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A Pilot *In Vivo* Proton Magnetic Resonance Spectroscopy Study of Amino Acid Neurotransmitter Response to Ketamine Treatment of Major Depressive Disorder

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Abstract

The NMDA receptor antagonist ketamine can improve major depressive disorder (MDD) within hours. To evaluate the putative role of glutamatergic and GABAergic systems in ketamine's antidepressant action, medial prefrontal cortical (mPFC) levels of glutamate + glutamine (Glx) and γ-aminobutyric acid (GABA) were measured before, during, and after ketamine administration using proton magnetic resonance spectroscopy. Ketamine (0.5 mg/kg i.v.) was administered to eleven depressed patients with MDD. Glx and GABA mPFC responses were measured as ratios relative to unsuppressed voxel tissue water (W) successfully in 8/11 patients. Ten of 11 patients remitted (50% reduction in 24-item Hamilton Depression Rating Scale and total ≤ 10) within 230 minutes of commencing ketamine. mPFC Glx/W and GABA/W peaked at 37.8%±7.5% and

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38.0%±9.1% above baseline in ~26 minutes. Mean areas under the curve (AUC) for Glx/W (p = 0.025) and GABA/W (p = 0.005) increased and correlated (r = 0.796; p=0.018). Clinical improvement correlated with 90-minute norketamine concentration (df=6, r=−0.78, p=0.023), but no other measures.

Rapid increases in Glx and GABA in MDD following ketamine administration support the postulated antidepressant role of glutamate and for the first time raises the question of GABA's role in the antidepressant action of ketamine. These data support the hypothesis[1] that ketamine administration may cause an initial increase in glutamate that potentially activates mammalian target of rapamycin (mTOR) pathway via AMPA receptors, since ketamine blocks NMDA receptors. The role of the contemporaneous surge in GABA remains to be determined.[2]

Keywords

proton magnetic resonance spectroscopy; glutamate/glutamine (Glx); Major Depressive Disorder

Introduction

Major depressive disorder (MDD) affects approximately 14.8 million American adults. Contributing to the disease burden is the multi-week lag in onset of antidepressant effect and the fact that only about one third of patients remit after 6–8 weeks. A single subanesthetic dose of ketamine, a glutamate N-methyl-D-aspartate (NMDA) receptor antagonist, based on two meta-analyses[3, 4] of ketamine's antidepressant effect in randomized placebo-controlled trials (10 trials and 246 patients total; 6 trials and 163 patients overlapped; 34 patients had bipolar disorder), produces an antidepressant effect in hours to days with standardized mean differences of −0.91 and 0.9. These findings held true even in previously treatment-resistant depression. Although studies have examined the duration of antidepressant response to a single ketamine dose[5] and following repeated ketamine infusions,[6, 7] the number of completed short and long-term controlled and dose-dependent studies is insufficient to establish ketamine as a treatment for general clinical use. However, given the promise of ketamine's efficacy as a rapid-acting antidepressant, identification of ketamine's mechanism of action may permit development of a new class of fast-acting antidepressants.

To date, most studies of ketamine's antidepressant action have been conducted in animals. Ketamine activates the mTOR pathway[8, 9] via glutamatergic α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors. mTOR activation increases synaptic proteins and sprouting of new synaptic spines in prefrontal cortex (PFC) within hours, consistent with the time-course of its antidepressant effect.[1] The mTOR signaling pathway in hippocampal neurons[10] activated by glutamatergic synaptic activity[11, 12] is required for long-term potentiation (LTP),[10, 12, 13] long-term depression (LTD)[14] and memory consolidation.[15] Deficits in mTOR expression and in several mTOR-dependent translation initiation factors are reported in prefrontal cortex (PFC) in MDD *postmortem,* suggesting that this ketamine target may also be part of the pathogenesis of MDD.[16] *In vivo* brain proton magnetic resonance spectroscopy (^{1}H MRS) studies in healthy volunteers report increased glutamine[17] and unchanged[18] or increased glutamate[19] levels in response to ketamine administration. A study in depressed patients[20] found no effect of ketamine on

glutamatergic compounds. Thus, it remains unclear how ketamine enhances glutamatergic signaling in MDD *in vivo*.

This pilot study sought to test the hypothesis that ketamine administration in depressed patients produces a rapid, robust surge in glutamatergic compounds in medial PFC (mPFC), as observed in rodent studies.[21, 22] ^{1}H MRS was used to dynamically measure the time-course of brain glutamatergic response to ketamine (via the combined resonance of glutamate + glutamine, or Glx) from baseline through 40 minutes of infusion to approximately 30 minutes after infusion in depressed DSM IV-defined MDD patients. An exploratory objective was to synchronously measure ketamine's effect on brain γ-aminobutyric acid (GABA), reported to be low in severe MDD.[23–25] GABAergic abnormalities associated with MDD include low GABA in cerebrospinal fluid[26] and plasma[27, 28] and are consistent with magnetic resonance spectroscopy (MRS) findings of low brain GABA levels.[23, 24, 29–31]

Materials and Methods

Patients

All subjects provided written informed consent as approved by the Institutional Review Board prior to participation. Eleven outpatients (eight female) with mean age of 38.8 ± 12.8 years participated and met DSM-IV criteria for major depressive episode (MDE) and MDD, scoring at least 16 on the 17-item Hamilton Depression Rating Scale (HDRS-17) (mean score= 20.7± 3.7). All participants were free of psychotropic medications for at least 14 days prior to scanning, off fluoxetine for 6 weeks and off serotonin depleting drugs for 3 months.

Patients were excluded for: lack of capacity; history of other major Axis I disorders; suicidal ideation with a plan or intent or attempted suicide within the preceding 6 months; current or past drug or alcohol dependence; prior use of ketamine; electroconvulsive therapy in the preceding three months; having a first-degree relative with a psychotic disorder (for subjects under age 33); any significant active physical illness; previous loss of consciousness for more than a few minutes that required medical evaluation; pregnancy or intent to conceive during study participation or having ferromagnetic implants or other magnetic resonance imaging (MRI) contraindications. Medical history, physical examination and standard blood tests (including urinalysis and toxicology) confirmed the absence of active physical illness, pregnancy and drug use. Prior treatment for depression was a requirement for inclusion; treatment resistance was not.

Study Design

Subjects fasted for approximately 8 hours prior to scanning. Baseline ratings were administered within 24-hours of scanning [24-item Hamilton Depression Rating Scale (HDRS-24), Beck Depression Inventory (BDI), Profile of Mood States (POMS), Young Mania Rating Scale (YMRS) and the Brief Psychiatric Rating Scale (BPRS)].

Prior to ketamine administration, patients were positioned in the MRI scanner. After structural MRI and baseline ^{1}H MRS scans, 0.5 mg/kg of ketamine hydrochloride in saline was administered intravenously over approximately 40 minutes. Six ^{1}H MRS data frames

were acquired, each of ~13-minute duration: one pre-ketamine, four during the 40-minute ketamine infusion, and one after the ketamine infusion. The POMS was completed 80 and 110 minutes after initiation of the ketamine infusion. Blood samples were obtained from a second venous line at 90 minutes and 120 minutes post-ketamine for levels of ketamine, norketamine and dehydronorketamine.

Baseline psychiatric ratings were repeated 230 minutes post-ketamine infusion, excluding items not expected to change (eg: sleep). HDRS-24 was also administered 24 hours post-infusion. Remission was defined as ≥50% improvement from baseline *and* a total score of ≤10. Response was defined as ≥50% improvement.[5] The HDRS-24 was the primary outcome measure, as in most other ketamine studies.[5] The BPRS was administered at baseline and at 230 minutes post-infusion to monitor potential adverse effects of ketamine. The POMS was used to measure clinical state during the first 230 minutes post-infusion because it is better suited for short-term (hours) re-administration.[32, 33]

MRI and MRS Data Acquisition

Neuroimaging data were acquired on a General Electric Signa EXCITE 3.0T MR scanner using commercial 8-channel phased-array head coil. A three-plane localizer imaging series was obtained, followed by a volumetric T_1 weighed spoiled gradient-recalled (SPGR) echo acquisition (TE=2.86ms, TR=7.12 ms, flip angle = 9°, field of view = 256×256 mm^2, image matrix size = 256×256, slice thickness 1 mm; voxel size 1×1×1 mm^3). Next, *in vivo* brain spectra of the GABA and combined resonance of glutamate and glutamine (Glx) were recorded from a 3.0×2.5×.2.5-cm^3 mPFC voxel (Figure 1A, B) using the standard J-edited spin echo difference method.[34, 35] A pair of frequency-selective inversion pulses was inserted into the standard point-resolved spectroscopy (PRESS) method and then applied to the GABA C-3 resonance at 1.9 ppm on alternate scans using TE/TR 68/1500ms. This resulted in two subspectra (Figure 1C, traces [a] and [b]) in which the GABA C-4 resonance at 3.03 ppm and Glx C-2 at 3.71 ppm were alternately inverted. Subtracting these two subspectra yielded a spectrum consisting of only the edited GABA C-4 and Glx C-2 resonances, with all overlapping resonances eliminated (Figure 1B). Data were acquired in 13-minute frames using 256 interleaved excitations (512 total) with the editing pulse alternatingly on or off. The resultant raw 8-channel phased-array coil data were combined into a single regular free-induction decay signal using the coil sensitivity factors derived from the unsuppressed water signal acquired with each receiver coil. The magnetic field homogeneity for all acquisitions was required to be less than ≤20 Hz, as assessed by the full width at half of the unsuppressed water resonance.

Areas under the Glx and GABA peaks, which are proportional to their concentrations, were obtained as illustrated in Figure 1C (traces [a-f]) by fitting each resonance to a Gauss-Lorentz (i.e., pseudo-Voigt) function in the frequency-domain using a Levenberg-Marquardt nonlinear least-squares minimization routine written in IDL (ITT EXELIS, McLean, VA). The levels of Glx and GABA in the edited spectra were then expressed as ratios of peak areas relative to the simultaneously acquired and similarly fitted unsuppressed voxel water signal (W)—a commonly used[36, 37, 38_40] method with high test-retest reliability.[41]

Plasma Ketamine, Norketamine and Dehydronorketamine

Plasma ketamine, norketamine and dehydronorketamine were assayed by liquid chromatographic (LC) procedure with UV detection. Within-day coefficient of variation of ketamine, norketamine and dehydronorketamine did not exceed 12.8% (range 2000–5ng/mL), (n=12 for each of seven concentrations). Day-to-day variation of ketamine and norketamine quality controls at 1250, 250, and 50ng/mL did not exceed 4.3 and 3.4%, respectively (n=11 days). For dehydronorketamine, day-to-day variation at 500, 100 and 20 ng/mL did not exceed 8.8% (n=11 days). The minimum quantifiable limits were set at 10ng/mL for both ketamine and norketamine, and 5ng/mL for dehydronorketamine (such low levels were not seen in this study).

Statistical Analysis

Linear mixed-effects models tested for: (1) effect of time point on POMS score with time point as a (categorical) fixed effect and subject as a random effect; (2) ketamine's effect on log-transformed Glx/W and GABA/W levels, with subject as a random effect and each 13-minute MRS frames as a fixed effect. Correlations were calculated using Pearson product moment. MRS outcome measures were log-transformed to ensure normality of distribution, sphericity and compound symmetry. A first-order autoregressive covariance structure was applied to account for time dependency within subjects.

Results

Effect of Ketamine on MDD Symptoms

Ten of 11 subjects met criteria for remission by 230 minutes post-infusion, 9/11 at 24 hrs post-infusion and 7/10 at three days. HDRS-24 and BDI scores declined dramatically 24 hours post-ketamine ($F=52.5$; $df=1,10$; $p<0.0001$ and $F=21.7$; $df=1,10$; $p=0.0009$, respectively) (Figure 2B–C).

POMS total score (Figure 2A) declined rapidly ($F=18.0$; $df=4,35$; $p<0.0001$), reached a nadir at 230 minutes, and remained low 24 hours later. Notably, POMS sub-score *vigor* gradually increased in contrast to total score and *confusion* decreased comparably to the total score (Table 3).

BPRS scores (Figure 3) declined from baseline to 230 minutes post-ketamine infusion ($F=26.2$; $df=1,10$; $p=.0004$). There were decreases in BPRS sub-scores [42] *anxiety-depression* ($F=33.5$; $df=1,10$; $p=.0002$) and *anergia* ($F=9.8$; $df=1,10$; $p=.01$),. Psychotic symptom sub-scores remained stable or declined after infusion. No patient's *thought disturbance* score changed from baseline after ketamine infusion. Subjects experienced mild or no adverse cognitive or dissociative effects (Table 3). There was no correlation between change in BPRS score and change in Glx or GABA levels.

Response of mPFC Glx and GABA to Ketamine

MRS data for three subjects were excluded from all analyses due to head motion, manifested as distorted peak phases and large residual water resonance in difference spectra, which degraded spectral quality. For the remaining subjects, there was a main effect of ketamine

infusion on Glx/W (F=6.16; df=5,31; p=0.0004), that post-hoc analysis attributed to higher Glx/W in MRS frame 1 (p=0.037), frame 2 (p=0.001), and frame 3 (p=0.027) compared with the baseline frame (Figure 3A). For GABA/W there was a main effect of ketamine infusion (F=2.90; df=5,31; p=0.029) and post hoc analysis indicated higher GABA/W in MRS frame 1 (p=0.036) and frame 2 (p=0.031) compared with the baseline frame (Figure 3A).

The peak frame for each subject revealed a 38% ± 8% (mean ± SD) increase in Glx/W from baseline and a 38% ± 9% increase in GABA/W from baseline. The time points for these peak values, obtained by averaging the mid-time point of the frame of each peak, were 28 ± 8 and 26 ± 21 minutes from the start of infusion for Glx/W and GABA/W, respectively. When responses to ketamine were quantified as area under the curve (AUC), both Glx/W and GABA/W increases were statistically significant: Glx/W AUC was 0.013 ± 0.01 (SD) (one sample t-test: t = 2.8479, df = 7, p = 0.025); and GABA/W AUC was 0.016 ± 0.01 (t = 4.0841, df = 7, p = 0.005) (Figure 3B). The correlation between AUCs of Glx/W and GABA/W was 0.796 (p=0.018).

Vital Signs and Clinical/Imaging/Ketamine Level Correlations

Neither Glx/W nor GABA/W changes correlated with clinical response to ketamine. Of ketamine and its two active metabolites, norketamine and dehydronorketamine, measured at two time points (see Table 3), only norketamine concentration at 90 minutes correlated with clinical outcome (df= 6; r= −0.78; p= 0.023; uncorrected) expressed as a percent change in HDRS-24 scores. No effects were observed on blood pressure, heart rate, oxygen saturation, or conscious state during and after the ketamine infusion.

Discussion

This is the first study to report rapid and robust *in vivo* increases in both mPFC Glx and GABA in response to intravenous administration of a single subanesthetic dose of ketamine for treatment of MDD. A prior study failed to detect an effect of ketamine in depressed subjects.[20] While differences in MRS methods cannot be ruled out as an explanation of this discrepancy, MRS data in the prior study were acquired *after* completion of the ketamine infusion, and comport with our study, which found that most of the Glx and GABA responses to be dissipated by the end of infusion. Consistent with our findings, two studies in healthy volunteers also reported increases in glutamine[17] and glutamate[19] levels, and one study did not.[18] The infusion methods used in the studies with positive findings may have produced higher ketamine blood levels than those achieved in the negative study and caused a more robust glutamatergic response in healthy volunteers. This highlights the need for future studies of the glutamatergic response.

A rapid increase in Glx in response to ketamine is consistent with microdialysis rodent studies[21,43] that found a 250% increase in extracellular glutamate levels in response to ketamine administration at 40 minutes compared to baseline, and using 13-C labeled glucose found that 13-C enrichment of Glu-C4 increased roughly 18% from baseline, significantly more than in saline-control rats. The relatively modest increase of *de novo* glutamate synthesis measured by incorporation of 13-C labeled glucose into the carbon backbone of

glutamate belies the substantial increase in extracellular glutamate by microdialysis. These findings support a role for the glutamatergic system in the rapid antidepressant action of ketamine.

We also observed a parallel increase in total tissue GABA (as measured by ^{1}H MRS) in MDD in response to ketamine in depressed subjects that is consistent with animal studies.[22] The potential role of GABA in the antidepressant action of ketamine is an area for future research.

We also confirm previous reports of rapid remission of MDD in response to ketamine.[44] POMS scores declined more than 50% improvement within one hour of initiating the ketamine infusion (Figure 2A). Remission rates observed in this open study are comparable with those in previous studies (e.g.[5]), or perhaps better because this study's subjects were younger, had shorter duration of illness, fewer previous MDEs, and were less treatment-resistant.

Norketamine levels, which showed substantial variance at 90 minutes (in contrast to ketamine), correlated negatively with HDRS-24 scores 24 hours after ketamine infusion. Plasma ketamine and dehydronorketamine did not correlate with clinical outcome or with Glx/W or GABA/W changes. Since norketamine levels were relatively high, their correlation with antidepressant effect suggests that norketamine may be a long-lived and active metabolite due to slow conversion to dehydronorketamine, and thus may have a more pronounced role in blocking NMDA receptors. The absence of a correlation of ketamine level with clinical response or the Glx or GABA response may be due, in part, to the standardized dose given to all subjects, which minimized variance in ketamine blood levels, and to the generally robust clinical response exhibited by most subjects in the study. A dose-finding study, using a wider range of doses, may clarify how ketamine and its active metabolite levels relate to glutamate and GABA responses and to antidepressant action.

The nearly 40% increase in the concentration of both Glx and GABA may be explained by changes in brain glucose utilization. Following ketamine administration in patients with bipolar disorder, an increase in regional metabolic rate of glucose (rMRGlu) correlated with improvement in depressive symptoms in the right ventral striatum.[45] The cerebral metabolic rate for glucose in human brain is approximately 0.4 μmol/min/g tissue and turnover of glutamate is approximately 0.8 μmol/min/g tissue.[46] Virtually all the glucose that enters the brain is metabolized through glutamate since one molecule of glucose gives rise to two molecules of acetyl-CoA, which enter the tricarboxylic acid cycle (TCA) to become α-ketoglutarate and then glutamate. In most cells of the body, glutamate is in equilibrium with a-ketoglutarate (a-KG) as it is continuously reconverted and then metabolized through the TCA cycle. However, in glutamatergic neurons, the enzyme aspartate aminotransferase (which aminates a-KG to glutamate) has much higher activity than the enzyme a-KG dehydrogenase, which can lead to an accumulation of intracellular glutamate.[47] In GABAergic neurons, this same process feeds GABA synthesis because glutamate is the precursor of GABA. The robust correlation between Glx and GABA increases supports the hypothesis that glucose utilization drives the increase in both neurotransmitters.

Preclinical studies indicate that ketamine's antidepressant action may depend on activation of glutamatergic AMPA receptors[8] and the downstream mTOR pathway.[1] Our findings support the model[1] positing that ketamine causes a rapid increase in cortical glutamate through an unknown mechanism that, in combination with blockade of NMDA receptors by ketamine, diverts glutamate signaling to AMPA receptors. AMPA activation leads to the downstream activation of the mTOR pathway, leading to increased BDNF release,[48_51] dendritic protein synthesis,[1] mushroom spine formation and associated downstream effects.[52_54] Other NMDA receptor antagonists exhibit antidepressant effects,[55_64] and monoaminergic antidepressants and modulators of metabotropic glutamatergic receptors with antidepressant effects also reduce NMDA signal transduction.[54, 65_71] The glutamatergic system may also be a target for treatment of depression because it is abnormal in major depression.[72, 73] A ^{1}H-MRS study reported glutamate deficits in anterior cingulate cortex (ACC) in MDD.[74] Conversely, higher glutamate in cerebrospinal fluid (CSF) has been reported and a cytotoxic role for glutamate transmission via NMDA receptors has been implicated in the loss of mature granule cells in dentate gyrus and glia in hippocampus and in amygdala.[75]

Our finding of a parallel GABA elevation following ketamine administration is a novel observation. Though the mechanism is uncertain, the synchronous surge in this inhibitory neurotransmitter could limit ketamine-mediated glutamate release and reduce excessive spread of glutamatergic excitation. CSF,[26] plasma[27, 28] and *in vivo* brain GABA levels[23_25, 30] are reported in MDD during depression; but not when not depressed.[78] GABA(B) receptor agonists and positive modulators have antidepressant-like effects in rodent depression models[79, 80] and antidepressant treatment (SSRI or ECT) normalizes GABAergic deficits in MDD patients.[81_84] Postmortem studies report fewer GABA neurons in MDD and bipolar disorder and a GABAergic deficit may be a part of the pathophysiology of major depressive episodes.[75,85, 86] GABAergic deficits are found postmortem with decreased density of calbindin immunoreactive GABA neurons in MDD compared with controls.[87] GABA(A) receptor deficits have also been demonstrated postmortem in the brains of MDD suicides.[88] Olfactory bulbectomy and learned helplessness models in rodents have shown deficits in GABAergic function.[76,77] GABAergic system involvement in the pathophysiology of MDD also may involve a complex interplay between GABA and Glu that is more abnormal in TRD depression.

This pilot proof of concept study has a small sample size, but all subjects were medication-free and the measured clinical effects of ketamine were robust. While overall neurochemical effect of ketamine was statistically significant, lack of effect on Glx or GABA in some patients (Figure 3B) calls for further investigation via larger randomized controlled trials. ^{1}H MRS measures total tissue levels, including intracellular, synaptic, and vesicular levels, but not transmission or shifts from one compartment to another, which limits interpretation. The tissue concentration of GABA requires relatively large voxels for reliable quantification; consequently, associated partial volume effects could make measurement more difficult, but since this is a within-subject design, partial volume effects were stable throughout the infusion. The Glx peak consists of the combined resonances of glutamate and glutamine; however, we recently reported that the Glx measured by the J-editing technique contains

mainly glutamate, and little or no glutamine.[92] The contribution of macromolecules known to co-edit with GABA was not taken into account and is a potential confound.

Our findings require replication in a larger sample using a wider range of ketamine doses, which will generate a more complete dose response curve and more effectively reveal correlations, including determining if Glx or GABA responses are predictors of antidepressant response. Such a multi-dose study with a larger sample size would also allow for further analyses of covariates in antidepressant response to ketamine such as sex differences, as a preclinical study[93] found gonadal hormones enhanced antidepressant-like effects of ketamine in female rats. The effect on Glx and GABA observed in this study is an early effect that may or may not be the initial step in antidepressant effect, though animal data with mTOR suggest that AMPA effects of glutamate initiate the antidepressant cascade. Lack of a relationship between a proximal drug effect and clinical response is common in the study of the action of psychotropic medications. For example, SSRIs require occupancy of at least 80% of transporter sites and MAOIs need to block at least 80% of MAO in order to work.[94,95] Greater occupancy does not correlate with antidepressant response but there is little doubt that this initial pharmacological effect is needed for an antidepressant effect.

Conclusion

This pilot study found rapid and comparably robust increases in glutamatergic compounds and GABA in most MDD patients in response to ketamine antidepressant treatment of MDD, which supports the potential involvement of these amino acid neurotransmitters in the antidepressant action of ketamine. Further studies are needed to clarify the relationship between clinical response, glutamate and GABA levels and ketamine dose.

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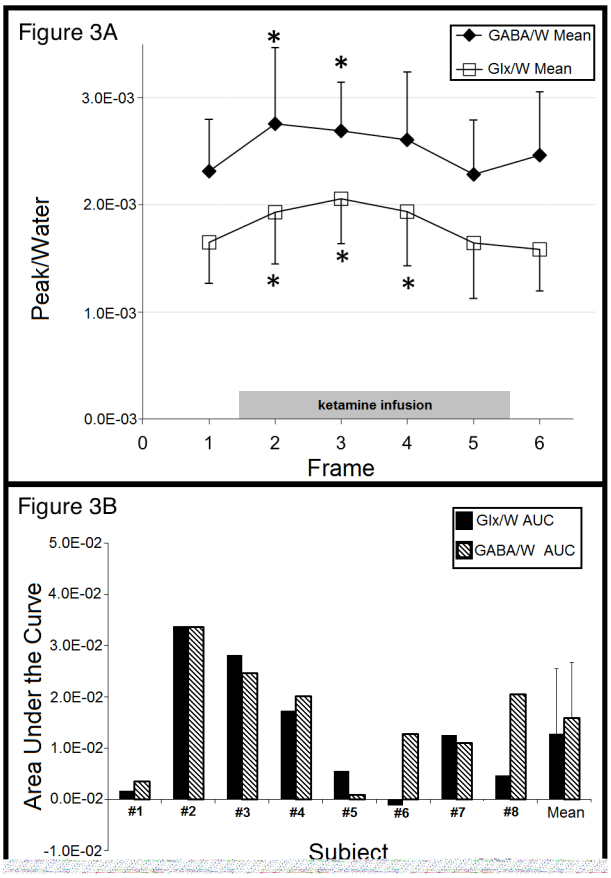

Figure 1.

(A) Axial and (B) sagittal localizer images showing the size and location of the mPFC voxel of interest. (C) Demonstration of *in vivo* human brain GABA and Glx detection by ^{1}H MRS: (a) and (b), single-voxel subspectra acquired in 13.4 minutes with the editing pulse on and off and 256 (512 total) interleaved averages; spectrum (c), difference between spectra (a) and (b) showing the edited brain GABA and Glx resonances; spectrum (d), model fitting of spectrum (c) to obtain the GABA and Glx peak areas; spectrum (e), individual components of the fits; spectrum (f), residual of the difference between spectra (c) and (d). Abbreviations: GABA, γ-aminobutyric acid; Glx, glutamate + glutamine; NAA, N-acetyl-aspartate; tCho, total choline; tCr, total creatine; MPFC, medial prefrontal cortex.

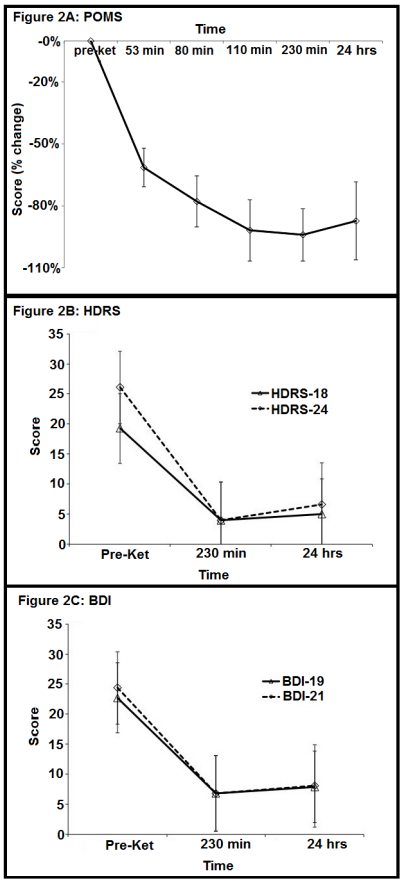

Figure 2.

Figure 2A. Profile of Mood States (POMS) total score, 24 hours pre-ketamine infusion, 80, 110 and 230 minutes after ketamine infusion and 24 hours after ketamine infusion. Error bars denote standard error of the mean.

Figure 2B. 18 Item Hamilton Depression Rating Scale (HDRS-18) total score, 24 hours pre-ketamine infusion, 230 minutes after ketamine infusion and 24 hours after ketamine infusion. Error bars denote standard error of the mean.

Figure 2C. 19 Item Beck Depression Inventory (BDI) total score, 24 hours pre-ketamine infusion, 230 minutes after ketamine infusion and 24 hours after ketamine infusion. Error bars denote standard error of the mean.

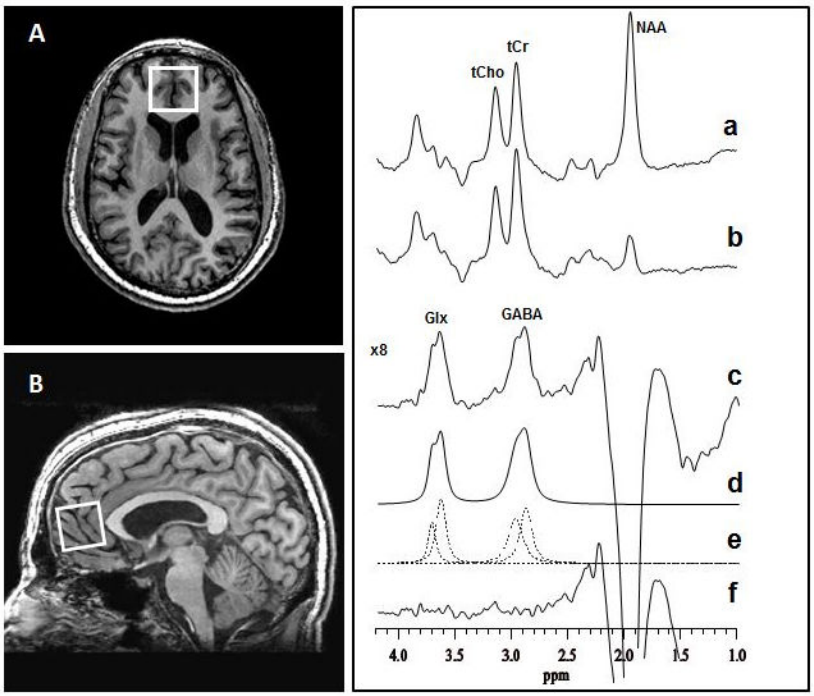

Figure 3.

Figure 3A. Magnetic Resonance Spectroscopy Measurement of GABA/water and Glx/water concentrations in Medial Prefrontal Cortex in Major Depressive Disorder before (baseline frame), during (frame 1–4-) and after (frame 5–6) an intravenous ketamine infusion (40 minutes duration). Frame duration was 13:20 minutes. Asterisks denote statistically significant group increases in GABA and Glx concentrations relative to pre-ketamine baseline levels.

Abbreviations: GABA/W, mean of water-corrected γ-aminobutyric acid level; Glx/W, mean of water-corrected glutamate + glutamine level. Error bars denote standard deviation from the mean.

Figure 3B. Individual subjects' Glx/W and GABA/W responses to ketamine as measured by the area under the curve. Abbreviations: AUC, area under the curve; GABA/W, water-corrected γ-aminobutyric acid level; Glx/W, sum of water-corrected glutamate + glutamine level.

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Table 1A

Demographic and clinical characteristics of subjects with Major Depressive Disorder.

(N=11)	**Table 1A: Demographic and Clinical Characteristics**							
	Gender		**Ethnicity**		**Race**		**Responder Status**	
	Male	**Female**	**Hispanic**	**Non-Hispanic**	**White**	**African American**	**At 230 minutes**	**At 24 hrs**
N	3	8	0	11	10	1	10	9
(Mean ± SD):	**Age**		**Age at MDD onset (yrs)**		**Duration of Current MDE (yrs)**	**Duration of MDD (yrs)**	**Number of Previous MDEs** *	
	38.8 ± 12.8		22.1 ± 14.0		8.9 ± 10.7	16.6 ± 9.4	1.1 ± 0.9	

Abbreviations: MDD, Major Depressive Disorder; MDE, Major Depressive Episode.

* 2 patients were excluded from this analysis because we were unable to ascertain the number of previous MDEs.

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Table 1B

Clinical severity measures of current Major Depressive Episode.

(N=11)	**Table 1B: Clinical Severity Measures of Current MDE**		
(Mean ± SD):	**Baseline**	**230 mins**	**24 hrs**
HDRS-18	19.3 ± 5.8	4.0 ± 6.3	5.0 ± 5.9
HDRS-24	26.1 ± 6.1		6.6 ± 6.9
BDI-19	22.7 ± 7.6	6.8 ± 5.8	7.9 ± 8.0
BDI-21	24.4 ± 8.3		8.1 ± 8.0
BPRS	33.1 ± 6.6	21.0 ± 4.5	
POMS	85.3 ± 42.2	14.6 ± 30.7	20.2 ± 38.8

Abbreviations: Baseline, 24 hours pre-ketamine infusion; 230 minutes, 230 minutes post-ketamine infusion; HDRS-17, 17 Item Hamilton Depression Rating Scale; HDRS-18, 18 Item Hamilton Depression Rating Scale; HDRS-24, 24 Item Hamilton Depression Rating Scale; BDI-21, 21 Item Beck Depression Inventory; BDI-18, 18 Item Beck Depression Inventory; POMS, Profile of Mood States; BPRS, Brief Psychiatric Rating Scale.

Table 2A

Correlation analyses between change in Glx over water and GABA over water, frame 1 to frame 4, and clinical outcome (HDRS and BDI, pre-ketamine to Day 1; POMS, pre-ketamine to 230 minutes post-ketamine).

Table 2A: Glx/W and GABA/W and Clinical Outcome						
df = 6	**Change in HDRS-24**		**Change in BDI-21**		**Change in POMS**	
	r	**p**	**r**	**p**	**r**	**p**
Glx/W	−0.21	0.60	−0.24	0.57	0.34	0.41
GABA/W	−0.05	0.90	−0.13	0.75	0.20	0.63

Abbreviations: GABA, γ-aminobutyric acid; Glx, glutamate + glutamine; HDRS-24, 24 Item Hamilton Depression Rating Scale; BDI-21, 21 Item Beck Depression Inventory; POMS, Profile of Mood States.

Table 2B

Correlation analyses between ketamine and its metabolites norketamine and dehydronorketamine and clinical outcome.

Table 2B: Ketamine and Its Metabolites and Clinical Outcome

df=6	% Change in HDRS-24		% Change in BDI-21		% Change in POMS	
	r	p	r	p	r	p
Ket 90 min	−0.17	0.68	0.24	0.56	−0.19	0.68
Ket 120 min	0.01	0.98	−0.02	0.97	−0.42	0.34
Norket 90 min	−0.78	**0.023***	−0.09	0.83	−0.06	0.90
Norket 120 min	−0.66	**0.07**	−0.36	0.38	−0.35	0.44
Deh 90 min	−0.10	0.81	0.07	0.87	0.09	0.85
Deh 120 min	−0.02	0.97	0.02	0.97	0.02	0.96

Abbreviations: ket, ketamine; norket, norketamine; deh, dehydronorketamine; HDRS-24, 24 Item Hamilton Depression Rating Scale; BDI-21, 21 Item Beck Depression Inventory; POMS, Profile of Mood States.

Table 3

Change over time in BPRS and POMS sub-scores.

Scale	Sub-Score	F-Value	DF	P-Value
POMS	Tension	16.1	3, 38	<0.0001
	Depression	16.5	3, 38	<0.0001
	Anger	9.5	3, 38	0.0001
	Fatigue	18.0	3, 38	<0.0001
	Confusion	16.1	3, 38	<0.0001
	Vigor	7.1	3, 38	0.0007
BPRS	Anxiety-Depression	33.5	1, 10	0.0002
	Anergia	9.8	1, 10	0.01
	Thought Disturbance	p=1, all values are equal.		
	Activation	1.9	1, 10	0.20
	Hostile-Suspiciousness	3.2	1, 10	0.10

Abbreviations: POMS, Profile of Mood States; BPRS; Brief Psychiatric Rating Scale.