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Ketamine-induced reduction in mGluR5 availability is associated with an antidepressant response: an [11C]ABP688 and PET imaging study in depression

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Abstract

The mechanisms of action of the rapid antidepressant effects of ketamine, an NMDA glutamate receptor antagonist, have not been fully elucidated. This study examined effects of ketamine on ligand binding to a metabotropic glutamatergic receptor (mGluR5) in individuals with major depressive disorder (MDD) and healthy controls. Thirteen healthy and thirteen MDD nonsmokers participated in two [^{11}C]ABP688 positron emission tomography (PET) scans on the same day – before and during intravenous ketamine administration – and a third scan 1 day later. At baseline, significantly lower [^{11}C]ABP688 binding was detected in the MDD as compared to the control group. We observed a significant ketamine-induced reduction in mGluR5 availability, (i.e. [^{11}C]ABP688 binding), in both MDD and control subjects (average of 14±9% and 19±22%, respectively; $p<0.01$ for both), which persisted 24 hours later. There were no differences in ketamine-induced changes between MDD and control groups at either time point ($p=0.8$). A

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Conflict of Interest
Other authors report no conflict of interest.

significant reduction in depressive symptoms was observed following ketamine administration in the MDD group ($p<0.001$), which was associated with the change in binding ($p<0.04$) immediately post ketamine. We hypothesize that glutamate released after ketamine administration moderates mGluR5 availability; this change appears to be related to antidepressant efficacy. The sustained decrease in binding may reflect prolonged mGluR5 internalization in response to the glutamate surge.

Depression is the psychiatric disorder with the greatest impact on the global disability burden[1]. This is because it is common[2] and because many individuals fail to respond to available treatments[3]. Moreover, currently available medications typically require weeks to months to exert their beneficial effects, which are commonly lost within the first year of treatment[4]. Identifying novel treatment targets and developing new tools that can rapidly ease the burden of depression to individuals and society is thus of high priority.

Glutamate signaling has an important role in regulation of affective processes[5–7]. A postmortem study reported elevated tissue glutamate levels in depression in the anterior cingulate cortex (ACC)[8] and magnetic resonance spectroscopy (MRS) study in the ACC[9] and occipital cortex detected elevated glutamate levels in individuals with MDD[10]. Elevations in glutamate may produce neuroadaptations in neural structure and function, including alterations in the regulation of glutamate receptors[11, 12]. There is, however, also MRS literature showing lower glutamate and glutamate/glutamine (GLX) levels in individuals with depression[13, 14].

The metabotropic glutamatergic receptor 5 (mGluR5) is a receptor target for glutamate that may show depression-related changes in regulation. MGluR5 is a G-protein coupled receptor located pre- and post-synaptically[15–18] and is present on neurons both at the cell surface and intracellularly[19–21]. Preclinical literature suggests that changes in mood states are associated with changes in mGluR5 levels. Knock out (KO) of mGluR5 in mice is associated with depressive-like behavior,[22] and reductions in mGluR5 protein occur in animal models of depression[23, 24]. In human postmortem brain tissue, reductions in mGluR5 protein levels have been reported in depressed individuals in the cerebellum[25] and prefrontal cortex (PFC, BA9)[26]. A different postmortem study, however, failed to demonstrate significant alterations in the anterior cingulate mGluR5 density in MDD[27]. *In vivo*, a positron emission tomography (PET) imaging study with [^{11}C]ABP688 reported lower mGluR5 density in MDD in several regions[26]. Chronic, but not acute, antidepressant treatment appears to increase mGluR5 expression,[28] raising the possibility that therapeutic effects might be mediated by mGluR5 regulation.

In the present study, we compared ketamine effects upon mGluR5 availability in depressed patients and healthy comparison subjects. Ketamine is an NMDA glutamate receptor antagonist that produces clinically meaningful improvement in depression symptoms within 24 hours of administration in a majority of patients[29, 30]. Previous work in rodent models have shown evidence suggesting rapid changes in glutamate cycling are related to the rapid antidepressant effects of ketamine and other rapidly acting agents[31, 32].

Using [^{11}C]ABP688, a PET tracer specific for the negative allosteric modulator (NAM) site on the mGluR5, our prior study demonstrated a rapid and large (20%) reduction in [^{11}C]ABP688 binding after ketamine infusion in healthy humans[11]. Since glutamate does not compete with radioligands that target the NAM site on mGluR5 (Novartis, personal communication; Lin, in preparation), the ketamine-induced decrease in ligand binding could be an indicator of reduction in mGluR5 availability (i.e., internalization) in response to glutamate released following ketamine administration[11] or a glutamate-induced conformational change in the receptor that reduces the likelihood of radioligand binding at the NAM site. Presently, we examined ketamine effects on mGluR5 availability in depressed and healthy subjects. We hypothesized that the administration of ketamine would lead to an immediate reduction in mGluR5 availability and that the magnitude of the reduction of mGluR5 in the PFC or hippocampus would predict improvement in depressive symptoms in the depressed cohort. Secondary exploratory analyses investigated whether the reduction in mGluR5 availability during infusion is maintained at 24 hours post-ketamine; the time point with highest antidepressant effect.

Methods and Materials

Subjects

The Yale University Institutional Review Board, the Yale Radiation Safety Committee and the Yale-New Haven Hospital Radiation Safety Committee approved this study. After providing informed consent, inclusion criteria were assessed by: a physical examination, routine blood tests, psychiatric and neurological examination. A drug screen (urine), ECG, and a pregnancy test (for female subjects) were performed as part of subject screening, before radiotracer administration. Inclusion criteria for both cohorts were: 1. Subjects are between 18 and 60 years old, 2. English speaking. Inclusion criteria for healthy controls were: 1. No current, or history of, any DSM-IV diagnosis as assessed by SCID, 2. No first-degree relative with history of psychotic, mood, or anxiety disorder, 3. No regular medication use within the past two months, and no history of psychiatric medication use. 4. No lifetime drug abuse or dependence disorder (including for alcohol and nicotine). Inclusion criteria for depressed subjects were: 1. Currently in a depressed mood state as defined by MADRS severity scores, 2. Primary diagnosis of MDD as assessed by DSM-IV SCID, 3. No other current DSM-IV diagnosis, besides anxiety disorder, 4. No use of psychiatric medication in the past month preceding the study. 5. No significant suicidal ideation. 6. No lifetime drug abuse or dependence disorder (including for alcohol and nicotine).

Thirty-three subjects were met eligibility criteria for the study (16 control and 17 depressed). Of those, one HC subject was withdrawn from the study after the baseline PET scan due to high blood pressure (prior to ketamine administration); two HC could not tolerate the full ketamine dose due to psychotomimetic effects and were unable to continue with the scanning procedures, thus their data were discarded; 2 subjects with depression were excluded for abnormally rapid plasma radiotracer metabolism, which prevented an accurate fit of the plasma data; and 1 subject exhibited too much motion during the ketamine scan for accurate analysis. In total, thirteen contemporaneous healthy non-smoking volunteers (6 males, 7 females) and 14 unmedicated mild to moderately depressed subjects (4 males, 10 females) completed this study (mean age: 33.5 ± 13.2 years) (Table 1). Data from the first 10 HC's (same day assessment only) were published previously[11].

Psychiatric Assessments

A structured clinical interview (SCID-NP) and psychiatric history were conducted at screening. Montgomery–Åsberg Depression Rating Scale (MADRS)[33] and Beck Depression Inventory (BDI-II)[34] were used to assess subjects' depressive symptoms both during intake and on PET scan day (before and 30 min and 24 hours after ketamine administration). The effects of ketamine on the subject's mental state were assessed using the Clinician Administered Dissociative State Scale (CADSS)[35] and Profile of Mood States (POMS)[36].

Positron Emission Tomography

[^{11}C]ABP688 of high *E/Z* ratio (70:1; $n = 20$) is produced from reacting [^{11}C]methyl iodide with desmethyl-ABP688 in the presence of tetrabutylammonium hydroxide using the loop method developed by Nabulsi[37]. The average radiochemical and chemical purities is 97% and 93% average, respectively. The *E/Z* ratio of [^{11}C]ABP688 conformers in the final PET drug product (range: 42/1 – 98/1) was determined for each produced batch by analytical radio HPLC area percent. The *E*-isomer exhibits a higher K_D *in vivo*[38]. PET imaging was performed and analyzed as described previously[11].

Subjects participated in 3 scans: 2 on same day - baseline [^{11}C]ABP688 scan and [^{11}C]ABP688 scan with ketamine infusion, and [^{11}C]ABP688 scan 24 hours after ketamine infusion. The radiotracer [^{11}C]ABP688 was administered as a bolus in all scans. After the baseline [^{11}C]ABP688 scan, subjects were given a short break (~ 1 hour). A second [^{11}C]ABP688 dose was administered (as a bolus over 1 min) and, following successful radioligand administration, ketamine was immediately administered. The study was designed as such to decrease subject burden in case of equipment failure. In addition, the design ensures the immediate effects of ketamine (rapid glutamate release) are captured. Blood pressure, pulse and peripheral capillary oxygen saturation (SPO_2) were acquired before and after ketamine administration, and during the ketamine infusion (at 5–10 minute intervals). Twelve (of 14) depressed and 10 (of 13) healthy control subjects returned for the 24-hour scan. Of these scans, five (2 from the depressed cohort, 3 from the healthy control cohort) were removed from analysis because arterial lines could not be placed.

The Yale New Haven Hospital Pharmacy provided racemic ketamine, which was administered intravenously[11, 39, 40] (initial bolus: 0.23 mg/kg over 1 minute, followed by constant infusion: 0.58 mg/kg over 1 hour). The dose administered here is considered a psychotomimetic dose, and not antidepressant dose.

T1-weighted Magnetic Resonance Images (MRIs) were acquired on a 3T Trio imaging system (Siemens Medical Systems, Erlangen, Germany) and used to delineate anatomical regions on the PET (coregistered to the MRI). The MRI voxel size was 1×1×1 mm.

Input Function Measurement

Prior to PET imaging, catheters were inserted in the forearm veins and radial artery for radioisotope injection and arterial blood sampling, respectively, as previously described[11]. Radioactivity was analyzed as previously described[41]. After correction for dispersion and delay[42], the automated and manual plasma concentration values were combined and convolved with a Gaussian function (FWHM = 24 s) for smoothing.

An HPLC assay of arterial blood samples (five samples at 0, 4, 12, 30, and 60 min) provided unmetabolized parent compound levels[43]. These levels were fit with a Hill function[44]. To calculate the input function, the fit parent fraction curve and merged plasma counts were multiplied. A combination of a straight line (before the peak) and the sum of three exponentials, (after the peak) was used to fit the combined data in order to generate the metabolite-corrected arterial input function. Free fraction (f_P) measurements were considered unreliable[45].

Image Analysis

Image analysis routines were implemented in MATLAB (The MathWorks, Natick, MA) as previously described[11]. Regions of interest were probabilistic, as determined by nonlinear registration of previously drawn regional atlases to the subject's MRI as previously described[46]. Cortical regions of interest were grey matter masked by voxel-wise multiplication (regional probability multiplied by the probability of that the voxel is in grey matter, as assessed by Statistical Parametric Mapping, SPM5; Institute of Neurology, University College of London, London, England). In this work, the striatum was defined as a weighted (by volume) average binding in the dorsal caudate, dorsal putamen and ventral striatum. A semi-automated technique was used to coregister the subject's mean PET image to the MRI[47]. The mean activity of a region, which was weighted by regional label probabilities, within each PET frame was then calculated to generate time activity curves.

Outcome Measure Calculation

An unconstrained two-tissue compartment (2TC) mode was used to calculate regional outcome measures[11, 48]. Given the unreliable f_P values and a non-ideal reference region[49], V_T (volume of distribution: ratio, at equilibrium, of the ligand concentration in the region of interest to that in the plasma[50]) was used as the main outcome measure. Percent change in binding was then calculated as $[(V_{T,baseline}-V_{T,ketamine\ or\ 24\_hour\_scan}) / V_{T,baseline}]*100$.

Statistical Analysis

To determine the significance of measured V_T differences due to ketamine administration or between groups or sexes at baseline, a linear mixed-effects model with region as a fixed effect was used. The dependence structure among regions and images from the same subject was modeled using the Kronecker product between unrestricted symmetry (to model the correlation among all regions) and compound symmetry (to model the correlation between two scans). The interaction term between region and scan was examined and, if appropriate, included in the model. To model change in subjects' vital signs following ketamine administration, linear mixed models for longitudinal data were also used. The dependence structure used for these models was compound symmetry. The paired comparisons of scores from subjective reports from two time points were performed using Wilcoxon's signed rank test. Both unadjusted p-values and False Discovery Rate (FDR) corrected values, based on Benjamini & Hochberg method, are provided for these paired comparisons. Tests were all two-sided. All analyses were performed using R 3.0.2 (http://www.r-project.org/) and SAS 9.2 (SAS Inc. Cary, NC).

Results

All mean results are presented as an average ± standard deviation.

Vital Signs/Subjective Report

Significant increases in blood pressure and heart rate were observed during the ketamine scan. Systolic and diastolic blood pressure and heart rate were significantly (p <0.05) higher than baseline until ~30 min post ketamine, similar to our previous report[11]. SPO_2 values differed from baseline only at the 4-min time point.

There were statistically significant, large reductions in depression scores following ketamine administration (45±70% on MADRS, 52±29% on BDI-II; all p's <0.001) and 24 hours post ketamine (53±69% on MADRS, 49±35% on BDI-II; all p's <0.001) in the MDD group, which is similar to previously reported[29, 30] (Table 1).

There was a significant increase in CADSS scores during ketamine administration in both the HC and MDD groups (amnesia: F(2,22)=15.08, p<0.001; depersonalization: F(2,22)=24.48, p<0.001; derealization: F(2,22)=25.85, p<0.001), which returned to baseline at 24 hours. No between-group differences were observed, however, suggesting that the immediate psychotomimetic effects of ketamine were similar between control and depressed groups (all F's<1.97, all p's>0.16).

Tracer Metabolism/Clearance

No significant differences were observed between scans in injected dose (568±143 MBq), specific activity (329.8±208.6 MBq/nmol), injected mass (0.7±0.7 μg) or delivery rate of tracer from arterial plasma to brain tissue (K_1[50]), in either cohort (listed values represent averages over all scans). Clearance rates (injected dose divided by the calculated area under the metabolite-corrected arterial input function[51]) were not significantly different across time points in the depressed cohort (baseline: 93.0±12.5 L/h, ketamine: 87.1±12.9 L/h, 24 hours: 91.7±14.4 L/h, $p = 0.37$). However, there was a significant decrease in clearance between the baseline and ketamine scans (−11.3±21.6% difference, on average) in the healthy control cohort. The average clearance values for the healthy control cohort were: baseline: 104.1±36.6L/h, ketamine: 89.5±29.2 L/h, 24 hours: 83.2±17.7 L/h, $p = 0.03$ (linear mixed effects model across times).

Baseline Analysis

Baseline mGluR5 availability (V_T) was significantly lower in the depressed group as compared to the healthy cohort in all regions assessed (Figure 1, $p = 0.01$, linear mixed effects model across all regions).

Ketamine-Induced Change in [^{11}C]ABP688 Binding

There were no significant between-group differences in the amount of ketamine injected (p=0.16). As indicated in Figure 2, significant binding (V_T) reductions from baseline were observed on average during the ketamine scan and the 24-hour scan, both within the depressed group and the healthy controls ($p < 0.01$, linear mixed effects model, in both cases). No significant differences were observed between the ketamine scan and the 24-hour scan in either group ($p > 0.8$, in both cases). The linear mixed effects model did not indicate a diagnosis by scan interaction ($p = 0.46$), showing that the binding *reductions* (V_T; from baseline to ketamine scan and from baseline to 24-hour scan) were not significantly different between depressed and control groups. Figures 2c and 2d show the average percent difference in V_T from baseline. Across all subjects and regions, the percent reductions from baseline in the control and depressed cohort, respectively, were: 19±22% and 14±9% in the ketamine scan and 31±30% and 14±13% in the 24-hour post ketamine scan. As indicated by this and Figure 2 (c and d), variability was particularly high for the 24-hour post ketamine scan in controls (grey bars).

To examine this variability further, Figure 3 shows the changes in V_T for each individual subject. On average, there was a significant decrease in binding during the ketamine scan, and this pattern was similar for individual brain regions. However, two healthy control subjects (Figure 3 Left) showed increases in binding. Further, there was high variability in the 24-hour scan in the healthy controls (subject binding differences from baseline ranged from −75±13% to 14±3%).

Associations with Mood during Ketamine PET Scan

We examined whether there was an association between ketamine-induced changes in mGluR5 availability and mood in the PFC and hippocampus. Given the sample size and exploratory nature of the analyses, we did not correct for multiple comparisons. Exploratory analyses (4 in total: MADRS and BDI-II as measures and PFC and hippocampus as regions) revealed that reduction in mGluR5 availability in the hippocampus was associated with a reduction in total depressive symptoms assessed by the MADRS (r= 0.52, p=0.035, Figure 4). The questions that MADRS and BDI-II include have several latent factors, including psychic anxiety and dysphoric apathy[52, 53]. In our data, the change in mGluR5 availability in the hippocampus was strongly correlated with the psychic anxiety symptoms on both the MADRS (r= 0.6, p=0.031, Figure 4) and the BDI-II (r= 0.68, p=0.010). We did not observe similar associations between change in mGluR5 availability and the dysphoric symptoms.

Discussion

The current study highlights the acute reduction in mGluR5 availability *in vivo* in humans following administration of ketamine, consistent with the capacity of this drug to enhance glutamate release[54]. Reductions in mGluR5 availability persisted for 24 hours following ketamine administration, suggesting that glutamate release triggers persisting adaptations in mGluR5s, perhaps through internalization. Interestingly, hippocampal mGluR5 changes during infusion correlated with ketamine's rapid antidepressant effects, in particular the latent factor of psychic anxiety symptoms.

At baseline, mGluR5 availability (measured as V_T) in the depressed cohort was significantly lower than in the controls. Lower mGluR5 availability in the depressed cohort was consistent with a previous report of lower mGluR5 levels in individuals with MDD in several brain regions and reduced prefrontal cortex mGluR5 protein expression in postmortem brains of depressed subjects.[26]

In this work, ketamine-induced reductions in mGluR5 availability were observed in both the depressed and control cohorts, and these reductions persisted 24 hours after the ketamine infusion. The immediate decreases in mGluR5 availability are likely due to the rapid surge in extracellular glutamate leading to mGluR5 downregulation or internalization. The glutamate surge hypothesis is consistent with rodent data showing that a single administration of ketamine rapidly, but only transiently, induces increases in glutamate efflux[54] and cycling[32, 55]. The hypothesis is also in line with other recent studies demonstrating that activation of postsynaptic AMPA receptors is a critical component related to the rapid antidepressant-like effects associated with several additional compounds[56, 57]. Evidence exists from other studies suggesting that activation of the AMPA receptors leads to changes in the mGluR5 signaling and facilitation of downstream effects[58], which could play a role in the current work.

Given the apparently similar ketamine-induced decrease in mGluR5 availability and similarity in the psychotomimetic response, it appears that the ketamine-induced surge in glutamate does not differ between these groups. Previous studies have found both elevated[8–10] and reduced[13, 14] levels of total tissue glutamate levels to be associated with MDD. These findings may suggest that some difference in ketamine-induced release would be expected between the MDD and healthy comparison subjects. However, it is critical to note that these studies are providing measures of total tissue glutamate levels, and do not truly inform us about glutamate transmission to any extent, thus making it very difficult to predict the effect on stimulated glutamate release.

As noted above, the allosteric relationship between the radioligand used and the endogenous neurotransmitter glutamate is different in our study as compared to many published PET studies where the radiotracer interacts with the orthosteric site and thus is subject to direct competition by endogenous neurotransmitter. In our study, however, the radioligand is a NAM that binds to a site in the transmembrane domain of the receptor and not to the N-terminal orthosteric site where glutamate binds. Consistent with its different sites of binding, glutamate does not directly impact the binding of [^{11}C]ABP688 in a membrane preparation but it does lead to receptor internalization (Lin et al., in preparation). Thus, the measured reduction in receptor availability is potentially due to rapid receptor internalization. It is also possible that the rapid surge in glutamate leads to a conformational change of the receptor, reducing the ability of the radioligand to bind to the receptor. The overall result would be an observation of lower receptor availability. Overall, the decrease in ligand binding observed here immediately following treatment likely represents an indirect *in vivo* measure of the ketamine-induced glutamate surge. However, the exact mechanism leading to mGluR5 downregulation in the current study cannot be assessed with PET imaging; nonetheless, our data suggest that long-term reduced mGluR5 availability may play an important role in relieving symptoms of anxiety specifically.

The glutamate surge has been shown to be transient in nature[32, 54, 59]. Thus the persistent mGluR5 downregulation at 24 hours is unlikely to reflect continued exposure of these receptors to elevated glutamate levels. We hypothesize that the persistent reduction in mGluR5 availability reflects an imbalance between mGluR5 internalization and the rate of recycling of internalized receptor to the plasma membrane within the 24-hour period between assessments. Preclinical studies are in line with this interpretation. mGluR5 receptors are anchored to the cytoskeleton via the proteins Homer and Shank. mRNA levels for Homer decline as early as 90 minutes after ketamine infusion, with downregulation of Homer 1B (although upregulation of Homer 1A has been reported)[60]. Persistent downregulation of mGluR5 may be among the persisting neuroadaptations to acute ketamine administration that are associated with the persistence of antidepressant effects beyond the brief occupancy of NMDA receptors by ketamine[57].

It is possible that we are underestimating the ketamine-induced decrease in mGluR5 density during infusion. In studies of test-retest reliability of mGluR5 radioligand binding, when test and retest studies are conducted on the same day, we have consistently found an *increase* in binding in the retest (afternoon) scan[61, 62] (average percent increase in V_T: 27±32%). In prior work, we also reported a significant correlation between percent change in V_T from baseline and percent change in K_1/clearance from baseline, using two tracers that bind to mGluR5 - [^{11}C]ABP688 and [^{18}F]FPEB[62]. We have hypothesized that the significant correlation between the percent change in V_T and the percent change in clearance / K_1 was due to the diurnal variation in glutamate underlying all three parameters: K_1 (as there are multiple pathways by which glutamate influences blood flow in the brain[63]), clearance and V_T changes. This may be the case in this study as well: namely, the post-ketamine surge in glutamate may similarly affect tracer binding, K_1 and clearance, since a similar correlation was observed (ρ = 0.3/0.6, p = 0.04/<0.01 for correlation of V_T with K_1 /clearance across all subjects and all time points). Given that diurnal glutamate changes appear to be in the opposite direction of ketamine-induced changes, it is likely that we are underestimating the ketamine-induced decreases in mGluR5 binding by ~25%, on average. Further, the ultimate goal of this study was to examine the change in specific binding (i.e. mGluR5 binding) due to ketamine. However, in this study, change in total binding (V_T = specific plus nonspecific binding) was evaluated. The ratio of nonspecific to specific binding in the cerebellum was estimated by Kagedal *et al* to be 1.33.[64] As this ratio has only been measured in controls, it is unknown whether the ratio is different in a depressed cohort. Assuming that it is not, based on cerebellar V_T, the non-specific binding of the depressed subjects is estimated to be ~1.3 mL/cc and ~1.1 mL/cc for the controls, on average. Therefore, in the high binding regions of Figure 1 (all regions shown excluding the cerebellum), the non-specific binding is approximately one-third of the total binding (~27–38%). Ketamine is not expected to change the non-specific binding. Therefore, following ketamine treatment, when specific binding is decreased, the non-specific binding is expected to become a greater portion of the total binding (~34–44%, in this case). Taken together, this means that examining percent change in V_T underestimates the total effect on specific binding. Given the values above, this underestimation is ~7% for the depressed cohort and ~10% for the healthy controls. As such, given these assumptions, using specific binding rather than total binding (V_T) should not greatly affect overall conclusions.

We observed that individuals who exhibited the greatest reduction in mGluR5 density during ketamine infusion also exhibited the greatest response, primarily on symptoms related to psychic anxiety (Figure 4), immediately after treatment. Although still preliminary, the results are consistent with prior evidence suggesting a model in which the ketamine-induced glutamate surge, and presumably mGluR5 internalization, is a critical step relating to the rapid acting antidepressant effects of the drug[65]. Briefly, blockade of NMDA receptors on tonically firing GABAergic interneurons is thought to lead to glutamatergic disinhibition, and thus a surge of glutamate in the PFC. This surge increases AMPA receptor activation. which, coupled with ketamine's inhibition of extrasynaptic NMDA receptors, initiates and enables postsynaptic activation of signaling pathways related to neuroplasticity. These pathways include those involving mTOR and BDNF[30, 66]. These neuroplasticity pathways are hypothesized to underlie ketamine's antidepressant response. mGluR5 antagonism and internalization have also been associated with rapid, although not long lasting, antidepressant effects, in preclinical studies[67, 68]. Furthermore, the functional interplay between mGluR5 and NMDA was previously related to antidepressant activity[69], potentially through facilitation of BDNF signaling pathways. A recent, phase 2b study (n = 333), examining the efficacy of a selective mGluR5 NAM (basimglurant) as adjunctive therapy for previously treatment non-responsive MDD, failed to show the drug to produce a statistically significant difference in the primary endpoint (mean change in clinician rated MADRS) compared to a placebo group.[70] However,,the study was complicated by very high placebo response rates and secondary analyses (at 1.5 mg) showed statistically significant differences in basimglurant adjuvant therapy versus placebo in multiple other scales including the patient rated MADRS and CGI-Improvement mean score. Thus, the findings suggest that future studies are required to more fully understand the clinical utility of this psychopharmacological approach.

mGluR5 antagonism has also been shown to be effective in reducing anxiety symptoms[71–74]. The rapid anxiolytic effects of mGluR5 antagonists such as MPEP do not appear to rely on new protein synthesis but may partially involve facilitation of mTOR signaling pathways and regulation of glutamate neurotransmission[71, 75]. Sustained effects of mGluR5 antagonism, however, as seen at the 24 h time point, may rely on new protein synthesis and mTOR signaling pathways that lead to normalization of synaptic plasticity[76, 77], as well as other factors that have yet to be elucidated.

Recently, it has been suggested that a metabolite of ketamine, (2S,6S;2R,6R)-hydroxynorketamine (HNK) may be responsible for the antidepressant response since in preclinical studies HNK was necessary and sufficient for this response.[56] The metabolite pathway is independent of NMDA receptors, acting through AMPA receptors to activate BDNF and mTOR pathways. However, even this independent pathway would affect mGluR5 downstream. Evidence from other studies suggests that activation of the AMPA receptors leads to changes in the mGluR5 signaling and facilitation of downstream effects[58], which could play a role in the current work. The exact mechanism leading to mGluR5 downregulation in the current study cannot be assessed with PET imaging; nonetheless, our data suggest that mGluR5 may play an important role in relieving symptoms of anxiety specifically.

Limitations

There are several limitations of this study. First, our study lacks a placebo control group. Ketamine is an increasingly well-known therapeutic agent, and individuals with MDD may have the expectation of reduction in symptoms post ketamine administration; some of the antidepressant response might be due to these expectations. However, the reduction in depressive symptoms was associated with a change in receptor availability, suggesting that the change in symptoms was not solely due to expectation, but potentially due to the therapeutic effects of the glutamate surge and mGluR5 downregulation. Second, hemodynamic ketamine effects may alter tracer delivery and washout. The kinetic models used assume equilibrium conditions[50]; however heart rate and blood pressure were elevated (transiently) during ketamine administration. Furthermore, ketamine has been associated with increased blood flow in frontal regions[78–80], measured with fMRI, although the effect reported was not great and isolated to a few regions. In this study, however, the delivery of tracer (K_1) did not significantly change post-ketamine, and clearance only differed post-ketamine in the control group. Thus, given the magnitude of the previously reported effects and the lack of difference in K_1 over the whole scan, even if ketamine were to cause transient changes in K_1, these are unlikely to affect outcome. Further, the outcome V_T, by incorporating the metabolite-corrected arterial input function, accounts for other potential changes induced by ketamine (e.g. metabolism).. Third, our study lacks long-term follow up. Repeat scanning about 2 weeks post ketamine administration, at the time when the antidepressant response wanes, would help determine the time course of mGluR5 re-insertion into the membrane and whether this corresponds to the increases in depressive symptoms. Fourth, only nonsmokers were studied in the present experiment. The extent of ketamine induced changes on mGluR5 availability (or [^{11}C]ABP688 binding) in smokers has yet to be determined. Although previous work showed that there is downregulation in mGluR5 with chronic tobacco exposure[81] and both mGluR5 NAMs and NMDA antagonists appear reduce smoking related behaviors, whether there are smoking-specific differences in the glutamate surge (and mGluR5 downregulation) post ketamine administration is not known. Thus, the current results might only apply to the nonsmoking MDD population. Last, the ketamine dose administered here is higher than the typical dose used for clinical purposes. Thus, the extent of change in receptor availability might be greater in this study as compared to the general practice.

Conclusion

We conducted a novel examination to evaluate ketamine-induced changes in mGluR5 availability using PET in healthy and depressed cohorts. Significant decreases in [^{11}C]ABP688 binding, a tracer specific for mGluR5, were observed in both cohorts. It is likely that this decreased binding reflects mGluR5 downregulation/internalization. Exploratory analyses suggest that mGluR5 downregulation (and/or the likely surge in glutamate) plays a role in the reduction of depressive symptomatology. These data support the development and implementation of mGluR5 therapies for the relief of depressive symptomatology.

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John Krystal: 1. Vladimir, Coric, Krystal, John H, Sanacora, Gerard – Glutamate Modulating Agents in the Treatment of Mental Disorders US Patent No. 8,778,979 B2 Patent Issue Date: July 15, 2014. 2. Charney D, Krystal JH, Manji H, Matthew S, Zarate C., - Intranasal Administration of Ketamine to Treat Depression United States Application No. 14/197,767 filed on March 5, 2014; United States application or PCT International application No. 14/306,382 filed on June 17, 2014. Also, consultant/advisory board for: AMGEN, AstraZeneca Pharmaceuticals, Biogen, Idec, MA, Biomedisyn Corporation, Forum Pharmaceuticals, Janssen Research & Development, Otsuka America Pharmaceutical, Inc., Sunovion Pharmaceuticals, Inc., Takeda Industries, Taisho Pharmaceutical Co., Ltd, Biohaven Pharmaceuticals, Blackthorn Therapeutics, Inc., Lohocla Research Corporation, Luc Therapeutics, Inc., Pfizer Pharmaceuticals, TRImaran Pharma

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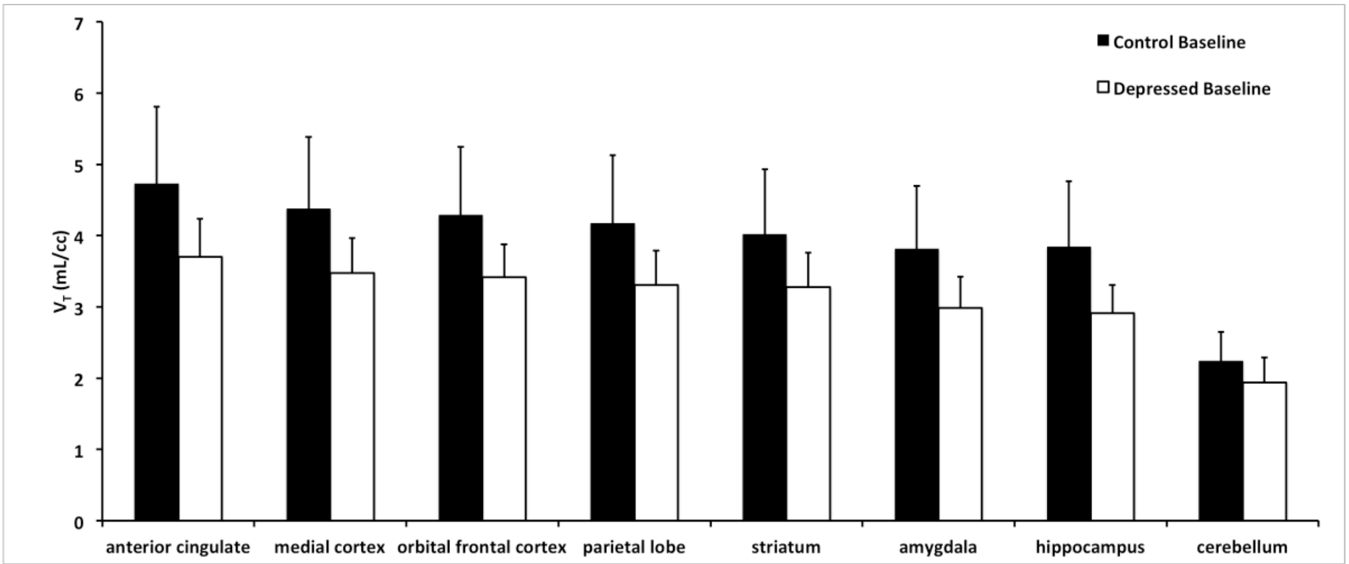

Figure 1.

Average Volume of Distribution (V_T) across healthy control (*n* = 13, black) and depressed (*n* = 14, white) subjects. All individual regional differences were significant in post hoc analysis. Error bars represent standard deviation across subjects.

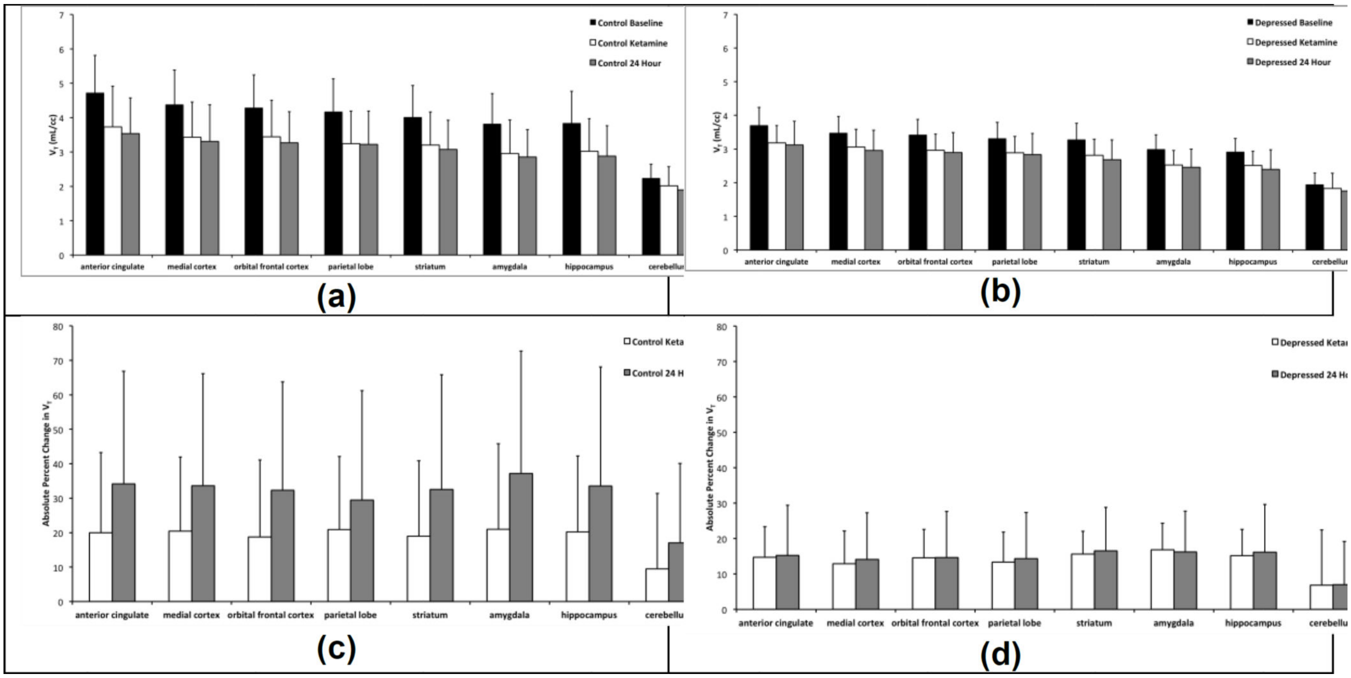

Figure 2.

Top: Average Volume of Distribution (V_T) across (a) healthy control (*n* = 13 total, 7 at 24 hours) and (b) depressed (*n* = 14 total, 10 at 24 hours) subjects. Average values from the baseline (black bars), ketamine challenge (white bars), and 24-hours post ketamine (grey bars) scans are shown. Regions are organized from left to right in order of highest to lowest mean baseline binding. Differences in all regions shown are statistically significant ($p<0.05$, uncorrected) in post hoc testing. Error bars represent standard deviation across subjects. For comparison, the depressed and control cohort data are displayed with the same scale.

Bottom: Average Percent Reduction in V_T from Baseline. Percent differences were measured across all subjects within a region and averaged. All average percent differences are negative. Absolute values are shown for easier visualization. Error bars represent standard deviation across subjects. For comparison, the control (c) and depressed (d) cohort data is displayed with the same scale.

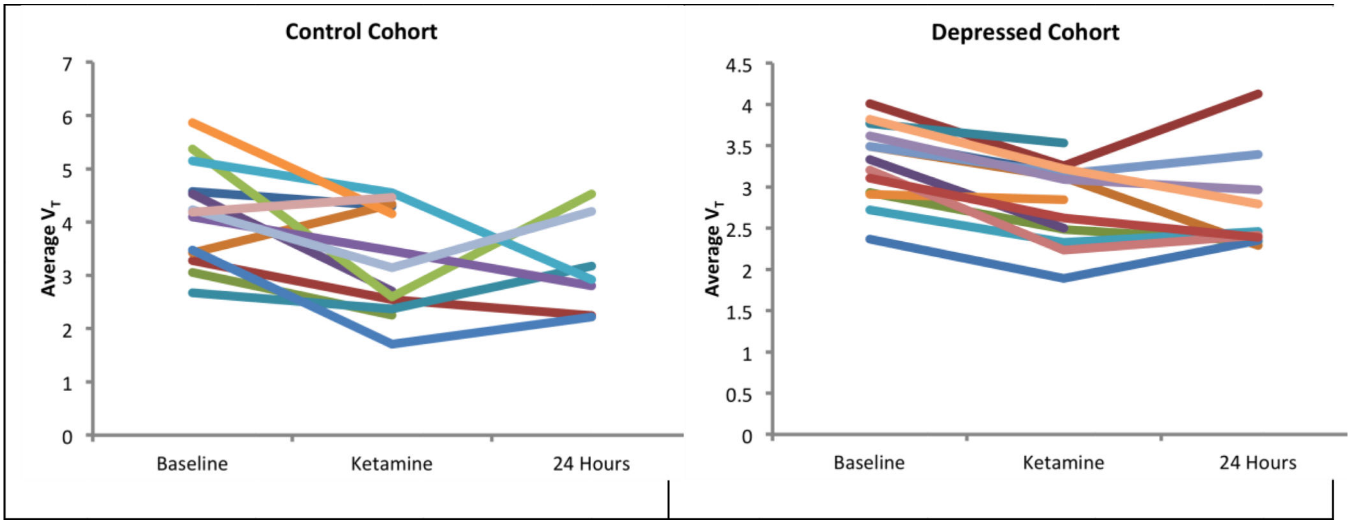

Figure 3.

Average Volume of Distribution (V_T) within a Subject. Each subject is represented by a single line. Plotted values are the average V_T values across all regions.

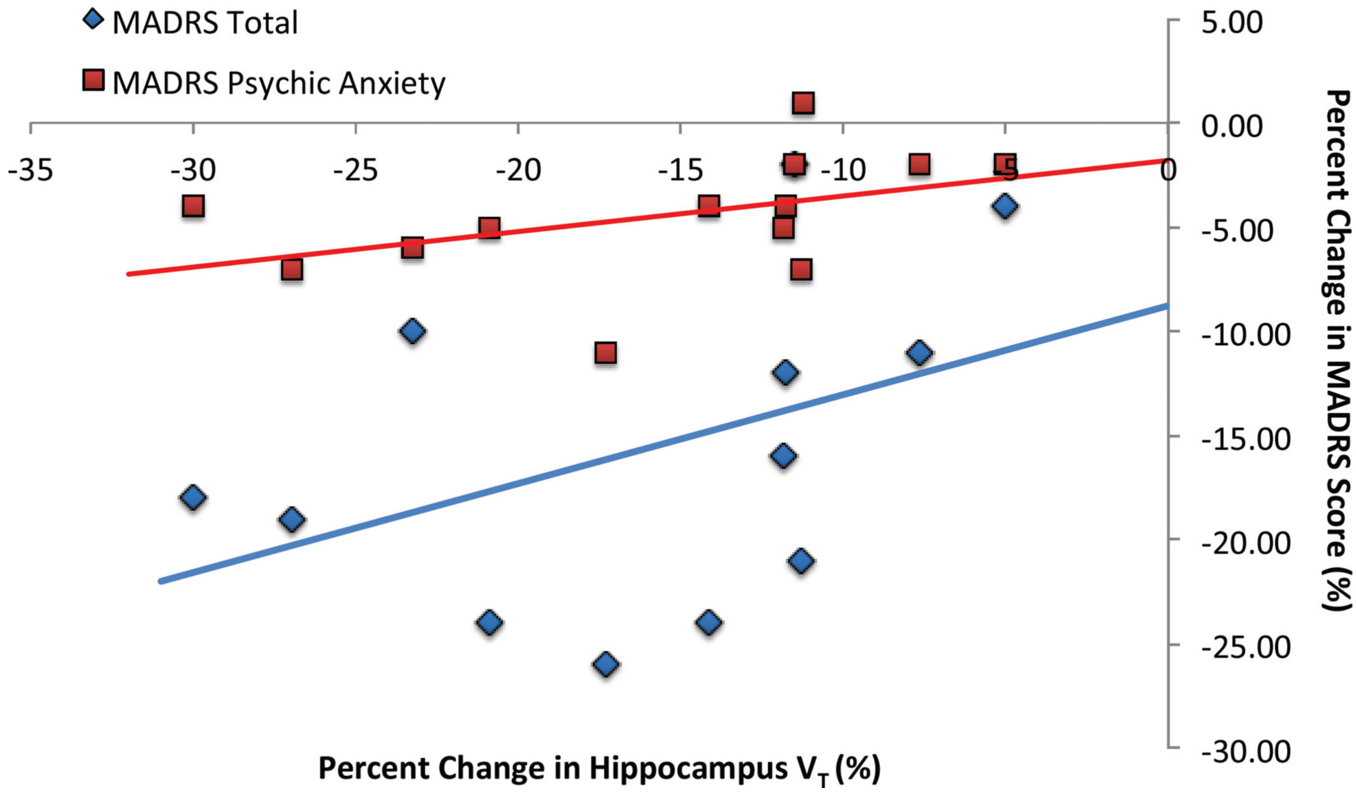

Figure 4.

Relationship between Ketamine-induced Change in Montgomery–Åsberg Depression Rating Scale (MADRS) Score [Total (blue) and Psychic Anxiety (red)] and Percent Change in Volume of Distribution (V_T) in the Hippocampus. Each symbol represents a different subject.

Table 1

Demographic characteristics

	MDD (n=14)			HC (n=13)		
Age, yrs	35.6±13.6			33.1±13.1		
Gender, male	46%			29%		
Age at illness onset, yrs	25.1±12.0			NA		
Duration of current episode, months	52.4±130			NA		
	Baseline	Post ket	24 hrs	Baseline	Post ket	24 hrs
MADRS	23.9±8.7	11.5±11.2	11.9±12.3	0.5±1.0	2.3±3.3	1.1±1.8
BDI-II	24.2±11.4	13.2±8.5	14.3±11.2	0.2±0.6	0.7±1.5	0.4±1.2

MDD: Cohort with Major Depressive Disorder; HC: Healthy Control Cohort; MADRS: Montgomery–Åsberg Depression Rating Scale; HAMD: Hamilton Depression Rating Scale; BDI-II: Beck Depression Inventory; NA: not applicable. Mean±SD is reported.