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Prefrontal Cortical GABA Abnormalities Are Associated With Reduced Hippocampal Volume In Major Depressive Disorder

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Abstract

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Contributors

C.G.A. and J.D.C. conceptualized the structural analysis. C.G.A. and D.C.S wrote the draft of the manuscript. C.G.A. and R.C. managed the literature searches and undertook the analysis. A.J. and J.R.S. performed the MRI image processing, segmentations, quality control and analyses. D.C.S. and X.M. conducted the MRS and MRI studies. D.C.S., X.M., and G.K. performed the MRS image processing and analysis. S.J.M. and D.C.S conceived the study, developed the protocol, oversaw the study, and contributed to the interpretation of the data. All authors critically reviewed the report and have approved the final manuscript.

Conflict of interest

CGA received research fund or consultation fee from Brain and Behavior Research Foundation (NARSAD), American Psychiatric Foundation, and Genentech. SJM received research funding or salary support over the last three years from the Banner Family Fund, Brain and Behavior Fund (NARSAD), The Brown Foundation, Inc., Bristol-Myers Squibb, Department of Veterans Affairs, Evotec, Johnson & Johnson, and the National Institute of Mental Health. He has received consulting fees or honoraria from Allergan, AstraZeneca, Cephalon, Corcept, Noven, Roche, and Takeda. He has received medication (Rilutek) from Sanofi-Aventis for a NIMH sponsored study. Dr. Mathew has been named as an inventor on a use-patent of ketamine for the treatment of depression. Dr. Mathew has relinquished his claim to any royalties and will not benefit financially if ketamine were approved for this use. JDC received grant support from NIMH, NYSTEM, GlaxoSmithKline, Pfizer, and Alexza Pharmaceuticals. He is on the Pfizer advisory board and gives talks for BMS, AstraZeneca, GSK, and Pfizer. JDC received grant support from NIMH, NYSTEM, GlaxoSmithKline, Pfizer, and Alexza Pharmaceuticals. He is on the Pfizer advisory board and gives talks for BMS, AstraZeneca, GSK, and Pfizer. No biomedical financial interests or potential conflicts of interest are reported for AJ, JRS, XM, GK, RC, and DCS.

Hippocampal volume reduction has been related to treatment-resistant depression (TRD) and is hypothesized to reflect impaired amino-acid neurotransmission. To better understand the role of amino acid neurotransmission in hippocampal volume deficits, and subsequent resistance to treatment, this study investigated the relationship between hippocampal volumes and GABA levels in the anterior cingulate cortex (ACC), previously associated with TRD. Thirty-three medication-free major depressive disorder (MDD; 14 TRD and 19 non-TRD) and 26 healthy controls (HC) subjects were studied. Participants underwent high-resolution magnetic resonance imaging (MRI) to estimate hippocampal volume and proton MR spectroscopy (^{1}H MRS) to measure ACC GABA levels. MDD patients, with known ACC GABA levels, were divided into two groups: MDD Low GABA and MDD High GABA. We found a significant reduction in hippocampal volume in the MDD Low GABA group compared to MDD High GABA ($p < 0.001$) and HC ($p = 0.01$). The relationship between hippocampal volume and cortical GABA was population (i.e. MDD group) and region specific (i.e. prefrontal cortex). Comparing TRD, non-TRD and HC groups, there was a main effect of group on hippocampal volume ($p = 0.04$), which *post hoc* analysis revealed as smaller hippocampal volume in TRD subjects than in non-TRD ($p = 0.05$) and HC groups ($p = 0.03$). No hippocampal volume differences between non-TRD and HC groups. The data provides insight into the role of prefrontal neurochemical deficits in the limbic structural abnormalities observed in MDD. In addition, it replicates the relationship between TRD and smaller hippocampal volumes.

Keywords

major depressive disorder (MDD); γ-Aminobutyric acid (GABA); hippocampal volume; magnetic resonance spectroscopy (1H MRS); MRI; treatment resistant depression

1. Introduction

Major depressive disorder (MDD) is a prevalent mental illness and a leading cause of disability worldwide (Collins et al., 2011). Yet, to date, the mechanisms underlying the neurobiology and treatment of MDD are not fully understood. Fewer than 50% of MDD patients show a clinically meaningful response following a 3-month treatment with a monoaminergic reuptake inhibitor (Trivedi et al., 2006). This high prevalence of treatment resistance to monoaminergic antidepressants suggests further insights into the neurobiology of MDD can be gained through scrutiny of neurobiological features associated with treatment resistance. For this report, we investigated two such features previously associated with antidepressant resistance in MDD, namely, decreased hippocampal volume (MacQueen and Frodl, 2011) and a deficit in the levels of the inhibitory amino acid neurotransmitter γ-Aminobutyric acid (GABA) in the anterior cingulate cortex (ACC) (Bhagwagar et al., 2008; Gabbay et al., 2012; Price et al., 2009).

Although meta-analyses of the totality of the data on hippocampal volume in MDD have consistently found a reduction, there are several studies that have reported normal hippocampal volume in MDD, and others that have shown left-but-not-right or right-but-not-left hippocampal volume changes [reviewed in (McKinnon et al., 2009)]. Clinical predictors have generally associated reduced hippocampal volume with a chronic and resistant course of illness (e.g. longer duration of illness or multiple depressive episodes)

(McKinnon et al., 2009; Sheline et al., 2012; Vakili et al., 2000). Together, these findings suggest that treatment-resistant depression (TRD) is likely a characteristic of a subgroup of MDD patients with hippocampal volume deficits. However, the neurochemical correlates of hippocampal volume alterations are not known.

Low ACC GABA levels have been reported in MDD (Bhagwagar et al., 2008; Gabbay et al., 2012; Price et al., 2009). In addition, low GABA has been recently proposed as one of the most promising endophenotypes in MDD (Hasler and Northoff, 2011). Preclinical and clinical evidence has increasingly associated TRD with dysregulation of the major inhibitory and excitatory amino acid neurotransmitter systems of GABA and glutamate, respectively, in mood and anxiety disorders. Studies in animal models of depression have shown reduced GABA and glutamate metabolism, abnormal glutamate release, reduced post-synaptic glutamate receptors, and glutamate uptake deficits (Sanacora et al., 2012). Furthermore, abnormal glutamate and GABA neurotransmission has been associated with TRD in humans (Levinson et al., 2010; Price et al., 2009; Zhang et al., 2013a). These GABAergic and glutamatergic abnormalities are believed to precipitate excitotoxicity and structural changes leading to hippocampal volume reduction (Drevets et al., 2008; Kassem et al., 2013), and several human studies have reported evidence of an association between smaller hippocampal volumes and poor response to standard antidepressants (Frodl et al., 2008; Frodl et al., 2004; Hsieh et al., 2002; Kronmuller et al., 2008; MacQueen et al., 2008; Sheline et al., 2012; Vakili et al., 2000).

Based on the preceding and because ACC (a) plays a critical role in stress and emotional regulation, (b) has been implicated in the pathophysiology and treatment (e.g. site for DBS) of MDD, (c) is well connected to the hippocampus, and (d) has been shown to have abnormally low GABA levels in MDD (Bhagwagar et al., 2008; Gabbay et al., 2012; Price et al., 2009), we aimed to characterize associations among hippocampal volume, ACC GABA levels, and treatment resistance in MDD. We hypothesized that MDD patients with low ACC GABA levels will have reduced hippocampal volumes compared to healthy controls (HC) and to MDD with high GABA levels. Additionally, we predicted that TRD patients would show reduced hippocampal volumes compared to HC and to non-TRD groups.

2. Experimental Procedures

Study Participants

Detailed description of the study sample and assessment procedures were previously reported (Price et al., 2009). In summary, 33 MDD and 26 HC with successful structural Magnetic Resonance Imaging (MRI) were included in this study; of which 26 MDD and 20 HC had concurrent prefrontal proton Magnetic Resonance Spectroscopy (^{1}H MRS). Institutional Review Boards approved all study procedures and all participants provided informed consents. Diagnoses of MDD were made according to DSM-IV-TR criteria, as established by the Structured Clinical Interview for DSM-IV Axis I Disorders-Patient Edition (First et al., 1995) and by independent interview by a psychiatrist. Using the Antidepressant Treatment History Form (ATHF), patients with three failed adequate antidepressant trials in the current episode were classified as treatment resistant (Sackeim,

2001). Eligible participants were psychotropic medication-free for at least 2 weeks prior to scan; were free of substance abuse/dependence for at least 6 months; had no lifetime history of psychotic disorder, mania, or hypomania; had no pervasive developmental disorder or mental retardation; had no current eating disorder; and had no clinically unstable medical or neurologic conditions. Day-of-scan clinical assessments included 17-item Hamilton Depression Rating Scale ($HDRS_{17}$) and Hamilton Rating Scale for Anxiety (HAM-A). Healthy subjects – with no lifetime Axis I disorder as confirmed by Structured Clinical Interview for DSM-IV Axis I Disorders-Nonpatient Edition – were enrolled as control group (Price et al., 2009).

MR acquisition and processing

MRI and ^{1}H MRS scans were acquired during the same session on a 3.0T GE EXCITE magnetic resonance (MR) system (General Electric Medical Systems, Waukesha, Wisconsin). The ^{1}H MRS scans were performed using a manufacturer supplied eight-channel phased-array head coil and the standard J-edited spin echo difference method (Price et al., 2009). The editing sequence was implemented to record the GABA spectra in 13 min from a 2.5 × 2.5 × 3.0 cm^3 anterior cingulate cortex (ACC) voxel, using TE/TR 68/1500 ms, a 5-KHz spectral width, 1024 sample points, and 256 interleaved excitations, or a total of 512 excitations with the editing pulses on or off (Fig. 1).

The derived raw eight-channel phased-array coil free-induction decay (FID) signals were combined into a single regular FID using a time-domain reconstruction algorithm. The unsuppressed ACC voxel water signal from each receiver channel provided a reference signal to compute the relative sensitivities of the eight array coil elements. Spectra obtained by Fourier transformation of the combined signals were model-fitted in the frequency domain to a pseudo-Voigt function. The derived GABA peak areas were expressed as ratios relative to the area of the unsuppressed ACC voxel tissue water resonance. All mentions of GABA levels should be considered as GABA + macromolecules over water signal unless explicitly stated otherwise. Commercial software, MEDx (Medical Numerics, Sterling, Virginia), was used to segment the brain tissue based on the signal-intensity histogram of each subject's volumetric MRI. From the histogram, a segmentation mask of the ACC voxel was generated and the proportions of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) for the voxel were computed.

Volumetric MR images were acquired using an Axial 3D T1 sequence (TR= 8.684 ms, TE= 1.844 ms, FOV=240 mm, 1.5-mm thickness with no gaps, totaling 210 slices per slab, matrix size 256 × 192, NEX=1, flip angle = 7°, voxel size = 0.9375 × 0.9375 × 1.5 mm). Hippocampal volumetric segmentation was performed with the recon-all pipeline from Freesurfer (FS) image analysis suite (http://surfer.nmr.mgh.harvard.edu/). This fully automated processing includes imaging intensity normalization, removal of non-brain tissue, segmentation of the gray/white matter and subcortical volumetric structures (including hippocampus). Further technical details of these procedures are as described previously (Fischl et al., 2002). Post-processing quality checking by visual inspection was performed, however, no manual interventions were required. A detailed description of the boundaries of the FS hippocampal segmentation can be found in Morey et al. (Morey et al., 2009).

All imaging analyses were performed while masked to the clinical status of the subjects. The GABA levels were previously reported (Price et al., 2009), however, the hippocampal volume data are reported here for the first time.

Statistical analyses

One-way analysis of variance (ANOVA), t-test and chi-square were performed to assess demographic and clinical differences between study groups. A general linear model (GLM) with repeated measures was conducted to study right and left hippocampal volumetric differences among groups while controlling for intracranial volume (ICV) and handedness. Providing there were no significant interactions ($p = 0.80$) between brain side (left or right) and group, right and left hippocampal volumes were combined for the correlational analysis to minimize Type I error due to multiple comparisons. Spearman's Rank Order was used for correlational analysis. All tests were two-tailed, with significance level set at $p \leq 0.05$.

3. Results

3.1 MDD vs. HC

Thirty-three MDD (mean age ±*SEM*, 41.4 ±*2.0;* 23 males) and 26 HC (mean age ±*SEM*, 37.3 ±*2.6;* 12 males) subjects had successful MRI. Age, gender, weight, years of education, and IQ were not statistically different between MDD and HC groups (all $p > 0.05$). There were no significant hippocampal volumetric differences between MDD and HC, while controlling for ICV and handedness, ($F_{(1,54)} = 2.7$, n = 59, $p = 0.11$; Effect size $\eta^2 partial = 0.05$; Fig. 2A). No hemispheric effect ($p = 0.21$) or hemispheric-by-group interactions ($p = 0.74$) were present. Age, gender, weight, education, and IQ were considered as covariates, but they had no significant effect (all $p > 0.05$).

3.2 TRD vs. non-TRD vs. HC

Fourteen TRD (mean age ±*SEM*, 43.9 ±*3.3;* 10 males), 19 non-TRD (mean age ±*SEM*, 39.6 ±*2.5;* 13 males), and 26 HC subjects had successful MRI. Age, gender, weight, and IQ were not statistically different among the three groups (all $p > 0.05$). Years of education differed between TRD (14.2 ±*0.8* years) and HC (16.4 ±*0.5* years; $p = 0.04$). Compared to non-TRD, TRD subjects had higher HAM-A (26 ±*1.6 vs. 19* ±*2.2; df*= 31; $p = 0.02$) and $HDRS_{17}$ scores (24 ±*1.2 vs. 17* ±*1.4; df*= 31; $p = 0.004$). Duration of illness did not differ between TRD and non-TRD. Comparison across the three groups showed a significant group effect ($F_{(1,52)} = 2.7$, n = 59, $p = 0.04$; Effect size $\eta^2_{partial} = 0.11$; Fig. 2B). Considering that this finding is a replication of previous evidence associating hippocampal volume with TRD, *post hoc* analysis was conducted with Fisher's LSD, which showed a significant reduction in hippocampal volume in TRD compared to non-TRD ($p = 0.05$) and HC subjects ($p = 0.03$), but no difference between non-TRD and HC ($p = 0.4$). No hemispheric effect ($p = 0.29$) or hemispheric-by-group interactions ($p = 0.68$) were present. Age, gender, weight, education, IQ, age of onset, duration of illness, HAM-A, and $HDRS_{17}$ were considered as covariates in the model. However, they had no significant effect (all $p > 0.05$).

3.3 MDD Low ACC GABA vs. MDD High ACC GABA vs. HC

In the MDD group, ACC GABA was positively correlated with standardized hippocampal volume (hippocampal/intracranial × 10000) [r_s = 0.42, n = 26, p = 0.03] (Fig. 3). However, there was no correlation between these two measures in the HC group (r_s = 0.27, p = 0.24). The positive correlation in the MDD group demonstrates smaller hippocampal volume in patients with low ACC GABA. However, it is not clear whether this subgroup of MDD has abnormal reduction in hippocampal volume compared to HC. Thus, we conducted a complementary analysis using the median split cutoff point of the anterior cingulate GABA level to divide MDD subjects into two groups: MDD Low ACC GABA (n = 13) and MDD High ACC GABA (n = 13) (Fig. 4).

Demographics and clinical characteristics did not differ between the three groups (Table 1). As shown in figure 2C, we found a significant group effect across the three groups ($F_{(2,45)}$ = 9.0, n = 52, p = 0.0005; Effect size $\eta^2_{partial}$ = 0.29). *Post hoc* analysis with Bonferroni correction revealed a significant reduction in hippocampal volume in MDD Low ACC GABA group compared to MDD High ACC GABA (p < 0.001) and HC (p = 0.01). Hippocampal volume did not differ between MDD High ACC GABA and HC groups (p = 1.0). No hemispheric effect (p = 0.50) or hemispheric-by-group interactions (p = 0.80) were present. Age, gender, weight, education, IQ, age of onset, duration of illness, treatment resistance and psychotropic-naïve status, HAM-A, and $HDRS_{24}$ were examined as covariates in the model. However, they had no significant effect on the model (all p > 0.05). Tissue compositions of the ACC voxel [i.e. percent of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF)] were not different among the three groups (all p > 0.05). Including percent GM, WM, and CSF as covariates had no effect on the model (all p > 0.05) and did not affect the significance of the group effect.

To examine whether the relationship between hippocampal volume and cortical GABA is specific to the prefrontal affective network, we investigated the relationship between occipital GABA and hippocampal volume. Occipital GABA levels were extracted from our previous report (Price et al., 2009). We found no correlation between standardized hippocampal volume (hippocampal/intracranial volumes) and Occipital GABA in the MDD (r_s = − 0.08, p = 0.67) and the HC groups (r_s = − 0.08, p = 0.73). Exploratory analyses showed a trend for an association between standardized hippocampal volume and $HDRS_{17}$ scores (r_s = − 0.41, n = 33, p = 0.02, Bonferroni corrected p = 0.08), but not HAM-A (p = 0.10), age of onset (p = 0.30), or duration of illness (p = 0.25).

Finally, although the pulse sequence used in the current study is optimized for GABA detection, it also achieved detection of the combined resonance for glutamate and glutamine (Glx). Thus, we examined the association between ACC Glx, and hippocampal volume. We found no correlation between ACC Glx and hippocampal volume in the MDD (p = 0.70) or HC groups (p = 0.82). However, there was a significant main effect of group (MDD Low ACC GABA, MDD High ACC GABA, HC) on ACC Glx ($F_{(2,41)}$ = 4.51, p = 0.02; Fig. 5). *Post-hoc* pairwise comparisons with Bonferroni adjustment showed reduced ACC Glx in the MDD Low ACC GABA as compared to MDD High ACC GABA group (p = 0.04) and HC (p = 0.03). In the current cohort, age and weight had an effect on ACC Glx, as covariates in

the GLM; therefore they were retained in the model. Post-hoc bivariate correlational analyses showed the following correlations between Age and Glx in MDD (r_S = 0.40, p = 0.04, *corrected* p = 0.16) and HC (r_S = − 0.02, p = 0.94, *corrected* p = 1), and between Weight and Glx in MDD (r_S = 0.24, p = 0.25, *corrected* p = 1) and HC (r_S = 0.40, p = 0.08, *corrected* p = 0.32).

4. Discussion

Consistent with our hypothesis, hippocampal deficits were most apparent in MDD subjects with low ACC GABA levels (Fig. 2C), an association which remained significant after controlling for demographic and clinical variables. The data also revealed two unforeseen yet intriguing findings: 1- the relationship between hippocampal volume and cortical GABA was limited to the prefrontal network, not involving the occipital cortex, highlighting a functional affective neurocircuitry in MDD. 2- the group effect size was close to three times larger when the biological measure of ACC GABA ($\eta^2_{partial}$ = 0.29) was used to classify MDD patients, compared to the classification based on history of antidepressant resistance ($\eta^2_{partial}$ = 0.11). The latter finding underscores the heterogeneity of MDD as well as the diffuse concept of TRD. Moreover, in light of the increased interest in the field for biomarker-based diagnostic classification (Insel et al., 2012; Kapur et al., 2012) and consistent with emerging evidence proposing GABA as the most promising endophenotype in MDD (Hasler and Northoff, 2011), the current findings underscore the utility of GABA as biological measure for patients' stratification to provide homogenous groups with increased effect sizes, which may better map underlying brain circuitry and facilitate the replication and interpretation of neuroimaging findings.

Smaller hippocampal volume is associated with poor treatment outcome in MDD patients treated with antidepressants (MacQueen and Frodl, 2011). Therefore, understanding the biological correlates of the hippocampal volume reduction in MDD could be critical to the development of novel therapeutics. To our knowledge, this is the first evidence relating prefrontal GABAergic abnormalities to hippocampal volume deficits in patients with MDD. This association was not confounded by the treatment-resistance status, the depression severity, the ACC voxel tissue composition, or the demographics of the study groups. Together, the data suggests a direct association between GABA abnormalities and the structural deficits beyond the effect of the severity of illness and treatment resistance.

In the same study cohort, we previously reported reduced cortical GABA levels in TRD subjects compared to non-TRD and HC (Price et al., 2009). Other studies have found low cortical, CSF, and plasma GABA levels to be associated with anhedonic symptoms and melancholic depression (Gabbay et al., 2012; Petty et al., 1992; Roy et al., 1991; Sanacora et al., 2004). Levinson and colleagues have shown GABA-A receptor neurophysiological activity to be selectively impaired in TRD patients compared to non-TRD, euthymic MDD, and HC (Levinson et al., 2010). In the current cohort, we found no direct association between ACC Glx and hippocampal volume. However, ACC Glx levels were abnormally low in the MDD Low ACC GABA group as compared to MDD High ACC GABA and HC groups (Fig. 5). Similarly, glutamate and glutamine deficits have been demonstrated in MDD (Auer et al., 2000; Bhagwagar et al., 2008; Hasler et al., 2007; Horn et al., 2010;

Merkl et al., 2011; Pfleiderer et al., 2003; Rosenberg et al., 2005; Walter et al., 2009) and were associated with TRD (de Diego-Adelino et al., 2013; Grimm et al., 2012; Merkl et al., 2011; Portella et al., 2011; Zhang et al., 2013a; Zhang et al., 2013b). The reduced GABA and glutamate neurotransmission the prefrontal cortex of MDD is consistent with prolonged elevation in extracellular glutamate activating presynaptic glutamate release inhibitors (i.e. mGlu2/3 receptors) and leading to excitotoxity, neuronal remodeling and structural deficits (Gorman and Docherty, 2010). It is also in line with preclinical evidence of reduced GABA and glutamate neurotransmission following repeated stress – an animal model of depression (Banasr et al., 2010; Yuen et al., 2012). The paradoxical maintenance of increased extracellular glutamate in the presence of low GABA and glutamate neurotransmission is believed to be the result of reduced astroglial glutamate uptake [evidence of glial impairment in MDD was recently reviewed by (Sanacora and Banasr, 2013)]. Agents that enhance astroglial glutamate uptake (e.g. riluzole) or increase glutamate neurotransmission (e.g. ketamine) oppose the effects of repeated stress on GABA and glutamate neurotransmission, and on brain structure (Banasr et al., 2010; Chowdhury et al., 2012; Li et al., 2011). Taken together, the data support a pathophysiological model in which impaired amino-acid neurotransmission precipitates volumetric deficits and confers treatment resistance to antidepressants, raising the question whether amino-acids neurotransmission modulating agents (such as ketamine or riluzole) would be particularly efficacious in patients with prominent amino-acid dysfunction, hippocampal volume deficits, and treatment-resistance to traditional antidepressants. Consistent with this hypothesis, we recently found that riluzole and ketamine, both of which modulate GABA and glutamate neurotransmission (Banasr et al., 2010; Chowdhury et al., 2012), are particularly effective in treating mood and anxiety disorders in patients with profound hippocampal volume deficits (Abdallah et al., 2013; Abdallah et al., 2014).

The pulse sequence used in the current study is optimized for GABA detection. However, it did not permit the separation between glutamate and glutamine signals. An optimal investigation of the relationship between glutamate neurotransmission and prefronto-limbic structural deficits would benefit from the use of ^{13}C MRS to measure glutamate/glutamine cycling (Rothman et al., 2011) and ^{1}H MRS specialized sequences along with higher strength magnetic fields (e.g. 7T) for more accurate glutamate and glutamine separation (Yang et al., 2008). Another limitation of the current study is that the determination of TRD status was based on historical report. Given the cross-sectional nature of the study, it is plausible that some of the non-TRD subjects are treatment-resistant yet at the time of the study they were either treatment naïve or did not receive three adequate trials of antidepressants. The strengths of the current study include a medication-free sample, the multimodal MR approach, the GABA editing methods, and the automated hippocampal volumetric, which reduces human bias and facilitates the biomarker implementation in larger samples.

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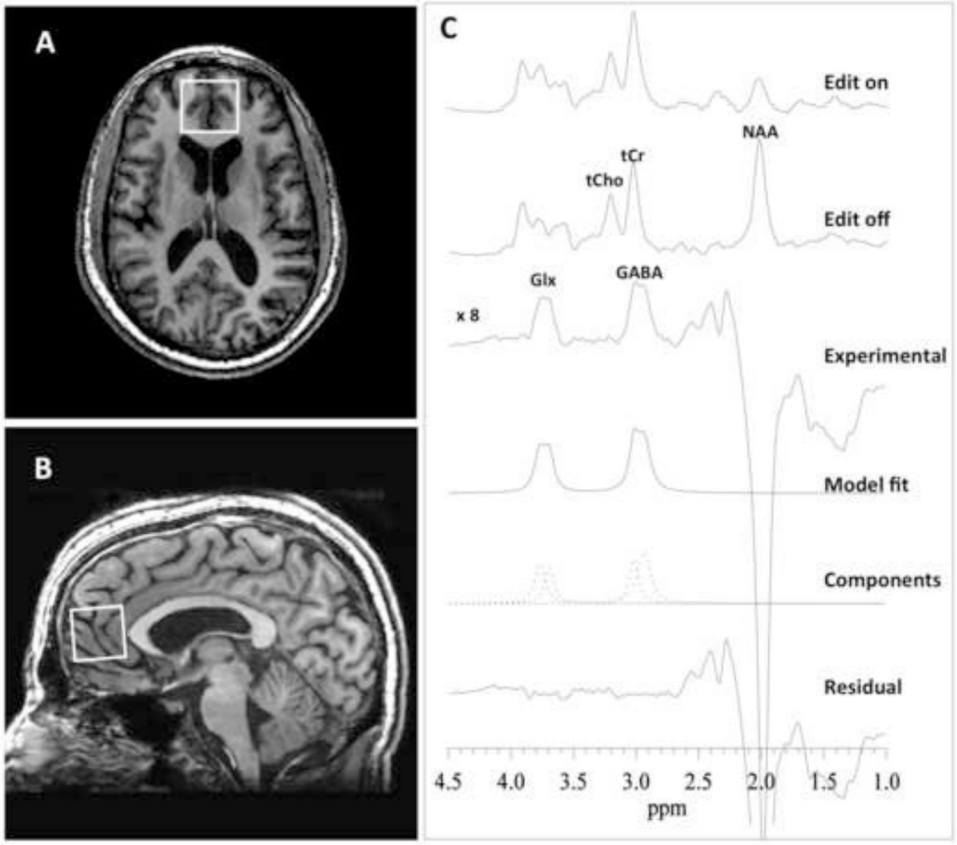

Figure 1.

Anterior cingulate cortex (ACC) voxel size and location are shown on axial (A) and sagittal (B) images. Spectra acquired in 13 minutes for the ACC voxel, as well as their model fit and residual, are shown (C).

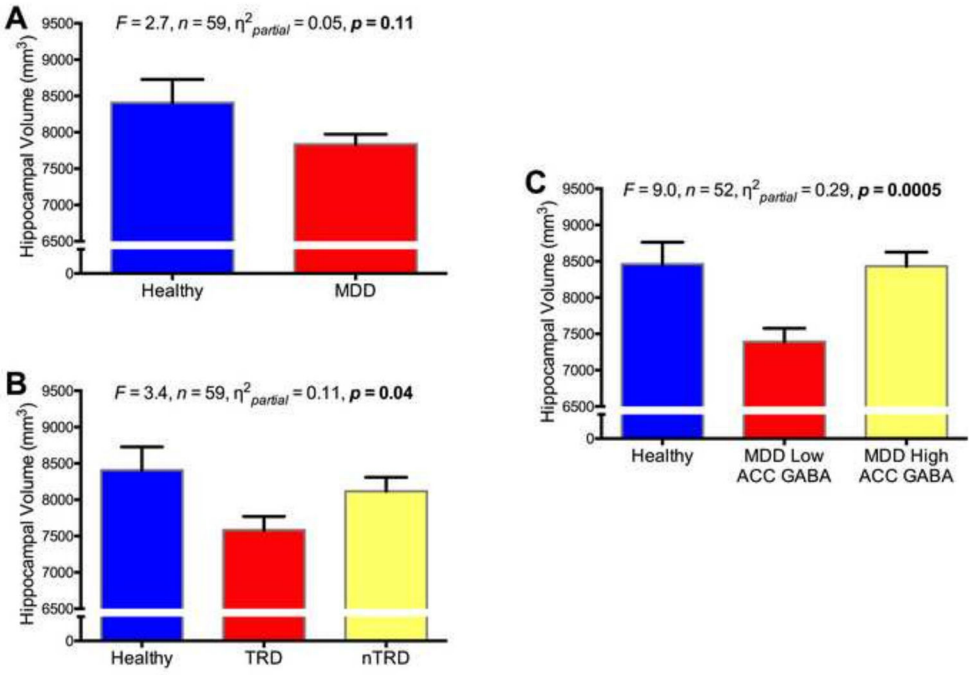

Figure 2. Hippocampal Volume in Major Depressive Disorder (MDD)

Comparison of hippocampal volumes across study groups showed no significant difference between MDD and HC (A). However, hippocampal volume reduction was observed in depressed subjects with (B) treatment resistance (TRD) or (C) low anterior cingulate GABA (MDD Low ACC GABA). No hippocampal volume differences were found between HC and non-TRD or HC and MDD High ACC GABA. Hippocampal Volume is the estimated marginal mean of right and left hippocampal volumes, controlling for intracranial volume and handedness. Error-bars represent the standard error of means. Effect sizes reported as $\eta^2_{partial}$.

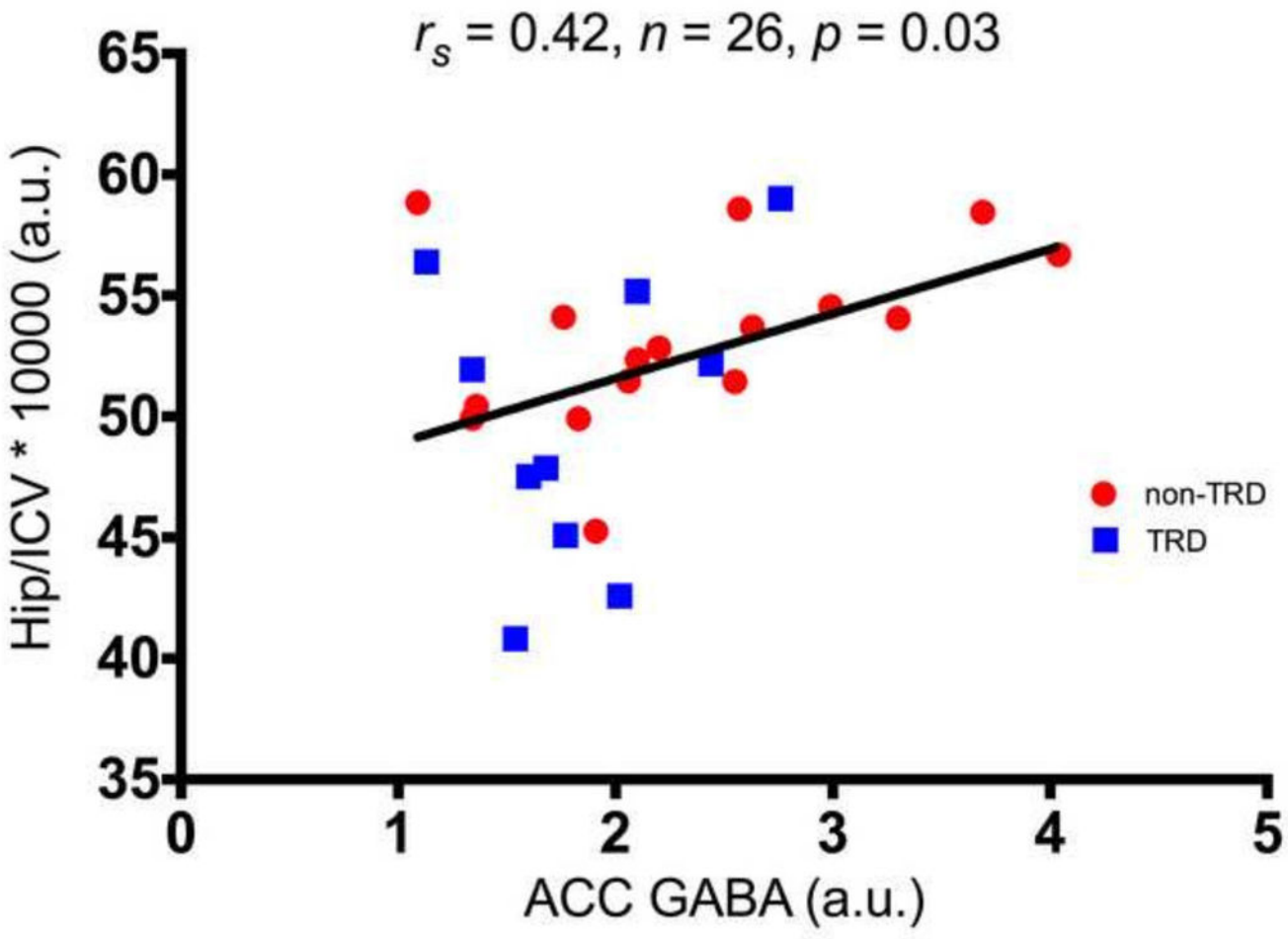

Figure 3. Hippocampal Volume And Anterior Cingulate GABA

Anterior cingulate cortex (ACC) GABA positively correlated with standardized hippocampal volume (hippocampal (Hip)/intracranial volume (ICV) * 10000) in the MDD, but not in the HC group (r_s = 0.27, p = 0.24).

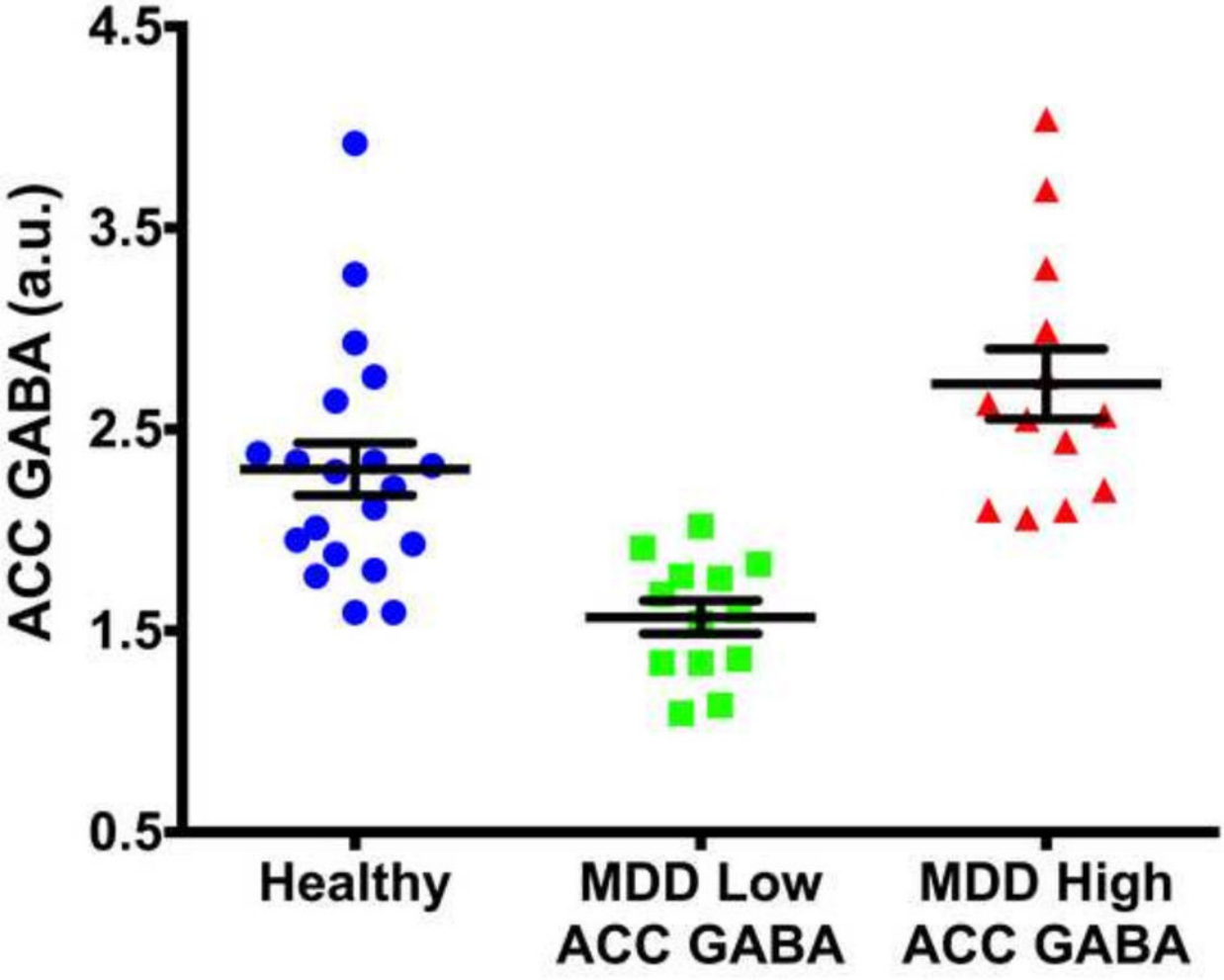

Figure 4. Scatter Of The Anterior Cingulate GABA Among The Study Groups
Bars represent means and the standard error of means (*SEM*).

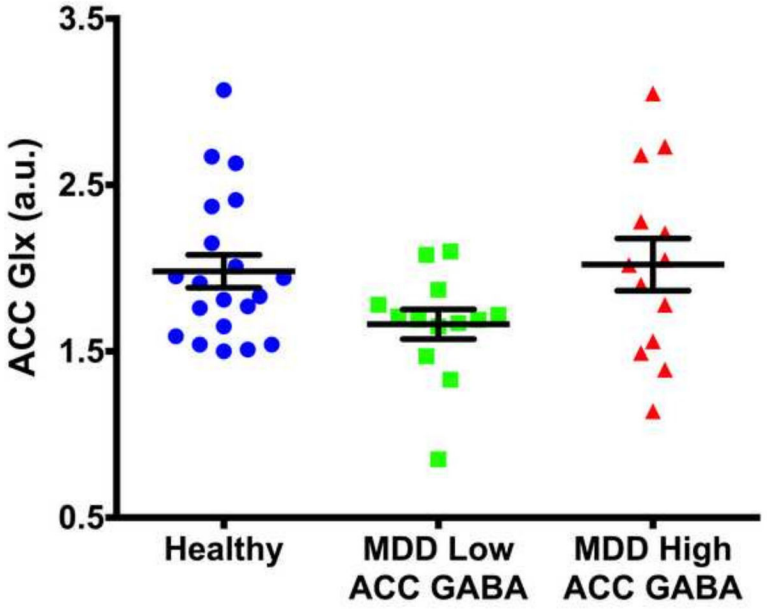

Figure 5. Scatter Of The Anterior Cingulate Glx Among The Study Groups
Bars represent means and the standard error of means (*SEM*).

Table 1

Demographic and Clinical Characteristics.

	MDD Low ACC GABA (n=13)	MDD High ACC GABA (n=13)	Healthy (n=26)		
	Mean ± *SEM*	**Mean ± *SEM***	**Mean ± *SEM***	***df.***	**Sig.[a]**
Age (y)	39.0 ± 2.7	39.2 ± 3.1	37.4 ± 2.6	2-49	0.88
Male (N; %)	9 (69%)	8 (62%)	12 (46%)	2	0.35
Weight (lbs)	175 ± 8.1	155 ± 8.6	172 ± 7.1	2-49	0.23
Education (y)	14.9 ± 0.7	15.4 ± 0.8	15.1 ± 0.5	2-49	0.19
IQ	113 ± 4.1	116 ± 4.4	114 ± 2.4	2-45	0.76
HAM-A	22.1 ± 2.9	22.0 ± 2.3		24	0.98
$HDRS_{17}$	19.2 ± 2.1	19.2 ± 1.4		24	1.00
Age of Onset (y)	15.1 ± 1.7	14.5 ± 2.6		24	0.86
Duration of Illness (y)	23.9 ± 3.6	23.8 ± 4.2		24	0.98
TRD (N; %)	7 (54%)	3 (23%)		1	0.11
Psychotropic-Naïve (N; %)	3 (23%)	5 (39%)		1	0.39

[a] ANOVA, Independent t-test or Chi square test (significance set at $p \leq .05$);

Abbreviations: MDD: Major Depressive Disorder; y: years; IQ: was determined with the Wechsler Abbreviated Scale of Intelligence; HAM-A, Hamilton Anxiety Rating Scale; $HDRS_{17}$, Hamilton Depression Rating Scale; TRD: Treatment Resistant Depression status was determined using the Antidepressant Treatment History Form (ATHF). Subjects with three failed adequate antidepressant trials in the current episode were considered TRD.