate at the 24-hour post-ketamine time point were below the sensitivity of the current technique; that these increases may occur in different brain regions than the pgACC; or that subgroups of MDD subjects may exist that have a differential glutamate response to ketamine.

Trial Registration: NCT#00088699

Introduction

Major depressive disorder (MDD) is a devastating illness with a poorly understood biological etiology. A growing body of pre-clinical and clinical evidence implicates the glutamatergic neurotransmitter system in the pathophysiology and treatment of MDD (Sanacora *et al*, 2012). In addition, recent data have consistently suggested that the glutamatergic modulator ketamine has striking and rapid antidepressant effects (Berman *et al*, 2000; Diazgranados *et al*, 2010; Dutta *et al*, 2015; Zarate Jr. *et al*, 2006), underscoring the role of glutamate in the treatment and pathophysiology of MDD (Jaso *et al*, 2017; Murrough *et al*, 2017).

Currently, the only technique capable of directly and non-invasively investigating underlying glutamatergic pathophysiology *in vivo* is magnetic resonance spectroscopy (MRS). Several previous proton MRS (^{1}H-MRS) investigations in MDD subjects detected lower levels of glutamatergic metabolites (glutamate (Glu) and glutamine (Gln)) compared to healthy volunteers (HVs) (Luykx *et al*, 2012; Yuksel *et al*, 2010); however, results have occasionally been inconsistent (Abdallah *et al*, 2014). Recent ^{1}H-MRS investigations also noted increased glutamatamatergic metabolite levels following the acute administration of ketamine: in HVs (Rowland *et al*, 2005; Stone *et al*, 2012) as well as MDD subjects (Glx = glutamate+glutamine) (Milak *et al*, 2015), and up to a day later in HVs (Gln/Glu) (Li *et al*, 2017). Nevertheless, other investigations found no significant changes in glutamate measurements one hour post-ketamine infusion in HVs (Glu) (Taylor *et al*, 2012), or three and 48 hours later in MDD subjects (Glu) (Valentine *et al*, 2011); variations in voxel location, medication status, timing of the scan, imaging parameters, and sample size may explain these discrepant findings (see Supplementary Table 1 which summarizes parameters for these papers). In addition, one study found that treatment response to ketamine was predicted by baseline levels of glutamatergic metabolites (Salvadore *et al*, 2012). Taken

together, these findings suggest that understanding the function of the glutamatergic system during major depressive episodes may be key to effectively treating MDD.

Most ^{1}H-MRS studies to date conducted in MDD subjects have used 1.5, 3, or 4T magnetic resonance imaging (MRI) scanners, which may not adequately distinguish glutamatergic metabolites. We recently developed a reliable (Lally *et al*, 2016) and effective (An *et al*, 2015) ^{1}H-MRS sequence capable of delineating glutamate and glutamine from each other and other neural metabolites at 7T. Here, we compared glutamate and glutamine metabolites in the pregenual anterior cingulate cortex (pgACC) in treatment-resistant, medication-free MDD subjects as well as in HVs using our ^{1}H-MRS sequence at 7T; glutamatergic metabolism is thought to be altered in this area of the cortex in MDD subjects (Walter *et al*, 2009). After baseline imaging assessments, MDD subjects and HVs received infusions of both ketamine and placebo (two weeks apart) and were scanned approximately 24-hours post-infusion (following both ketamine and placebo administration) using the same imaging parameters.

Given the recent positive findings in the ^{1}H-MRS depression literature (Li and Walter, 2016; Luykx *et al*, 2012; Taylor, 2013; Yuksel *et al*, 2010), we expected to observe decreased glutamate levels in MDD subjects relative to HVs. We further anticipated that ketamine would increase levels of glutamatergic metabolites in the pgACC 24 hours post-infusion to a degree correlated with drug response in MDD subjects and that would not affect other metabolites.

The present study is part of a larger study designed to identify clinical and neurobiological correlates of ketamine treatment in unmedicated inpatients with treatment-resistant MDD. Here, we assessed the effects of ketamine on mood and brain glutamate changes measured at baseline and at 24 hours post-ketamine/post-placebo infusion.

Materials and Methods

Participants

All subjects were recruited as part of a larger study (NCT#00088699, NIH Protocol #04-M-0222, substudy 4). Twenty MDD subjects and 17 HVs who had ^{1}H-MRS scans were included in this analysis. All participants were between 18 and 65 years old (see Table 1). Each subject provided written informed consent as approved by the National Institutes of Health (NIH) Combined Central Nervous System Institutional Review Board.

All subjects were physically healthy, as determined by medical history, physical examination, blood labs, urinalysis, and toxicology, and free of any serious medical conditions, comorbid substance abuse (within the preceding three months), or lifetime dependence (excluding caffeine and nicotine). HV and MDD subjects were evaluated and diagnosed via the structured clinical interview for DSM-IV Axis I disorders (First *et al*, 2002a; First *et al*, 2002b) and an unstructured interview with a board-certified psychiatrist. HVs were excluded if they had any first-degree relatives diagnosed with a major (Axis I) psychiatric disorder. MDD subjects were admitted to the 7SE inpatient unit at the National Institutes of Health Clinical Research Center for the duration of the study. HVs were outpatients but received all study-related interventions on the unit. Three MDD and one HV were smokers at the time of the study.

All MDD subjects were currently experiencing a major depressive episode lasting at least four weeks and had been medication-free for at least two weeks prior to study randomization. A Montgomery-Åsberg Depression Rating Scale (MADRS) (Montgomery *et al*, 1979) score ≥20 at the time of screening and also prior to each infusion was an inclusion criterion for MDD subjects. In addition, MDD subjects were refractory to pharmacological treatment; treatment-resistance was defined as not having responded to at least one adequate

antidepressant dose/duration trial (as defined in (Sackeim, 2001)).

Design

Figure 1A illustrates the randomized, double-blind, placebo-controlled, crossover design of this study. After a medication taper (if necessary) and a minimum two-week drug-free period, participants received one intravenous infusion of a sub-anesthetic dose of ketamine hydrochloride (0.5 mg/kg) and one infusion of placebo (0.9% saline solution), with two weeks between infusions. Infusion order was randomized across participants. The infusions were administered by an advanced cardiac life support-licensed practitioner over 40 minutes on the inpatient unit; infusion solutions appeared identical to ensure blinding. No psychotherapy or other treatment was permitted during the entire trial period.

Psychometric Scales

Mood ratings were obtained using the following clinician-administered rating scales: the MADRS, the Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960), and the Snaith–Hamilton Pleasure Scale (SHAPS) (Snaith *et al*, 1995). Ratings were obtained 60 minutes prior to both ketamine and placebo infusions and on the days of the ^{1}H-MRS scans.

MRI

MRI scans were acquired using a Siemens 7T Magnetom MRI scanner (Erlangen, Germany) with a 32-channel head coil. Scans took place at baseline (about three days before the first infusion) and 24 hours after both ketamine and placebo infusions. Standard 1mm^{3} isotropic resolution magnetization-prepared rapid gradient echo sequence (MPRAGE) images (repetition time (TR): 3 seconds, echo time (TE): 3.9 milliseconds) were acquired on each of

the scanning days and used to create anatomical images that were used to plan the location of the ^{1}H-MRS voxel.

^{1}H-MRS

The precise parameters of our imaging protocol have been detailed elsewhere (An *et al*, 2015; Lally *et al*, 2016). Briefly, a 2 cm isotropic voxel was acquired from the pgACC (Figure 1B) using a TE-optimized point resolved spectroscopy (PRESS) sequence with TE_1=69 milliseconds, TE_2=37 milliseconds with an inserted 90 degree J-suppression radio-frequency (RF) pulse to minimize the N-acetyl-aspartate (NAA) multiplet at 2.5 ppm (An *et al*, 2015) (additional sequence parameters: TR:2.5 seconds, spectral width:4000 Hz, number of data points:2048, number of transients:128, with water suppression using eight RF pulses of 350 Hz bandwidth). The time-averaged 32-channel free induction decay (FID) signals were merged into a combined single-channel metabolite FID using a generalized least squares method (An *et al*, 2013). Metabolite levels were then estimated from the spectrum (Fourier transform of the combined FID) using a custom-written linear combination fitting program (Li *et al*, 2015). Basis sets included glutamate, glutamine, glutathione, γ-aminobutyric acid (GABA), N-acetyl aspartate (NAA), N-acetylaspartylglutamate (NAAG), choline, and creatine. For statistical analyses, NAA was summed with NAAG (henceforth known as total NAA (tNAA)) because the sequence used here is not optimal for reliably detecting NAAG. Similarly, because the sequence is not ideal for measuring GABA, GABA was modelled but not reported.

Metabolite values are presented relative to creatine because creatine levels are considered relatively constant across individuals, and because no known differences in creatine levels have been observed between MDD subjects and HVs (Buonocore and Maddock, 2015).

A water proton peak-line broadening criterion of 16 Hz was selected, and only spectra with less than this value were included in the analyses to ensure high quality data. A representative ^{1}H-MRS spectrum obtained using our sequence is presented in Figure 1C.

Tissue segmentation and voxel localization

The anatomical image was segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) components using the FSL tool FAST (Zhang *et al*, 2001). The MRS voxel region of interest (ROI) was generated by creating a 20 mm cube mask centered at the voxel coordinates in subject space (3dcalc). The tissue composition of the voxel was then calculated using the segmented anatomical (3dROIstats).

In order to visualize all voxel centroids in a single reference space, each scan's voxel coordinates were first obtained from the MRS raw data file. The high-resolution anatomical image from which the MRS voxel was prescribed was then warped to standard Montreal Neurological Institute (MNI)-152 space (@auto_tlrc in AFNI (Cox, 1996)). The resulting linear transformation matrix was then applied to the center coordinate of the voxel to obtain the coordinate in standard MNI space.

Statistical analyses

A linear mixed effects model (LME) was performed in R (R Core Team, 2013) (using lme from the package nlme with restricted log-likelihood (REML) model fitting) at the metabolite level in order to evaluate the group and scan effects with subject as a random variable. Further models including age, grey matter (GM), and MADRS as covariates were also explored. Post-hoc contrasts evaluating pairwise metabolite differences between scans (ketamine-baseline, placebo-baseline, ketamine-placebo) were carried out using testInteractions from the package phia, using a chi-square test for this type of model (Fox and

Weisberg, 2011), with multiple comparison correction 'holm' (i.e. Holm-Bonferroni correction). A separate model was used to evaluate behavioural measures. Pearson product moment correlation coefficients were used to assess the relationship between behavioural measures and metabolite levels. Significance was set at $p<0.05$.

Results

Data descriptors and demographics

Table 2 shows the number of scans completed per session along with the average water line width and signal to noise ratio (SNR). Two HV placebo scans were excluded for having line widths greater than 16 Hz. The HV and MDD groups did not differ in age ($F(1,35)=0.04$, $p=0.83$) or water line width ($F(1,35)=0.97$, $p=0.33$). No interaction effect of scan by group for water line width was observed.

Behavioural response to ketamine

The mean change in mood rating scores for both groups at each scan timepoint is summarized in Table 1. Significant differences in behavioral measures were noted in the MDD group between the post-ketamine infusion and baseline scans (MADRS:$\chi^2=23.7$, $p<0.001$, HAM-D:$\chi^2=25.7$, $p<0.001$, SHAPS:$\chi^2=5.1$, $p<0.04$), as well as between post-ketamine and placebo scans (MADRS:$\chi^2=12.5$, $p<0.001$, HAM-D:$\chi^2=4.8$, $p=0.02$, SHAPS:$\chi^2=9.1$, $p<0.001$). No significant differences in mood were observed for these rating scales in the HV dataset. Results from the full dataset demonstrated a slight but significant increase in depressive symptoms in HVs; for details, please see (Nugent *et al*, in press).

^{1}H-MRS

Figure 2A illustrates the individual and HV and MDD group mean metabolite values for glutamate across scans (all other modeled metabolites are shown in Supplementary Figure S1 for completeness). Figure 2B displays the glutamate values across scans for each MDD subject; these are linked by lines to better illustrate individual variations in reponse to ketamine. Subjects were colour coded depending on whether they exhibited increased or decreased glutamate post-ketamine from baseline.

The results below were drawn from the simplest model with no covariates, given that all exploratory models returned similar group comparison statistics. No significant effects were noted for mean glutamate levels at baseline (HV=1.25±0.02; MDD=1.29±0.02; HV-MDD=-0.04, p=0.48, uncorrected), post-ketamine administration (HV=1.28±0.03; MDD=1.36±0.03; HV-MDD=-0.08, p=0.2, uncorrected), post-placebo administration (HV=1.27±0.03; MDD=1.32±0.03; HV-MDD=-0.05, p=0.4, uncorrected), or any other modelled metabolites (full listing appears in Table 3). In addition, no significant relationship was observed in the MDD group between baseline glutamate levels and pre-infusion MADRS score ($r_{(16)}$=-0.15, p=0.53).

Pairwise scan differences (baseline-ketamine, baseline-placebo, ketamine-placebo) were compared within each group in order to identify changes resulting from ketamine administration (see Table 4 for details). Although the MDD group showed a nominal increase in glutamate in the post-ketamine scans in comparison to both baseline (value=0.07, p=0.1) and placebo scans (value=0.04, p=0.1), these were not significant changes. Similarly, tNAA levels were somewhat increased following ketamine administration tNAA compared to both baseline (value=0.04, p=0.05) and post-placebo value (value=0.04, p=0.05).

Figure 2B shows that of the five subjects exhibiting an unexpected decrease in glutamate levels post-ketamine compared to baseline (dark blue lines), four also had the highest baseline glutamate levels. The subjects exhibiting increased glutamate levels post-ketamine administration compared to baseline (light blue dashed lines) generally had lower baseline glutamate levels. This preliminary and exploratory observation may potentially indicate functional subgroups of MDD subjects.

Discussion

This 7T ^{1}H-MRS study investigated glutamate and glutamine levels in the pgACC in both unmedicated, treatment-resistant MDD subjects and HVs at baseline and at 24 hours after a single infusion of intravenous ketamine and placebo. Contrary to our expectations, we found no significant differences in metabolites, either between groups or between scan sessions, although MDD subjects did have a trend towards increased glutamate post-ketamine infusion. In addition, no correlations were observed between glutamate levels and mood, and baseline glutamate levels were not associated with subsequent response to ketamine.

Previous studies have reported decreased glutamate levels in subjects with severe MDD, as defined using HAM-D scores, compared to HVs in the anterior cingulate cortex (ACC) (Horn *et al*, 2010; Luykx *et al*, 2012), but these results may vary by depression subtype (Horn *et al*, 2010) and in response to the exact location of the ^{1}H-MRS voxel. Horn and colleagues (Horn *et al*, 2010) separated MDD subjects into two groups using a HAM-D score of 15 and found that only the more severely depressed subjects showed reduced glutamate levels. Given that our MDD group had an average HAM-D score of 21.8 at baseline, it should be comparable to the more severely depressed HAM-D>15 group of Horn and colleagues; however, in contrast to the results of Horn and colleagues and to our own

expectations, we found a trend toward increased glutamate levels in MDD subjects compared to HVs. Several differences between the MDD subjects in our study and that of Horn and colleagues may have contributed to these results, including the unmedicated and psychotherapy-free status of our MDD group.

Unlike most previously published studies, the sequence used here at 7T permits resolution of the glutamate peak as distinct from glutamine. Furthermore, the J-edited PRESS sequence was optimized for glutamate detection (An *et al*, 2015), such that our measurements should be relatively free of confounding effects from surrounding metabolites, with additional benefits from increased signal-to-noise at this higher field strength. Despite these strengths, we found no significant differences in any measured metabolite levels between MDD subjects and HVs at baseline or between post-ketamine and baseline for either group; we observed only a trend-level increase in glutamate levels post-ketamine relative to both baseline and the post-placebo scan. Our results agree with a study by Valentine and colleagues (Valentine *et al*, 2011) that found no changes in metabolite levels either three hours or two days post-ketamine infusion in the occipital cortex of 10 subjects with MDD. Taylor and colleagues (Taylor *et al*, 2012) measured ACC metabolite levels in HVs at 3T at baseline as well as during and immediately after a 40-minute ketamine infusion and similarly found no changes in metabolite levels. Interestingly, an estimate for the Cohen's d effect size of the difference in glutamate levels post-ketamine vs post-placebo in the present study was 0.15, which is similar to that obtained by Valentine and colleagues (Valentine *et al*, 2011) in the occipital cortex. The present study also identified a trend-level increase in tNAA, which has not previously been noted in response to ketamine. Given that NAA is frequently taken as a marker of neuronal integrity (Baslow, 2003), this finding may be worthy of future study. It should also be pointed out that we are measuring steady state glutamate levels averaged over approximately 15 minutes from a relatively large (2 cm^3) volume of cortex which reduces our

ability to specifically localize the source of the glutamate changes. Approaches using ^{13}C MRS to measure directly measure the glutamate-glutamine cycle (Abdallah *et al*, 2014) and increasing the sensitivity of the MRS technique may be helpful in elucidating these details.

A noteworthy observation about the glutamate changes seen in our group of MDD subjects is the apparent difference in response to ketamine between the group of subjects with higher and lower baseline glutamate values. This suggests the existence of distinct MDD subgroups: one that experiences the expected increase in glutamate levels after ketamine administration and one that does not. This finding raises a potential avenue for future investigation into using baseline glutamate values as a way to identify MDD subgroups.

Interestingly, Li and colleagues (Li *et al*, 2017) found an increased glutamine/glutamate ratio measured at 7T in the pgACC in HVs 24 hours post-ketamine administration, a finding not reproduced here. Their study used a stimulated echo acquisition mode (STEAM) sequence with a short TE where the value measured for the glutamine peak may have been more affected by the presence of macromolecules than our measurement due to the long TE used in our sequence. It should be noted that other brain regions may exhibit larger glutamate changes than the pgACC. Futhermore, some, but not all, studies have found reduced GM volumes in the pgACC and subgenual ACC (sgACC) in MDD subjects (Drevets *et al*, 2008), which may also account for some variance; however, including GM as a covariate in the LME model did not result in a significant main effect of scan session (data not shown).

Several possibilities may account for our observed lack of a measurable difference at 24 hours post-ketamine. First, the glutamate difference induced by ketamine at this time point may be lower than the 8% sensitivity of our MRS sequence (Lally *et al*, 2016); second, the ketamine-induced proposed glutamate burst may happen before 24 hours, may be acute, and may be responsible for ketamine's acute rather than sustained antidepressant effects; and

third, glutamate differences might not occur in the pgACC. One study did find combined glutamate and glutamine (Glx) changes within the first 30 minutes post-ketamine administration in the pgACC (Milak *et al*, 2015), which supports the idea that the glutamate burst happens quite early post-ketamine infusion. While the sgACC is more frequently implicated in the pathophysiology of MDD than the pgACC, spectra with acceptable linewidths are generally not possible in this brain region due to its proximity to the sinuses. Other studies have found ketamine-induced glutamatergic differences in the hippocampus (Kraguljac *et al*, 2017) and thalamus (Stone *et al*, 2012). Thus, it is possible that future experiments should focus on earlier time points or other brain regions.

This study is associated with several strengths. First, we studied both unmedicated treatment-resistant MDD subjects as well as HVs. Second, we acquired ^{1}H-MRS measurements at the high field strength of 7T, where glutamate and glutamine are separable, in both groups. Third, both subject groups received the same scans and administration of ketamine and placebo infusions, which enabled direct comparison of their responses. However, and despite the intriguing nature of the results, the present study is also associated with several limitations. First, the cohort size was modest, which limited our ability to draw a definitive conclusion from our results. Second, a small amount of variance was incurred from manually positioning the voxel; however, the measured voxel placement difference was much smaller than the voxel size itself. Third, we did not monitor motion—which might have decreased the measured metabolite values—during these long acquisitions; nevertheless, the variance within the MDD and HV data was comparable, suggesting similar measurement conditions between the groups. Last, we chose to reference our metabolite values to creatine which may not be ideal as there may be inter individual, regional or disease variance. However, motion will have a larger effect on metabolite/water ratio than metabolite/creatine ratio as the water signal is obtained in separately and as such may not constitute a better

reference. Future studies may consider measuring the stability of creatine values in the pgACC in MDD.

Conclusion

In order to assess underlying glutamate changes associated with ketamine administration in both unmedicated, treatment-resistant MDD subjects and HVs, we acquired ^{1}H-MRS measurements at 7T in both groups. We found that 7T ^{1}H-MRS pgACC glutamate differences between HVs and MDD subjects in this study were less than the measurement sensitivity of the pulse sequence (~8% change). The data suggest that larger effects may occur earlier than 24 hours or in a different brain region, or that subgroups of MDD subjects may exist that have a differential response to ketamine. Our findings may help guide future investigations of the neurochemical changes after post-ketamine administration in individuals with MDD.

Funding and Disclosures

Funding for this work was supported by the Intramural Research Program at the National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH; ZIA MH002857), by a NARSAD Independent Investigator Award to Dr. Zarate, and by a Brain and Behavior Mood Disorders Research Award to Dr. Zarate. Dr. Zarate is listed as a coinventor on a patent for the use of ketamine and its metabolites in major depression and suicidal ideation. Dr. Zarate is listed as a co-inventor on a patent for the use of (2*R*,6*R*)-hydroxynorketamine, (*S*)-dehydronorketamine, and other stereoisomeric dehydro and hydroxylated metabolites of (*R*,*S*)-ketamine metabolites in the treatment of depression and neuropathic pain. Dr. Zarate is listed as co-inventor on a patent application for the use of (2*R*,6*R*)-hydroxynorketamine and (2*S*,6*S*)-hydroxynorketamine in the treatment of depression, anxiety, anhedonia, suicidal ideation, and post-traumatic stress disorders; he has assigned his patent rights to the U.S. government but will share a percentage of any royalties that may be received by the government. All other authors have no conflict of interest to disclose, financial or otherwise.

Acknowledgements

The authors thank the 7SE research unit and staff for their support. Ioline Henter (NIMH) provided invaluable editorial assistance

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Figure Legends

Figure 1. A) Schematic of the double-blind, placebo-controlled, crossover study illustrating the timing of the medication taper, ketamine and placebo infusions (triangle), and magnetic resonance spectroscopy (MRS) imaging (circle). B) i) Example voxel location (yellow box) shown overlaid on an anatomical image slice in the saggital (left), axial (middle), and coronal directions. ii) Centroid locations of all MRS voxels overlaid on a glass brain. iii) Example segmentation into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) with overlaid voxel location in red. C) Example voxel spectrum labeled with modeled metabolites.

Figure 2. A) Group mean glutamate (Glu) concentrations referenced to creatine for the healthy volunteer (HV, red) and major depressive disorder (MDD, blue) groups for each scan. Each filled circle represents the value for a subject. The open circle is the group mean, and the error bars are the standard error. B) Glutamate values for each MDD subject are shown joined with a line across scans. Solid dark blue lines are subjects where the glutamate value was larger at baseline than after ketamine administration and the light dotted blue line are subjects whose glutamate levels increased after ketamine (only subjects with completed baseline scans are shown).

Table 1. Mean subject demographics (and standard error) and behavioral results across baseline, ketamine, and placebo scans.

		HV		MDD		
	N	17		20		
	Gender (N Female, %)	12, 71%		12, 60%		
	Age	34.7	(2.9)	36.2	(2.5)	
	BMI	27.0	(1.0)	27.5	(1.5)	
Race (%)						
	Caucasian	71		85		
	Other	29		15		
Family history (%)						
	Alcoholism			45		
	Substance Abuse			70		
Length of						
	Illness (years)			21	(2.7)	
	Current episode (months)			47	(17.3)	
MADRS	baseline	1.2	(0.3)	32.9	(1.1)	*
	ketamine	2.3	(0.8)	22.9	(2.3)	*
	placebo	0.7	(0.4)	30.9	(1.1)	*
HAM-D	baseline	1.42	(0.3)	21.8	(1.1)	*
	ketamine	1.54	(0.5)	15.6	(1.5)	*
	placebo	0.25	(0.2)	18.7	(0.9)	*
SHAPS	baseline	18.2	(0.3)	39.6	(0.2)	*
	ketamine	18.0	(0.5)	35.1	(0.5)	*
	placebo	17.3	(0.4)	41.4	(0.3)	*

Abbreviations: BMI: Body mass index; HV: healthy volunteer; MDD: major depressive disorder; MADRS: Montgomery-Asberg Depression Rating Scale; HAM-D: Hamilton Depression Rating Scale; SHAPS: Snaith-Hamilton Pleasure Scale.
* indicates a significant ($p<0.05$) difference between the two groups (HV-MDD)

Table 2. Subject counts, mean line widths, and SNR for each scan for both groups. The voxel fraction as an average across scans is also shown for both groups.

		HV			MDD			t-test
	Scan	N	Mean	SE	N	Mean	SE	p-value
water line width	baseline	14	12.5	1.6	18	11.9	1.2	0.28
(Hz)	ketamine	11	12.2	1.3	18	12.2	1.6	0.37
	placebo	12	11.4	0.9	16	11.0	1.4	0.99
SNR	baseline		216.8	41.7		206.0	39.4	0.47
	ketamine		213.3	45.8		200.2	36.4	0.43
	placebo		229.6	44.4		214.1	48.8	0.38
Voxel fraction	GM		0.60	0.08		0.58	0.07	0.15
	WM		0.18	0.04		0.18	0.09	0.47
	CSF		0.22	0.07		0.23	0.06	0.79

Abbreviations: HV: healthy volunteer; MDD: major depressive disorder; SNR: signal to noise ratio; GM: grey matter: WM: white matter; CSF: cerebrospinal fluid; SE: standard error.

Table 3. Group mean and standard deviation of metabolite concentrations for HV and MDD subjects across all scan dates referenced to creatine.

		HC		MDD		difference		
metabolite/Cr	ScanType	adjusted mean	std. error	adjusted mean	std. error	value	$\chi 2$	p-value
Glutamate	baseline	1.25	0.02	1.29	0.02	-0.04	0.5	0.7
	ketamine	1.28	0.03	1.36	0.02	-0.08	1.4	0.7
	placebo	1.27	0.03	1.32	0.02	-0.05	0.9	0.7
Glutamine	baseline	0.29	0.02	0.29	0.02	0.00	0.0	n.s.
	ketamine	0.31	0.03	0.30	0.02	0.01	0.1	n.s.
	placebo	0.28	0.03	0.31	0.02	-0.03	1.0	n.s.
Glutamine/ Glutamate	baseline	0.23	0.02	0.23	0.02	0.00	0.0	n.s.
	ketamine	0.25	0.03	0.22	0.02	0.02	0.5	n.s.
	placebo	0.23	0.03	0.23	0.02	0.00	0.0	n.s.
Glutathione	baseline	0.24	0.02	0.25	0.02	-0.01	0.1	n.s.
	ketamine	0.24	0.03	0.26	0.02	-0.01	2.5	0.3
	placebo	0.25	0.03	0.25	0.02	0.00	0.0	n.s.
GABA	baseline	0.19	0.02	0.19	0.02	0.00	0.0	n.s.
	ketamine	0.17	0.03	0.20	0.02	-0.04	1.0	0.9
	placebo	0.17	0.03	0.17	0.02	0.00	0.0	n.s.
tNAA	baseline	1.58	0.02	1.59	0.02	0.00	0.0	0.9
	ketamine	1.57	0.03	1.63	0.02	-0.06	0.6	0.9
	placebo	1.66	0.03	1.59	0.02	0.06	1.4	0.7
Choline	baseline	0.29	0.02	0.31	0.02	-0.02	1.9	0.3
	ketamine	0.30	0.03	0.30	0.02	-0.01	1.3	0.3
	placebo	0.28	0.03	0.30	0.02	-0.03	5.2	0.1

Abbreviations: HV: healthy volunteer; MDD: major depressive disorder; tNAA: total N-acetyl aspartate; Cr: creatine; p (u.c.) uncorrected p-value; p (corr): p-value corrected for multiple comparisons; n.s: not significant.

Table 4. Pairwise mean differences between scans for metabolite concentrations for HVs and MDD groups referenced to creatine.

		HV			MDD		
		Value	χ^2	p-value	Value	χ^2	p-value
Glutamate	k-b	0.030	0.350	0.560	0.070	2.750	0.100
	k-p	0.020	0.090	0.760	0.040	0.700	0.400
	p-b	0.010	0.070	0.790	0.030	0.570	0.450
Glutamine	k-b	0.020	0.960	0.330	0.010	0.320	0.570
	k-p	0.030	2.050	0.150	0.000	0.000	0.950
	p-b	-0.010	0.270	0.600	0.010	0.370	0.540
Glutamine /	k-b	-0.010	0.420	0.520	0.003	0.070	0.791
Glutamate	k-p	0.020	0.920	0.340	-0.006	0.294	0.588
	p-b	0.010	0.130	0.720	-0.003	0.082	0.774
Glutathione	k-b	-0.030	1.900	0.170	0.010	0.870	0.350
	k-p	-0.010	0.290	0.590	0.020	2.770	0.100
	p-b	-0.020	0.670	0.410	-0.010	0.600	0.440
tNAA	k-b	0.000	0.020	0.880	0.040	3.820	0.050
	k-p	-0.050	2.040	0.150	0.040	3.910	0.050
	p-b	0.050	1.820	0.180	0.000	0.010	0.910
Choline	k-b	0.000	0.070	0.790	0.000	0.250	0.620
	k-p	0.000	0.170	0.680	-0.010	1.920	0.170
	p-b	0.000	0.470	0.490	0.010	0.820	0.360

Abbreviations: HV: healthy volunteer; MDD: major depressive disorder; tNAA: total n-acetyl aspartate; b: baseline; k: ketamine; p: placebo