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Amino Acid Neurotransmitters Assessed by ^{1}H MRS: Relationship to Treatment-Resistance in Major Depressive Disorder

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Abstract

Background—Significant alterations in γ-aminobutyric acid (GABA) and glutamate levels have been previously reported in symptomatic and remitted major depressive disorder (MDD); however, no studies to date have investigated potential associations between these amino acid neurotransmitters and treatment-resistance.

Methods—The objective of this study was to compare occipital cortex (OCC) and anterior cingulate cortex (ACC) GABA and glutamate+glutamine ("Glx") levels measured *in vivo* by proton magnetic resonance spectroscopy (^{1}H MRS) in 15 medication-free treatment-resistant depression (TRD) patients with those in 18 non-treatment-resistant MDD (nTRD) patients and 24 healthy volunteers (HVs).

Results—Levels of OCC GABA relative to voxel tissue water (W) were decreased in TRD patients compared to both HV (20.2% mean reduction; p=.001; Cohen's *d*=1.3) and nTRD subjects (16.4% mean reduction; p=.007; Cohen's *d*=1.4). There was a similar main effect of diagnosis for ACC GABA/W levels (p=.047; Cohen's *d*=0.76) with TRD patients exhibiting reduced GABA in comparison to the other two groups (22.4–24.5% mean reductions). Group differences in Glx/W

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were not significant in either brain region in primary ANOVA analyses. Only GABA results in OCC survived correction for multiple comparisons.

Conclusions—Our findings corroborate previous reports of decreased GABA in MDD and provide initial evidence for a distinct neuronal amino acid profile in patients who have failed to respond to several standard antidepressants, possibly indicative of abnormal glutamate/glutamine/GABA cycling. Given interest in novel antidepressant mechanisms in TRD that selectively target amino acid neurotransmitter function, the translational relevance of these findings awaits further study.

Keywords

glutamate; GABA; magnetic resonance; spectroscopy; depression

INTRODUCTION

Previous research using proton magnetic resonance spectroscopy (^{1}H MRS) has documented decreased concentrations (20–50%) of the inhibitory amino acid neurotransmitter γ-aminobutyric acid (GABA) in the occipital cortex (OCC) of medication-free patients with major depressive disorder (MDD), as well as smaller reductions (8.5–11%) in remitted MDD patients (1–4). Reductions in GABA levels of approximately 10% have been observed in the anterior cingulate cortex (ACC) of remitted unipolar depressed patients (4) and decreased prefrontal GABA [in a dorsomedial/dorsal-anterolateral region] was observed in unmedicated symptomatically depressed patients (5). Alterations in glutamate and/or glutamate+glutamine ("Glx") concentrations have also been reported in MDD samples relative to healthy volunteers, with increases reported in OCC and decreases reported in prefrontal regions encompassing the ACC (2–5).

Agents that initially target amino acid neurotransmitter receptors have been experimentally tested in treatment-resistant depression (TRD) patients who have failed to respond to conventional antidepressant medications (6–8). The neurobiological substrates of TRD are obscure, in part due to definitional differences across studies and confounds of concurrent medication use. The clinical course and long-term prognosis for these patients could suggest a distinct neurobiology contributing to insufficient therapeutic response to monoaminergic antidepressants. While previous studies have classified MDD subgroups based on recovery status (remitted, symptomatic) and DSM-IV subtypes (melancholic, atypical), no study to our knowledge has examined subgroups determined by antidepressant medication resistance.

Strategies for operationally defining TRD vary widely and there is no well-validated consensus definition (9–11). We selected a categorical cut-off of insufficient response to at least three adequate antidepressant trials in the current episode based on especially poor remission rates for these patients following subsequent antidepressant treatment in a large outpatient MDD sample (12). Capturing treatment-resistance as a categorical rather than a continuous variable has both clinical and regulatory relevance, and reflects the observation that patients who surpass a threshold of treatment failures appear markedly more likely to fail subsequent treatment trials. OCC and ACC were selected as ROIs based on the above MRS findings, feasibility of obtaining high-quality spectroscopic data in these regions, and the integral role of ACC's subregions in mood disorder pathophysiology (13). We predicted that TRD patients, in comparison to healthy volunteers and non-TRD patients with MDD (nTRD), would exhibit decreased GABA levels in OCC and ACC. Glutamate+glutamine (Glx) levels were hypothesized to be increased in OCC and decreased in ACC of TRD patients.

MATERIALS AND METHODS

Study Subjects

Treatment-seeking MDD patients were recruited via media advertisement or clinician referral. All participants signed informed consent after being given a complete description of the study, which was approved by the Institutional Review Boards of Mount Sinai School of Medicine and Weill Cornell Medical College. Diagnoses of MDD, including characterization of atypical, melancholic, and psychotic features during the current episode, and comorbid Axis I conditions were made according to DSM-IV-TR criteria, as established by the Structured Clinical Interview for DSM-IV (SCID-I/P) (14) and by independent interview by a board-certified psychiatrist. SCID interviewers were master's-level or higher clinicians trained to criterion for diagnostic reliability. Eligible participants were psychotropic medication-free for $\geq$ 2 weeks prior to scan; free of substance abuse/dependence for $\geq$ 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. Of 35 MDD patients receiving scans, 33 participants had interpretable data from the OCC ROI while data from 2 patients were discarded due to poor spectral quality. An additional 5 MDD patients had unusable data from the ACC ROI and were included in OCC analyses only. Healthy volunteers (HVs) were recruited via media advertisement and were eligible if, in addition to exclusion criteria outlined above, they had no lifetime axis I disorder (per SCID-I/NP). Of 26 HVs scanned, data of adequate spectral quality was obtained from 24 participants in OCC and 21 participants in ACC. Subjects with unusable data had significantly greater age (p=.01) and body mass index (p=.048) than subjects with complete datasets, but did not differ on sex distribution or clinical variables (all p's>.4).

The number of past antidepressant trials was determined by a physician-conducted phone or in-person interview using the Antidepressant Treatment History Form (ATHF), a validated instrument for assessing the strength and adequacy of past FDA-approved treatments with respect to type and dosage of treatments and adherence (15,16). Consistent with the recommendations of the ATHF manual, and in light of evidence that accuracy in patient-reported treatment history declines as a function of time (17), we focused on the current episode only. Three failed adequate antidepressant trials in the current episode was the minimum threshold for the designation of TRD (n=15); the nTRD group included treatment-naïve patients and those with <3 past trials (n=18).

Day-of-scan clinical assessments were performed using the Hamilton Rating Scale for Depression ($HRSD_{17}$), Quick Inventory of Depressive Symptomatology ($QIDS\text{-}SR_{16}$), Hamilton Rating Scale for Anxiety (HAM-A), Sheehan Disability Scale (SDS), Hollingshead Four-Factor Index of Social Position (HH-SES), and Family History Screen (18–23). Clinical and demographic characteristics of the sample are presented in Table 1.

^{1}H MRS Data Acquisition Methods

The GABA and Glx ^{1}H spectra were recorded on a 3.0T GE 'EXCITE' MR system, using the standard J-edited spin echo difference method (24), as modified by Sailasuta et al (25), and a manufacturer-supplied 8-channel phased-array head coil to enhance detection sensitivity, as recently described (26). Our implementation of the J-editing sequence consisted of incorporating a pair of frequency-selective cosine-modulated Shinar-LeRoux inversion or "editing" rf pulses—flanked by spoiler gradients of opposite signs—before and after the second 180° rf pulse of the standard PRESS sequence, to turn it into a volume-selective J-edited spin echo difference spectroscopy method (24,25). Application of these frequency-selective editing pulses at 1.9 ppm (the resonance frequency of the GABA C-3 protons) on alternate scans inverts the outer lines of the C-4 GABA triplet proton resonance at 3.0 ppm after a spin echo time,

TE, of 68 ms on alternate scans by, respectively, inhibiting and allowing its J-modulation (Fig. 1C). Subtracting the pair of sub-spectra thus acquired yields the desired GABA difference spectra, consisting of the outer lines of the GABA C-4 triplet at 3.0 ppm, while the much stronger overlapping Cr resonance—a singlet that is not J-modulated—is eliminated (Fig, 1D). Although this pulse sequence is optimized for GABA detection, it also achieves detection of the combined resonance for glutamate and glutamine (Glx) at 3.7 ppm (Fig. 1D), due to the high structural, chemical and magnetic similarities between these neurometabolites.

In the present study, the described editing sequence was implemented to record the GABA and Glx spectra in 13 min from a 2.0×3.0×3.0-cm^3 OCC voxel (Fig. 1A,B) and from a 2.5×2.5×3.0-cm^3 ACC voxel (Fig. 1A,B), using TE/TR 68/1500 ms, a 5-KHz spectral widths, 1024 sample points, and 256 interleaved excitations, or a total 512 excitations with the editing pulses on or off.

Spectral Data Processing, Analysis and Quantitation

The derived raw 8-channel phased-array coil time-domain free-induction decay (FID) signals were combined into a single regular FID using a published time-domain reconstruction algorithm (27), with the unsuppressed voxel tissue water signal from each receiver channel providing a strong and reliable reference signal to compute the relative sensitivities of the eight array coil elements. The single-voxel 1D spectra obtained by Fourier transformation of the combined signals were model-fitted in the frequency domain to a pseudo-Voight function (Fig. 1D), which permits more precise analysis of lineshapes that consist of mixtures of Lorentzian and Gaussian functions (28). Finally, the derived GABA and Glx peak areas were expressed semi-quantitatively as ratios relative to the area of the unsuppressed ACC or OCC voxel tissue water resonance (W), which is automatically and simultaneously acquired as part of single-voxel MRS acquisitions on our MR system. Tissue water resonance values did not differ across the three groups in either the OCC ($p=.70$) or ACC ($p=.51$). All reported values represent GABA/W and Glx/W ratios, which will simply be referred to as GABA and Glx, henceforth.

Statistical Analysis

MDD vs. HV group comparisons were made by unpaired t-tests, and 3-group comparisons (TRDs/nTRDs/HVs) were made by one-way analysis of variance (ANOVA). For post-hoc analysis, Tukey's HSD adjustment for multiple comparisons was used unless otherwise noted. Based on previously observed sex and age effects on MDD amino acid neurotransmitter levels (1) and non-equivalencies across our groups in age and ethnic distribution, additional analyses of covariance (ANCOVA) were performed for each group comparison with age, sex, and a dichotomous ethnicity variable (non-Hispanic Caucasian vs. any other ethnicity) entered as covariates. Effect sizes (Cohen's *d*) were calculated for all statistically significant results and trends (29). Tests of associations between continuous variables were performed using Pearson's product-moment correlations and rank-order correlations were performed with Spearman's rho. Multiple linear and logistic regression analyses were performed to investigate relationships between the TRD/nTRD dichotomous categorization and MDD patients' OCC GABA levels, while adjusting for clinical and demographic factors. Multicollinearity among predictors was assessed using a conventional tolerance threshold of $(1-r^2)>.20$ (30); all predictors showed tolerance values above this threshold. All tests were two-tailed, with alpha level set at .05, unadjusted. A Bonferroni correction for multiple comparisons was subsequently applied to the four primary ANOVA and ANCOVA analyses, yielding a critical p value of 0.05/4=0.0125.

RESULTS

Comparisons of TRD, nTRD MDD, and HV participants

A one-way ANOVA revealed a main effect of group (HV/TRD/nTRD) on OCC GABA levels ($F(2,54)=9.76$; $p<.001$). Post hoc pairwise comparisons showed that the TRD group (n=15; $M=2.44\times10^3$; $SD=0.39\times10^3$) had significantly reduced GABA in comparison to both nTRDs (n=18; $M=2.92\times10^3$; $SD=0.33\times10^3$; p=.007; Cohen's d=1.3) and HVs (n=24; $M=3.06\times10^3$; $SD=0.52\times10^3$; $p<.001$; d=1.3), while nTRDs and HVs did not differ (p=.57) (Figure 2). ANCOVA controlling for age, sex, and ethnicity did not alter significance for the main effect of group [$F(2,51)=10.62$; $p<.001$] and revealed no significant main effects of any covariate.

A one-way ANOVA revealed a similar main effect of group on ACC GABA [$F(2,46)=9.76$; p=.047]. The TRD group (n=12; $M=1.79\times10^3$; $SD=0.38\times10^3$) showed reduced GABA in comparison to both nTRDs (n=16; $M=2.36\times10^3$; $SD=0.85\times10^3$) and HVs (n=21; $M=2.30\times10^3$; $SD=0.57\times10^3$), although the differences did not reach the threshold for significance (TRDs vs. nTRDs: p=.06, d=0.88; TRDs vs. HVs: p=.08, d=1.03; nTRDs vs. HVs: p=.95) (Fig. 2). ANCOVA controlling for age, sex, and ethnicity did not alter significance for the main effect of group [$F(2,43)=3.26$; p=.048] and revealed no significant main effects of any covariate.

Identical one-way ANOVAs performed on Glx revealed no significant main effect of group in either the OCC (p=.46) or ACC (p=.22) regions. In the OCC, ANCOVA revealed a main effect of age which was negatively related to OCC Glx [$F(1,51)=6.38$; p=.02]; however the significance of group was unaltered by the covariates. In ACC, no single covariate was significant in the ANCOVA (p's>.12); however removal of the combined variance from these sources increased the explanatory power of group [$F(2,43)=3.70$; p=.03], revealing decreased Glx levels in the TRD group only in comparison to HVs (TRDs vs. HVs: p=.03; TRDs vs. nTRDs: p=.18; nTRDs vs. HVs: p=.99].

Applying a Bonferonni correction to the four primary ANOVAs and the four secondary ANCOVAs did not alter significance for the main effect of group on OCC GABA (adjusted p's<.004), but resulted in loss of significance for group effects on ACC GABA and Glx. Exclusion of antidepressant treatment-naïve patients (n=9) from the nTRD group altered significance in ACC GABA only, where the main effect of group was slightly reduced [$F(2,40)=3.21$; p=.052].

A confirmatory 2×3 ANOVA (with region as a within-subjects variable and group as a between-subjects variable) was performed on GABA levels from the 49 participants who had interpretable data for both ACC and OCC. There were main effects of region [$F(1,46)=39.82$; $p<.001$] and group [$F(2,46)=7.98$; p=.001]. The TRD group showed GABA reductions in comparison to both nTRDs (p=.005, d=1.30) and HVs (p=.001, d=1.59), while nTRDs and HVs did not differ (p=.97). The group × region interaction was not significant [$F(2,46)=.307$; p=.74], suggesting the pattern of GABA decreases was relatively consistent across regions.

Ratios of Glx:GABA levels were computed and compared across the three groups with one-way ANOVAs. In the OCC only, there was a main effect of group on Glx:GABA ratios [$F(2,54)=3.28$; p=.045]. Glx:GABA ratios were elevated in the TRD group (M=.84; SD=.22) in comparison to HVs (M=.71; SD=.15; p=.04; d=0.74) while nTRDs did not differ significantly from either group (p's>0.15). Glx:GABA ratios in the ACC did not differ across the three groups [$F(2,46)=.51$; p=.60].

Comparisons of MDD and HV participants

In the full sample of 33 MDD patients (TRD and nTRD) compared to the 24 HVs, unpaired t-tests revealed significantly decreased OCC GABA in MDD participants ($M=2.70\times10^{-3}$; $SD=0.43\times10^{3}$) compared to controls ($M=3.06\times10^{3}$; $SD=0.52\times10^{3}$) [$t(55)=2.85$; $p=.006$; $d=0.77$]. No significant group differences were found for ACC GABA, OCC Glx, ACC Glx, or Glx:GABA ratios (all p's>=.10). ANCOVAs controlling for age, sex, and ethnicity did not alter significance in any analysis.

Correlation Analyses

In the full sample of patients and controls (n=57 for OCC; n=49 for ACC), age was negatively associated with Glx in the OCC (r=−.30; p=.02), but not ACC Glx (r=.21; p=.16), OCC GABA (r=−.09; p=.45), or ACC GABA (r=−.13; p=.36). Sex was not related to any MRS variable (p>.40). GABA and Glx were positively correlated in the ACC across all participants (r=.34; p=.02) as well as within the MDD patients only (r=.42, p=.02), but not in the OCC in either the full sample (r=.13; p=.32) or the patients alone (r=.31; p=.10). GABA, Glx, and Glx:GABA in both ROIs were unrelated to depression or anxiety rating scales in either the MDD patient sample or the HV sample (p's>.10).

In MDD patients, a rank-order correlation between the number of failed trials and OCC GABA revealed a negative relationship (Spearman's rho=−.44; p=.01). No other MRS variable was related (p's>.09). The relationship between specific numbers of failed trials and OCC GABA is further explored in the Supplementary Material.

Regression Analyses in MDD Participants

Follow-up regression analyses in the 33 MDD participants were designed to explore the robustness of the TRD vs. nTRD dichotomous categorization in predicting OCC GABA levels when additional clinical and demographic features were added to the model (Table 2). In the full sample of 33 MDD patients, the TRD vs. nTRD dichotomous categorization was a highly significant predictor ($\beta = -0.57$; p=.001) of occipital GABA levels. In each of the four models tested, TRD status was entered in step 1. In Model 1, measures of clinical symptom severity ($HRSD_{17}$, HAM-A) and anxiety disorder comorbidity were entered in step 2. In Model 2, measures of sociodemographic status (Caucasian vs. non-Caucasian; HH-SES; years of education) were entered in step 2. In Model 3, measures of patient life history (age, age of illness onset, single-episode vs. recurrent depression) were entered at step 2. In Model 4, dichotomous measures of psychotropic exposure [antidepressant treatment-naïve vs. non-treatment-naïve; 2-week psychotropic washout (the study minimum) vs. > 2-week washout period] were entered at step 2. The change in R^2 at step 2 was not significant for any model [Model 1: $F(3,28)=.15$; p=.93; Model 2: $F(3,27)=.36$; p=.79; Model 3: $F(3,28)=.23$, p=.88; Model 4: $F(2,29)=.43$; p=.66], and TRD status was the only significant predictor in each final model.

A logistic regression predicting TRD status showed that OCC GABA levels correctly classified 83% of nTRDs and 73% of TRDs (Model $\chi^2=12.64$; p<.001). In addition, OCC GABA significantly improved the prediction of TRD/nTRD group status when clinical and demographic features that differed across groups (age, ethnic distribution, HAM-A, $HRSD_{17}$) were entered at Step 1. While these control variables correctly classified 94% of nTRDs and 80% of TRDs (Step 1 $\chi^2=21.25$; p<.001), the addition of OCC GABA significantly improved the model (Step 2 $\chi^2=9.02$; p=.003) resulting in correct classification of 94% of nTRD and 93% of TRD patients [Model $\chi^2=30.27$; p<.001]. OCC GABA (Wald's $\chi^2=4.38$; p=.04) and ethnicity (Wald's $\chi^2=4.12$; p=.04) were the only significant predictors in the final model.

DISCUSSION

Consistent with predictions, GABA levels in OCC were decreased in medication-free TRD patients. Reductions were evident compared to two concurrently recruited groups–a healthy volunteer sample as well as a medication-free MDD sample without history of treatment-resistance. Notably, 14 of 15 TRD patients exhibited GABA levels below the group mean for nTRD (Fig. 2). Reductions in TRD patients could not be explained by any clinical or demographic factor examined, including age, sex, ethnicity, SES, symptom severity, comorbid anxiety, age of illness onset, number of episodes, treatment-naïve status, or duration of psychotropic washout. Though a similar pattern of GABA reduction was present in the ACC region, with TRD mean percentage decreases (22.4–24.5%) larger than those observed in OCC (16.4–20.2%), the effect was statistically less robust, and did not survive correction for multiple comparisons.

Our data suggest that stratification based on systematic assessment of prior antidepressant treatment response is a salient variable for biological investigations of MDD and reliably distinguishes patients with more pronounced GABA deficits from those who resemble healthy participants. Previous ^{1}H MRS studies of occipital GABA in medication-free MDD patients (Table 3) have not distinguished TRD from nTRD patients. The largest published effect size to date was observed in a primarily inpatient MDD sample, 57% of whom (8 of 14) were identified as failing 3 or more antidepressant trials (1). In a follow-up investigation combining data from this inpatient sample with a larger sample of outpatients, GABA decreases were most pronounced in patients exhibiting melancholic features (2). In our outpatient sample, the low frequency of patients meeting specific DSM-IV subtype criteria did not permit similar analyses.

Although the occipital cortex has not been given a prominent role in neural circuitry models of MDD (13,31), serotonin$_{1A}$ receptor binding and glucose metabolism abnormalities in this region have been reported in PET studies (32–34). Examination of additional brain regions will be needed to determine whether GABA abnormalities are specific to occipital and prefrontal ROIs, or more broadly distributed throughout the brain, though at present technological limitations constrain the number of voxels that can be assessed via MRS within a feasible time frame. GABA decreases previously reported in the plasma and cerebrospinal fluid of depressed patients (35–37) might suggest a broad distribution of decreases. Decreased GABA in TRD patients is consistent with amino acid neurotransmitter dysfunction in mood disorder pathophysiology (7,38). Disruption of glutamate-glutamine neuronal-glial cycling in MDD is thought to result in excessive build-up of extracellular glutamate and decreased glutamate release, leading to a decrease in cortical GABA (39), a hypothesis consistent with the elevated OCC Glx:GABA ratios observed in our TRD group. However, the extent to which alterations in steady-state GABA and Glx levels relate to alterations in neurotransmitter function remains to be clarified, as altered MRS levels might reflect changes occurring in neurons or glia, and could index diverse physiological abnormalities including altered neurotransmitter metabolism, recycling/production rates, and/or synaptic or extra-synaptic accumulation (40).

We did not find strong evidence of Glx alterations in either our full sample of MDD patients or in our TRD subgroup. Diminished ACC Glx levels in TRD patients emerged when age, sex, and ethnicity were covaried, but the effect did not survive Bonferonni correction. Consistent with a previous report (4), we found that Glx and GABA were positively correlated in ACC but not OCC. OCC Glx levels, unlike GABA levels, were negatively correlated with age across all participants, which might have influenced results given that age-matched samples were not obtained, although we adjusted for age in ANCOVA analyses. At the current study's field strength (3.0T), the Glx resonance is predominately comprised of glutamate, with a smaller contribution from glutamine. Glutamate and glutamine alterations in MDD samples have been

more variable than GABA findings in previous ^{1}H MRS studies conducted at differing field strengths (1.5T-3T), with inconsistent direction of change reported across cortical regions (2–5,41,42). Additional studies are therefore needed to clarify the nature of regional glutamate/glutamine alterations in MDD.

Conventional antidepressants such as selective serotonin reuptake inhibitors (SSRIs) have broad effects on amino acid neurotransmission and have been reported to increase occipital GABA in MDD (43). These findings have generated interest in developing novel antidepressants that normalize amino acid neurotransmitter metabolism and neurotransmission (44) and enhance neuronal plasticity and cellular resilience in patients who do not respond to conventional approaches (38,45). Our data suggest that regional GABA dysfunction may be particularly prominent in MDD patients with a history of non-response to conventional antidepressants. Though our cross-sectional study does not permit us to disambiguate cause from effect in the relationship between treatment-resistance and GABA reductions, our findings support the potential utility of direct targeting of amino acid neurotransmitter function in TRD patients. We cannot rule out the possibility that GABA reductions in TRD are a cumulative result of previous antidepressant medication exposure, though the previous finding that chronic administration of SSRIs increases occipital GABA is inconsistent with this interpretation (43). Thus, our findings might suggest that decreased GABA levels confer risk for poor response to conventional antidepressants or, alternatively, that a third factor results in both GABA reductions and treatment-resistance. It is important to note that the present nTRD sample likely comprises both patients who would respond well to standard antidepressants and those who would emerge as TRD given adequate exposure, particularly given the inclusion of antidepressant treatment-naïve participants for whom treatment responsivity is not determinable. Our principle findings were maintained when treatment-naïve participants were excluded from analysis. However, a randomized, placebo-controlled, prospective design exploring the relationship between cortical GABA levels and subsequent specific antidepressant response would be necessary to corroborate a role for GABAergic deficits in increasing the risk of treatment-resistance.

The present study examined well-characterized, medication-free cohorts using high field-strength MRS and quantification methods with demonstrated reliability (46). The use of unsuppressed voxel tissue water resonance as a referencing standard, as opposed to total creatine ratios as in previous studies, permits more precise inference to be made regarding amino acid neurotransmitter levels *per se*. The present samples of TRD and nTRD patients were notably well-matched in terms of anxiety disorder comorbidity, sex distribution, IQ, BMI, SES, family history of mood disorder, self-reported disability level, illness duration, age of onset, chronicity, and number of episodes. However, differential sample characteristics included age, ethnicity, modal duration of washout period, and current anxiety and depression symptom severity. Regression analyses including these factors, as well as additional demographic and clinical predictors selected for conceptual or empirical relevance to TRD and GABA, consistently failed to reduce the predictive power of TRD status. However, it should be noted that regional GABA alterations are not specific to MDD and have been observed in other disorders, including bipolar disorder, social phobia, panic disorder, and alcohol dependence (3,47–50), suggesting that GABA decreases may relate to clinical features that cut across diagnostic boundaries. Future studies should aim to delineate the relationships between specific clinical characteristics with relevance to TRD status and potential neurochemical substrates of treatment non-response.

Our conclusions are further limited by small sample size, which constrained power to detect small and medium effects. The effects of partial volume averaging cannot be ruled out given that tissue segmentation was not performed. The generalizability of the present findings may be limited to patients sharing the specific clinical characteristics of our TRD and nTRD

samples, such as high rates of illness chronicity, anxiety disorder comorbidity, and self-reported disability. Although we used a validated instrument (ATHF) to quantify failed antidepressant trials in the current episode, retrospective assessment of treatment response is constrained by the accuracy of patient reports (17) and medical records. In addition, our TRD classification did not take into account trials occurring during previous depressive episodes, the total duration of exposure, nor exposure to medications not FDA-approved for MDD.

In summary, occipital GABA levels were reduced in TRD patients in comparison to both HVs and MDD patients without history of treatment-resistance. A similar main effect of diagnosis was present in the ACC, though evidence for TRD-specific reductions was less conclusive. These findings are possibly consistent with a role for occipital GABA reductions in conferring risk of inadequate antidepressant medication response and may suggest the potential utility of amino acid neurotransmitter-modulating agents for TRD. Large-scale prospective designs are necessary to corroborate this interpretation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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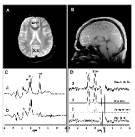

FIGURE 1.

(A) axial and **(B)** sagittal images showing ACC and OCC voxel sizes and locations; **(C)** PRESS ^{1}H MR spectra with "editing" rf pulse **(a)** off and **(b)** on. **(D)** The difference of the spectra in **(C)** showing: **(a)** the detected GABA and Glx peaks, with **(b–d)** best-fit model curves and residuals, which yield the areas under the peaks. The depicted data were acquired in 13 min from a 2.5×2.5×3.0 cm^3 ACC voxel, using TE/TR 68/1500 ms, and 256 interleaved excitations (total 512) with editing pulse on or off.

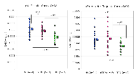

FIGURE 2.

GABA/W ratios [arbitrary units (a.u.)] in (A) the occipital cortex (OCC) and (B) the anterior cingulate (ACC) of healthy volunteers (HV), non-treatment-resistant major depression patients (nTRD), and treatment-resistant depression patients (TRD). Group means are displayed to the right of data from individual subjects. In the OCC, TRD patient values are 20.2% lower than HVs (p=.001; Cohen's *d*=1.3) and 16.4% lower than nTRD subjects (p=.007; Cohen's *d*=1.4). In the ACC, TRD values are 22.4% lower than HVs (p = .08, Cohen's *d*=1.03) and 24.5% lower than nTRDs (p = .06, Cohen's *d*=0.88). P-values derived by Tukey's HSD post-hoc tests.

Table 1

Descriptive and Clinical Characteristics of Patients and Healthy Volunteers

	Healthy Volunteers (n=24)[†]	**nTRD MDD (n=18)**[†]	**TRD (n=15)**[†]
Age, mean (SD), y	37.25 (13.5); range: 21–60	38.3 (12.3) range: 23–65	46.80 (11.9)[a] range: 28–68
Female, No. (%)	13 (54%)	6 (33%)	7 (47%)
Non-hispanic Caucasian, No. (%)	13 (54%)	8 (44%)	12 (80%)
IQ, mean (SD)	114.95 (10.3)	111.12 (16.0)	112.64 (12.1)
Years of education, mean (SD)	16.38 (1.9)	15.38 (2.9)	14.00 (2.6)[a]
Median household annual income	$35,000–49,999	$15,000–34,999	$15,000–34,999
HH-SES, mean (SD)	50.29 (10.7)	43.67 (15.3)	46.47 (9.8)
Body Mass Index, mean (SD), kg/m^2	26.27 (4.6)	26.83 (4.3)	27.72 (4.9)
Family History of Mood Disorder, No. (%)	1 (4%)	5 (31%) [c]	6 (40%) [a]
Any Comorbid Anxiety Disorder, No. (%)	-	13 (72%)	11 (73%)
Social Phobia		9 (50%)	5 (33%)
Obsessive Compulsive Disorder		2 (11%)	1 (7%)
Post-Traumatic Stress Disorder		2 (11%)	2 (13%)
Panic Disorder		0 (0%)	1 (7%)
Specific Phobia		1 (6%)	0 (0%)
Generalized Anxiety Disorder		5 (28%)	1 (7%)
Anxiety Disorder Not Otherwise Specified		3 (17%)	5 (33%)
MDD Subtypes, No. (%)	-		
Atypical		1 (6%)	3 (20%)
Melancholic		1 (6%)	1 (7%)
Psychotic		2 (11%)	1 (7%)
Antidepressant NaÏve, No. (%)	-	9 (50%)	0 (0%)[b]
2-week Washout Period, No. (%)	-	2 (11%)	7 (47%)[b]
Time Since Illness Onset, mean (SD), y	-	21.80 (16.4)	26.93 (10.8)
Age-of-Onset, mean (SD), y	-	17.73 (14.3)	22.20 (15.4)
Number of Episodes, mean (SD)	-	2.73 (4.8)	2.00 (2.1)
Single Episode (Non-remitting), No. (%)	-	11 (61%)	10 (67%)
Duration of Current Episode >= 2y, No. (%)	-	15 (83%)	15 (100%)

	Healthy Volunteers (n=24)†	nTRD MDD (n=18)†	TRD (n=15)†
Number of Failed Trials in Current Episode, mean (SD)	-	0.89 (0.9)	5.43 (2.8)[b]
$HRSD_{17}$, mean (SD)	0.84 (0.9)	17.94 (5.5)[c]	24.40 (5.1)[a,b]
$QIDS\text{-}SR_{16}$, mean (SD)	1.34 (1.5)	13.11 (4.0)[c]	18.13 (4.1)[a,b]
HAM-A, mean (SD)	1.42 (1.3)	19.44 (6.9)[c]	27.00 (7.9)[a,b]
SDS, mean (SD)	-	17.06 (5.7)	19.14 (10.5)

Note: Continuous variables compared by ANOVA w/LSD post-hoc comparisons, dichotomous variables compared by Fisher's exact test, 2-tailed; HV = Healthy Volunteer; nTRD MDD = Non-Treatment-Resistant Major Depressive Disorder; TRD = Treatment-Resistant Depression; HH-SES = Hollingshead Four-Factor Index of Social Position; $HRSD_{17}$ = Hamilton Rating Scale for Depression, 17-item; $QIDS\text{-}SR_{16}$ = Quick Inventory of Depressive Symptoms-Self-Report; HAM-A = Hamilton Anxiety Rating Scale; SDS = Sheehan Disability Scale (18–22)

† Descriptives are presented for subjects with interpretable occipital lobe MRS data; for ACC analyses, 3 HVs, 2 nTRD MDDs, and 3 TRD participants had uninterpretable data due to motion-degraded spectral quality

[a] TRDs differ from HVs, $p < .05$

[b] TRDs differ from nTRD MDDs, $p < .05$

[c] nTRD MDDs differ from HVs, $p < .05$

Table 2

Multiple Regression Statistics for Models Predicting Occipital GABA in Patients with Major Depressive Disorder

	β	Adjusted R^2	ΔR^2
Step 1: All Models		.301 (p = .001)	.323 (p = .001)
TRD vs. nTRD	−.57 (p =.001)		
Step 2: Model 1		.238 (p = .019)	.011 (p = 0.93)
TRD vs. nTRD	−.59 (p = .003)		
$HRSD_{17}$	−.09 (p = 0.72)		
HAM-A	.14 (p = 0.57)		
Comorbid anxiety disorder	−.06 (p = .73)		
Step 2: Model 2		.233 (p = .024)	.026 (p = 0.79)
TRD vs. nTRD	−.60 (p = .001)		
Non-hispanic Caucasian vs. any other ethnicity	.16 (p = 0.38)		
HH-SES	.03 (p = 0.88)		
Years of education	.05 (p = 0.84)		
Step 2: Model 3		.245 (p = .017)	.016 (p = 0.88)
TRD vs. nTRD	−.58 (p = .001)		
Age	.10 (p = 0.60)		
Age of illness onset	−.14 (p = 0.44)		
Single episode vs. recurrent	−.02 (p = 0.93)		
Step 2: Model 4		.274 (p = .006)	.019 (p = 0.66)
TRD vs. nTRD	−.49 (p = .01)		
2-week washout vs. > 2-week washout	.05 (p = 0.81)		
ADM-naïve vs. any exposure	−.14 (p = 0.42)		

Note: TRD = Treatment-Resistant Depression; nTRD = Non-Treatment-resistant Major Depressive Disorder; $HRSD_{17}$ = Hamilton Rating Scale for Depression, 17-item; HAM-A = Hamilton Anxiety Rating Scale; HH-SES = Hollingshead Four-Factor Index of Social Position; ADM = antidepressant medication

Table 3

Occipital GABA Findings in Medication-Free MDD samples

Study	Measurement	Field Strength	MDD Characteristics	Samples Compared	Effect Size: Cohen's *d* [95% CI]
Sanacora et al. 1999 (1)	GABA/Creatine	2.1 *Tesla*	primarily inpatient, 57% TRD	14 MDD vs. 18 HV	*d*=2.28 [1.42–3.25]
Sanacora et al. 2004 (2)	GABA/Creatine	2.1 *Tesla*	primarily outpatient, 24% TRD	33 MDD vs. 38 HV	*d*=0.64 [0.13–1.08]
Bhagwagar et al. 2007 (3)	GABA/Creatine	3.0 *Tesla*	fully remitted, recurrent	15 MDD vs. 18 HV	*d*=0.89 [0.14–1.58]
Current study	GABA/Water	3.0 *Tesla*	outpatient, categorized as TRD or nTRD by ATHF	15 TRD vs. 24 HV	*d*=1.32 [0.59–2.01]
				15 TRD vs. 18 nTRD MDD	*d*=1.35 [0.57–2.10]
				18 nTRD vs. 24 HV	*d*= 0.31[−0.31–0.92]
				33 MDD vs. 24 HV	*d*=.77 [0.22–1.30]

Note: TRD = treatment-resistant depression, defined as 3 or more failed antidepressant trials; nTRD= non-treatment-resistant depression; HV = healthy volunteer; ATHF = Antidepressant Treatment History Form (15); effect size (*d*) calculated from reported means and SDs (29)