

Reduced Prefrontal Glutamate/Glutamine and γ -Aminobutyric Acid Levels in Major Depression Determined Using Proton Magnetic Resonance Spectroscopy

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Context: Increasing evidence indicates that major depressive disorder (MDD) is associated with altered function of the major excitatory and inhibitory neurotransmitters glutamate and γ -aminobutyric acid (GABA), respectively. A recently developed magnetic resonance spectroscopy method allows for reliable measurement of glutamate/glutamine (Glx) and GABA concentrations in prefrontal brain regions that have been implicated in the pathophysiologic mechanisms of MDD by studies using other neuroimaging and postmortem techniques.

Objective: To measure Glx and GABA levels in 2 regions of the prefrontal brain tissue in unmedicated adults with MDD.

Design: Cross-sectional study for association.

Setting: Psychiatric outpatient clinic.

Participants: Twenty unmedicated, depressed patients with MDD and 20 age- and sex-matched controls.

Intervention: Participants underwent scanning using

a 3-T whole-body scanner with a transmit-receive head coil, providing a homogeneous radiofrequency field and the capability of obtaining spectroscopic measurements in a dorsomedial/dorsal anterolateral prefrontal region of interest (ROI) and a ventromedial prefrontal ROI.

Main Outcome Measures: Glx and GABA levels derived from magnetic resonance spectroscopy signals.

Results: Depressed patients had reduced Glx levels in both ROIs. The GABA levels were reduced in the dorsomedial/dorsal anterolateral prefrontal ROI. Levels of GABA and Glx were positively correlated in both ROIs.

Conclusions: For the first time, GABA and Glx concentrations were compared between unmedicated depressed adults and controls in prefrontal ROIs. The abnormal reductions in Glx and GABA concentrations found in the MDD sample were compatible with findings from postmortem histopathologic studies, indicating that glial cell density is reduced in the same areas in MDD.

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INCREASING EVIDENCE EXISTS TO suggest that major depressive disorder (MDD) is associated with perturbations in the metabolism of the major excitatory and inhibitory neurotransmitters glutamate and γ -aminobutyric acid (GABA), respectively. Early studies^{1,2} reported that the concentrations of glutamate/glutamine (Glx) and GABA are abnormally decreased in the plasma of patients with unipolar depression. Postmortem studies of frontal cortical N-methyl-D-aspartate receptor complex in suicide victims³ and of cerebrospinal fluid GABA concentrations also identified abnormalities in these systems in depression.^{4,5} More recently, advances in proton magnetic resonance spectroscopy (MRS) have allowed for direct, noninvasive, in vivo measurement of cerebral Glx (mea-

sured as the combined Glx peak in the MRS spectra) and GABA concentrations in patients with mood disorders.

Sanacora et al⁶ initially applied an editing MRS protocol to assess brain GABA concentrations in the occipital lobe of patients with MDD and found reduced GABA levels in unmedicated depressive individuals (imaged after a minimum 2-week medication washout) vs controls. The same group⁷ replicated this finding in the occipital lobe in a sample of moderately depressed individuals but noted that the magnitude of the reduction in mean GABA levels was smaller than that found in their earlier study of more severely depressed patients and observed that the differences found with respect to controls largely were accounted for by depressive patients meeting the criteria for melancholic subtype. Hasler et al⁸ applied a 3-T

MRS technique to assess GABA concentrations in prefrontal regions implicated more consistently in the pathophysiological mechanisms of depression but found no differences in GABA levels between controls and fully remitted individuals with a history of MDD. Because the data from Sanacora et al⁷ suggested that the sensitivity for detecting abnormal GABA concentrations in MDD may be affected by the extent of current depressive symptoms, the present study applied the 3-T MRS technique to measure prefrontal GABA levels in an independent sample of currently depressed patients with MDD.

In addition to the reductions observed in cerebral GABA concentrations using MRS, previous spectroscopic studies of depression also had reported that the Glx concentrations were abnormal in the anterior cingulate gyrus, the amygdala/hippocampus, and the occipital brain. Auer and colleagues⁹ conducted an MRS study in 18 patients with MDD, most of whom were medicated, and 18 age-matched controls and found a 10.4% decrease in Glx levels in the anterior cingulate gyrus in depressive patients vs controls. Pfleiderer et al¹⁰ replicated this result in a sample of treatment-resistant depressed patients with a mean age of 60 years who were not taking psychotropic drugs other than lorazepam for 3 to 8 days before scanning. The same group reported that an overlapping sample of treatment-resistant depressive patients free of psychotropic drugs other than lorazepam for 3 to 8 days showed reduced Glx concentrations in the amygdala/anterior hippocampus region¹¹ and found that Glx levels correlated negatively with depression severity in a dorsolateral prefrontal cortical region.¹² The Glx levels in a subset of these patients increased significantly after successful electroconvulsive therapy.^{10,11} Furthermore, an MRS study¹³ in patients with metastatic breast cancer showed that chemotherapy-induced reductions in Glx levels in the white matter of the center semiovale region were associated with combined suicidal ideation and depressive symptoms. Although the results of these studies potentially were confounded by the effects of currently or recently administered psychotropic medications, Mirza et al¹⁴ and Rosenberg et al¹⁵ also found reduced anterior cingulate gyrus Glx levels in unmedicated children with major depression. These results do not generalize to other patient populations with affective illness or to other brain regions. Elderly patients with MDD did not differ from controls regarding prefrontal Glx levels,¹⁶ and in patients with bipolar disorder, prefrontal Glx concentration was found to be increased.¹⁷ In the occipital lobe, Sanacora et al¹⁸ found that unmedicated depressed adults also had increased Glx levels vs controls. Moreover, a limitation of the literature on nonelderly adults with MDD has been that none of the studies on Glx concentrations in the limbic and prefrontal cortical areas where function seems to be more clearly relevant to the pathogenesis of the major depressive syndrome involved unmedicated depressed adults.

Recent findings from postmortem studies of abnormally decreased glial cell density, numbers, and markers in MDD has increased interest in the measurement of Glx and GABA concentrations in vivo in MDD. These MRS spectra reflect the combined intracellular and extracellular pools of glutamate, glutamine, and GABA but are dominated overwhelmingly by the intracellular

pools in neurons and glia.^{19,20} The abnormal reductions in glial cell counts and density found post mortem in MDD thus may account for the abnormalities reflected by the Glx spectra measured in vivo in MDD.²¹ If so, then the anatomical distribution of these abnormalities may be limited to areas that contain abnormalities in glial cells in MDD. The anatomical extent of the regions where reductions in glial cell counts, density, and gene expression have been identified has thus far been limited to limbic and prefrontal areas implicated by histopathologic and neuroimaging studies in the modulation of emotional behavior. These areas include the dorsal anterolateral prefrontal cortex, the anterior cingulate cortex, the orbitofrontal cortex, and the amygdala.²²⁻²⁹ The objective of the present study thus is to apply the 3-T MRS technique to compare Glx and GABA levels between unmedicated currently depressed patients with MDD and controls in the prefrontal regions of interest (ROIs) that encompass areas where glial cell abnormalities have been reported in MDD. Based on the previous MRS, histopathologic, and neurochemical studies reviewed previously herein, we hypothesize that Glx and GABA levels would be reduced in the dorsomedial/dorsal anterolateral ROI and the ventromedial ROI in depressive patients relative to controls.

METHODS

PARTICIPANTS

Individuals who met the DSM-IV criteria for a current major depressive episode and for MDD and who had a Montgomery-Asberg Depression Rating Scale score greater than 18 (n=20; 13 females; mean \pm SD age, 34.0 \pm 11.2 years; age range, 19-60 years) and controls (n=20; 13 females; mean \pm SD age, 34.8 \pm 12.4 years; age range, 19-58 years; mean \pm SD Montgomery-Asberg Depression Rating Scale total score, 0.1 \pm 0.4; range, 0-2) were included. **Table 1** gives additional clinical characteristics of the MDD sample. The participants were recruited through advertisements in local newspapers and posters at the National Institutes of Health campus and were evaluated during screening visits in the outpatient psychiatry clinic of the National Institutes of Health Clinical Center. Psychiatric diagnoses were established using an unstructured clinical interview by a psychiatrist and via a structured interview using the *Structured Clinical Interview for the DSM-IV*.^{30,31} The clinical evaluation also included a physical examination, electrocardiography, and laboratory tests, including liver and kidney function tests, hematology profile, thyroid function tests, urinalysis, and toxicology (drug screen). Exclusion criteria included current medical or neurologic disorders, exposure to psychotropic medications within 4 weeks of scanning (8 weeks for fluoxetine), cigarette smoking, and pregnancy. Participants were entered into the study after a full explanation of the purpose of the study and the study procedures and after written consent was obtained as approved by the National Institute of Mental Health institutional review board.

MAGNETIC RESONANCE SPECTROSCOPY

Participants underwent scanning in a single session using a 3-T whole-body scanner and a transmit-receive head coil (General Electric Medical Systems, Milwaukee, Wis) capable of providing a homogeneous radiofrequency (RF) field and spectroscopic measurements from prefrontal brain tissue. Based on pre-

Table 1. Clinical Characteristics of the 20 Individuals With MDD

Clinical Characteristic	Value
MADRS score, mean (SD) [range]	27 (4.3) [20-37]
HDRS score, mean (SD) [range]	22 (6.0) [14-33]
Age at onset, mean (SD) [range], y	16 (9.4) [4-43]
Episode duration, mean (SD) [range], mo	140 (130) [15-480]
Total illness duration, mean (SD) [range], y	18.8 (13.5) [2-55]
MDD course, No.	
1 MDE	3
2-3 MDEs	3
>3 MDEs	12
Chronic course	2
Time medication free	
Mean (SD) [range], mo	41 (61) [1.0-216]
Never medicated, No.	4
Comorbid diagnoses, No.	
Generalized anxiety disorder	7
Panic disorder	6
Social phobia	4
Posttraumatic stress disorder	1
History of suicidality, No. of suicide attempts	4
MDD subtypes	
Melancholic, current	2
Melancholic, lifetime	9
Atypical, current	4
Family history, No.	
MDD	9
Bipolar disorder	3
Anxiety disorder	4

Abbreviations: HDRS, Hamilton Depression Rating Scale; MADRS, Montgomery-Asberg Depression Rating Scale; MDD, major depressive disorder; MDE, major depressive episode.

vious imaging and histopathologic studies in major depression,³² proton MRS spectra were acquired from 2 voxels: the dorsomedial/dorsal anterolateral prefrontal DM/DA-PF ROI voxel and the ventromedial prefrontal (VM-PF) ROI voxel. The DM/DA-PF ROI voxel extended $5 \times 3 \times 2$ cm and was positioned with the posterior border 1 mm anterior to the anterior pole of the caudate and with the ventral border 1 mm superior to the dorsal border of the putamen. This cortex included portions of the dorsal and pregenual anterior cingulate gyrus, the adjacent medial frontal gyrus, and the DM/DA-PF cortex (ie, portions of Brodmann areas 9, 24, and 32³³). **Figure 1A** shows the placement of this voxel in the horizontal plane and the brain tissue segments. The VM-PF ROI voxel extended $3 \times 3 \times 2$ cm, was positioned with the posterior edge 1 mm anterior to the rostrum of the corpus callosum, and was centered on the midline in horizontal planes and on the bicommissural line in sagittal planes. This voxel included portions of the perigenual anterior cingulate gyrus and the adjacent frontal polar cortex (ie, portions of Brodmann areas 10, 24, and 32³³). **Figure 1B** shows the placement of this voxel in the horizontal plane and the brain tissue segments. **Figure 2** shows the placement of the voxels in the midsagittal plane.

The level of GABA was measured using an interleaved PRESS-based J editing method.^{8,34} This method uses PRESS for spatial localization. The GABA H-3 at 1.9 ppm is inverted in alternating scans. When GABA H-3 is inverted, the J evolution between GABA H-3 and GABA H-4 is refocused. When GABA H-3 is not inverted during the control scan, the 2 outer resonance lines of GABA H-4 at 3.0 ppm are in antiphase with respect to its central resonance line and creatine. Creatine methyl proton signals at 3.0 ppm and other overlapping resonances

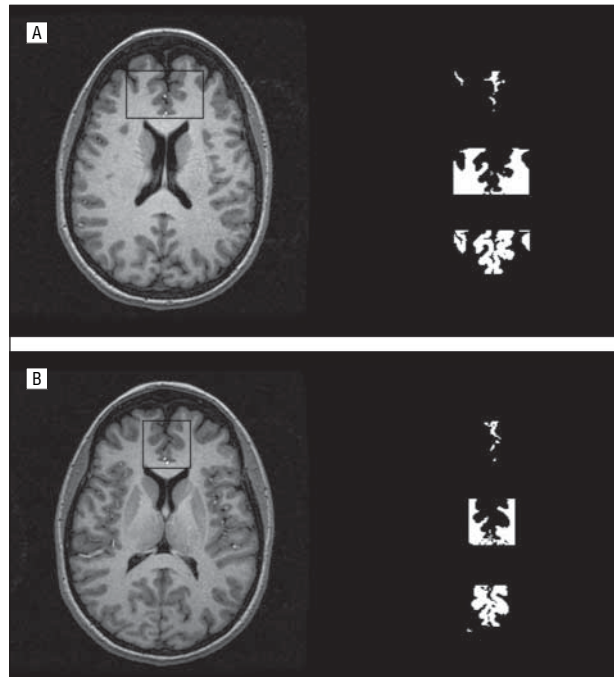


Figure 1. Voxel placement and tissue composition in the dorsomedial/dorsal anterolateral prefrontal region of interest (DM/DA-PF ROI) (A) and in the ventromedial prefrontal (VM-PF) ROI (B). Image on the left shows placement of the voxel in the horizontal plane through the middle of the voxel in the superoinferior direction; right, corresponding cerebrospinal fluid segment (top), white matter segment (middle), and gray matter segment (bottom).

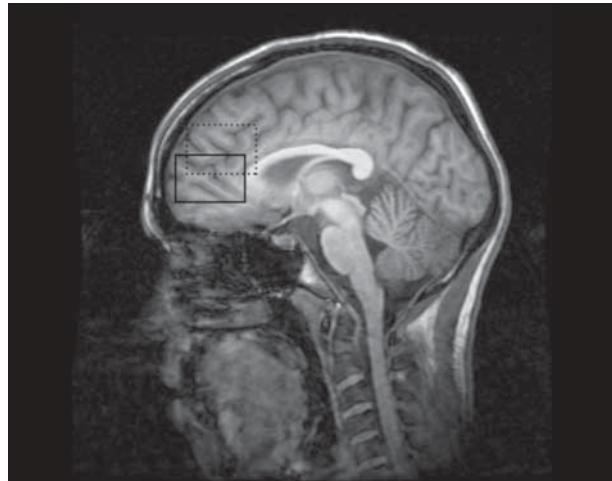


Figure 2. Voxel placement in the dorsomedial/dorsal anterolateral prefrontal region of interest (DM/DA-PF ROI) (upper voxel: dotted box) and the ventromedial prefrontal (VM-PF) ROI (lower voxel: solid box) shown in the midsagittal plane. Because of the large voxel size needed to provide a sufficient signal-to-noise ratio and the anatomical vicinity of the 2 brain areas of interest, the 2 voxels overlapped. The DM/DA-PF ROI was larger than the VM-PF ROI in the left/right dimension, reducing the amount of overlap.

are cancelled during subtraction to reveal the edited GABA H-4. The frequency profile of the editing pulse was flat over the frequency range of the resonances of the metabolites that were affected by the editing pulse. This made the method insensitive to slight variations in frequency during the scan, that is, the editing efficiency was not changed by slight variations in frequency, making the method robust for scanning in vivo. Echo time was 68 milliseconds, repetition time was 1.5 seconds, the number of excitations was 2, and the number of acquisition

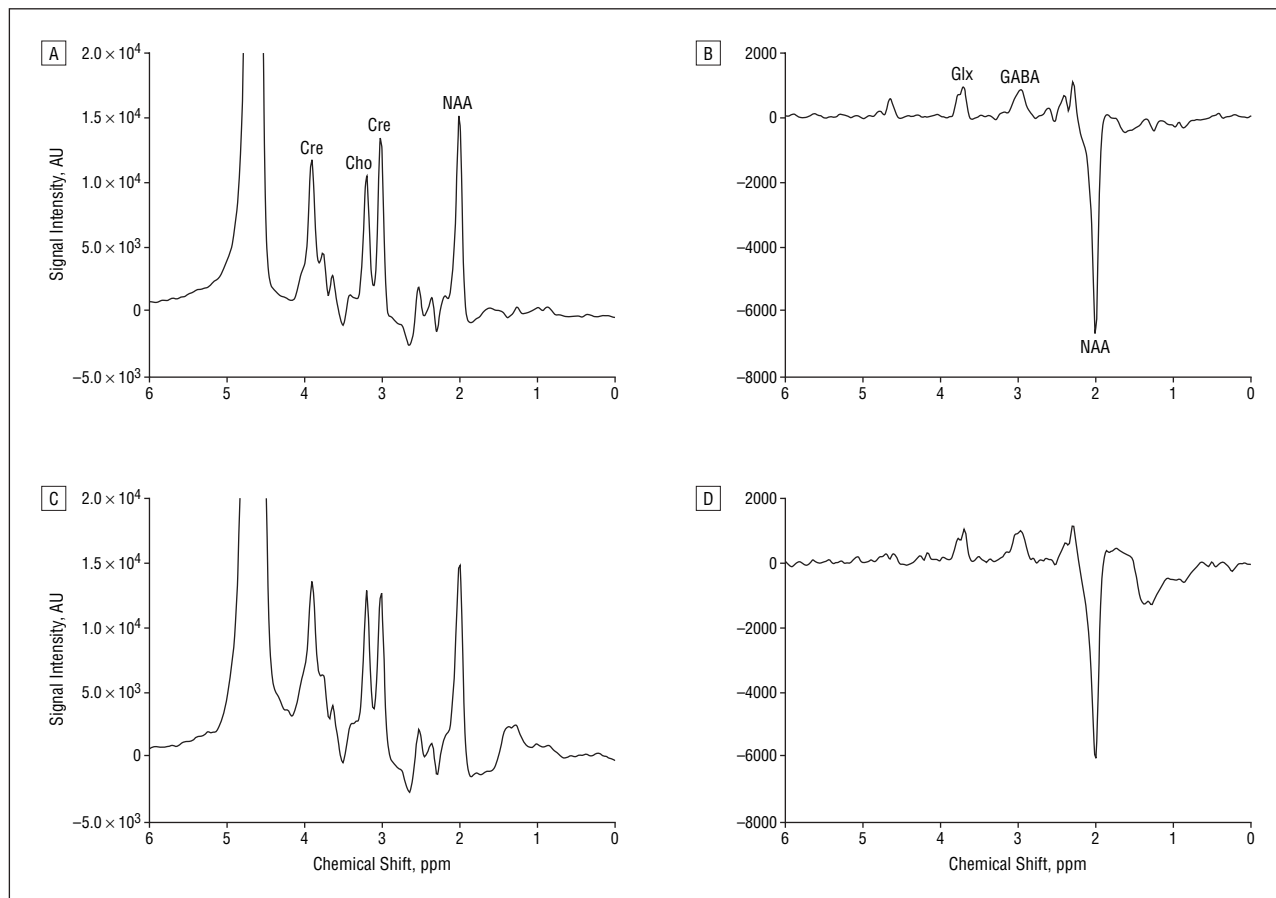


Figure 3. A typical set of γ -aminobutyric acid (GABA) editing spectra obtained at 3 T from a patient with major depressive disorder (A and B) and a control (C and D). A and C show intact subspectra at an echo time of 68 milliseconds (No. of scans=1024) with no GABA editing. B and D show edited spectra. The large *N*-acetylaspartate (NAA) signal at 2.0 ppm was inverted owing to the effect of the GABA editing pulse. The glutamate/glutamine (Glx)-2 signal at 3.8 ppm and the Glx-4 signal at 2.4 ppm also were detected owing to their J-coupling to Glx-3 at 2.1 ppm near the left edge of the flat portion of the GABA editing pulse. The coedited Glx-4 peak partially overlapped the negative NAA signal. However, the Glx-2 signal at 3.8 ppm was clearly coedited, allowing simultaneous determination of Glx without GABA contamination. The edited GABA-4 signal was located at 3.0 ppm and was used for quantification of the GABA concentration. The coedited GABA-2 signal at 2.3 ppm was largely overlapped by the residual Glx-4 signal at 2.4 ppm and the dominant NAA signal at 2.0 ppm. Excellent water and outer volume suppression was achieved. AU indicates arbitrary units; Cho, choline; and Cre, creatine.

points was 2048, with a sample frequency/spectral width of 5000 Hz. The scan time was 27 minutes for 1024 scans for each voxel.

In contrast to a previous study,⁸ individual peak areas were fitted using a nonlinear fitting program written in IDL (Research Systems Inc, Boulder, Colo) that performed time domain spectral analysis to determine the amplitude of the spectroscopic peaks in a fully automated manner (eliminating the possibility of rater bias). The concentrations of GABA, choline, *N*-acetylaspartate (NAA), and coedited Glx (ie, glutamate and glutamine) are expressed in millimoles per liter referenced to the concentration of creatine that was set at 93 mg/dL (7100 μ mol/L) because this value represents an average concentration from literature reports of creatine in gray and white matter.^{35,36} This conventional creatine referencing method was previously validated.^{6,37} Spectroscopic data were processed in 2 steps. First, the unedited spectra were fitted for the amplitudes of choline, creatine, and NAA. At the echo time optimal for GABA editing, the unedited macromolecule background is approximately in antiphase and cancels itself.³⁷ Second, GABA at 3.0 ppm and coedited Glx-2 at approximately 3.8 ppm were extracted from the edited spectra and fitted accordingly. The GABA intensity then was corrected for contamination from coedited macromolecules.³⁴ At experimental conditions optimized for GABA editing, fractions of Glx-2 at 3.8 ppm and Glx-4 at 2.4 ppm were coedited because of their J-coupling to the Glx-3 signal at 2.1 ppm. The cleanly coedited Glx-2 signal was used for

measurement of Glx because its intensity is proportional to the total concentration of Glx.³⁴ The GABA signal closest to the Glx-2 peaks resonates at 3.0 ppm, which does not overlap with Glx-2. **Figure 3** shows unedited and edited spectra measured in a participant with MDD and the corresponding control subject.

STATISTICAL ANALYSIS

We used 2-tailed *t* tests for independent samples to test for reductions in brain chemicals in depressed patients relative to controls. Because we tested in 2 regions, we used a Bonferroni-adjusted significance level of $P = .025$ for hypothesis testing. One-way analysis of covariance including age, sex, and gray matter fraction as covariates revealed that these covariates did not have significant effects on Glx or GABA concentrations at $P = .05$ and that they did not affect the main results of this study in either voxel. The Glx and GABA measures were not correlated with gray matter fraction in either voxel ($P > .25$). We did not find any significant diagnosis \times sex interactions. In secondary analyses we computed Pearson correlation coefficients to assess the relationship between Glx and GABA concentrations in each region and the associations between these concentrations and depression severity (6 tests). We used Pearson correlations to test for associations between NAA and choline and between age and

Table 2. Metabolite Concentrations in the Prefrontal Brain Regions*

Voxel and Participants	Concentration, Mean (SD), mmol/L			
	GABA	Coedited Glx†	Choline	NAA
DM/DA-PF ROI				
Patients with MDD (n = 20)	0.89 (0.11)	2.30 (0.38)	1.55 (0.18)	10.31 (1.10)
Controls (n = 20)	1.00 (0.11)	2.67 (0.54)	1.55 (0.29)	9.97 (0.99)
Statistics (df = 38)	t = 2.54 P = .02	t = 2.25 P = .02	t = -0.12 P = .90	t = -1.63 P = .11
VM-PF ROI				
Patients with MDD (n = 20)	0.94 (0.14)	2.65 (0.39)	1.59 (0.21)	9.57 (0.76)
Controls (n = 20)	0.95 (0.12)	2.96 (0.47)	1.57 (0.25)	9.75 (0.74)
Statistics (df = 38)	t = 0.35 P = .72	t = 2.30 P = .02	t = -0.29 P = .77	t = 0.76 P = .45

Abbreviations: DM/DA-PF, dorsomedial/dorsal anterolateral prefrontal; GABA, γ -aminobutyric acid; Glx, glutamate/glutamine; MDD, major depressive disorder; NAA, *N*-acetylaspartate; ROI, region of interest; VM-PF, ventromedial prefrontal.

*The creatine concentration was set at 93 mg/dL (7100 μ mol/L).

†Coedited Glx represents a fraction of the total Glx concentration (see the "Methods" section).

sex in each voxel (8 tests). We used *t* tests to compare tissue compartments and creatine/NAA ratios in each voxel (8 tests). Given the large number of statistical comparisons performed in assessing these correlations, the results of the secondary analyses were considered exploratory; therefore, we did not apply Bonferroni adjustments and we set the significance level at *P* = .05. Because of the small number of participants with current melancholic and atypical subtypes, we did not detect associations between depressive subtypes and spectroscopic measures.

RESULTS

A typical set of GABA editing spectra comparing patients and controls is shown in Figure 3. In the DM/DA-PF ROI, Glx and GABA levels were reduced in patients with MDD relative to controls (**Table 2**). Numerically, differences were more prominent in women than in men. The Glx and GABA levels were positively correlated with each other ($r=0.71$; $P<.001$). In patients with MDD, Glx and GABA levels were not correlated with depression severity as rated by the Montgomery-Asberg Depression Rating Scale total score ($r=0.18$ and 0.24 , respectively).

In the VM-PF ROI, Glx levels were reduced in depressed patients relative to controls, whereas GABA levels did not differ between groups (Table 2). Numerically, differences were more prominent in women than in men. The Glx and GABA levels were positively correlated with each other ($r=0.31$; $P=.04$). In patients with MDD, the Glx and GABA levels were not correlated significantly with the Montgomery-Asberg Depression Rating Scale scores ($r=-0.04$ and -0.07 , respectively).

Secondary analyses revealed a negative correlation between age and NAA level in the DM/DA-PF ROI ($r=-0.49$; $P=.001$) and in the VM-PF ROI ($r=-0.41$; $P=.008$) and higher choline concentrations in men than in women in the VM-PF ROI ($t_{38}=2.85$; $P=.007$). These differences were found in patients and controls. The mean \pm SD creatine/NAA ratio did not differ significantly between patients with depression (0.86 ± 0.08) and controls (0.88 ± 0.08).

Table 3 provides measures of tissue composition in patients with MDD and controls. There was no statistical difference in tissue composition in the VM-PF ROI. In the DM/

Table 3. Tissue Composition in the Prefrontal Brain Regions*

Voxel and Segment	Patients With MDD (n = 20)	Controls (n = 20)
DM/DA-PF ROI		
CSF	2.60 (1.07) [1.13-5.51]	2.83 (1.69) [0.94-6.96]
Gray matter	39.79 (3.92) [33.00-46.47]	41.88 (2.79) [36.29-49.89]
White matter	57.61 (4.15) [50.14-64.72]	55.30 (3.23) [48.18-62.18]
VM-PF ROI		
CSF	3.07 (1.24) [1.37-6.43]	3.41 (2.18) [0.98-9.19]
Gray matter	50.75 (5.64) [40.95-61.82]	52.69 (3.63) [44.69-58.83]
White matter	46.19 (6.11) [33.71-56.68]	43.90 (3.85) [36.74-53.55]

Abbreviations: CSF, cerebrospinal fluid; DM/DA-PF, dorsomedial/dorsal anterolateral prefrontal; MDD, major depressive disorder; ROI, region of interest; VM-PF, ventromedial prefrontal.

*Data are given as mean (SD) [range].

DA-PF ROI, individuals with MDD showed a nonsignificant trend toward having a smaller proportion of gray matter ($P=.06$) and a larger proportion of white matter ($P=.06$) than controls; there was no difference in the proportion of cerebrospinal fluid between groups.

COMMENT

This study constitutes the first comparison of regional cerebral GABA and Glx concentrations in prefrontal brain tissue between unmedicated depressed adults and controls. The depressive patients had abnormally reduced Glx levels in the DM/DA-PF and VM-PF regions where histopathologic and neurophysiologic abnormalities previously were identified in depression using postmortem and neuroimaging approaches.²¹ The GABA levels also were reduced in the DM/DA-PF ROI. Regional GABA and Glx levels correlated positively with each other in both of the brain regions studied. Neither the GABA nor the Glx concentrations correlated significantly with illness severity. Finally, although NAA and choline levels did not differ significantly between depressive patients and controls, negative correlations between age and NAA level were found in the DM/DA-PF ROI ($r=-0.49$; $P=.001$) and in the VM-PF ROI

($r = -0.41$; $P = .008$), and the choline concentrations in men exceeded those in women in the VM-PF ROI ($t_{38} = 2.85$; $P = .007$). Both findings are in line with previous studies^{38,39} on the effects of sex and age on spectroscopic measures. Although higher GABA levels in cortical gray matter compared with white matter have been reported,⁴⁰ we did not find a correlation between GABA concentration and gray matter fraction. This lack of correlation may be accounted for by the narrow range of the gray matter fraction across individuals because the voxel was too large to get a predominantly gray voxel and a predominantly white voxel. The potential contribution from the creatine referencing method, which used a single mean creatine concentration, was expected to be small because of the narrow range of changes in tissue composition.

Several methodological strengths of the present study merit comment. First, the MRS data were acquired using a 3-T MRS scanner. In contrast, previous studies of Glx and GABA concentrations in depressed samples were performed using 1.5-T^{9-11,13,14} or 2-T^{6,7,18} magnets. This is relevant because higher fields provide a higher signal-to-noise ratio and better separation between metabolite peaks. Second, the individuals studied herein either were medication naive ($n = 4$) or had been unmedicated for a minimum of 1 month and a mean of 41 months, so their MRS data were unlikely to have been confounded by the effects of psychotropic drug treatment on the GABAergic and glutamatergic systems. Third, given the potential effects of sex and age on spectroscopic measures,^{38,39} we used carefully selected samples of depressed individuals and controls matched subject-by-subject for age and sex. Fourth, all the participants manifested an early age at depression onset, avoiding the potentially confounding effects of cerebrovascular disease and structural brain abnormalities associated with late-onset depression.⁴¹ Fifth, the pattern of comorbidity with anxiety disorders is consistent with large-scale community-based studies on MDD⁴² suggesting that the patient sample was representative of nonelderly adult patients with MDD.

An important strength of the methods for measuring GABA concentrations was that this study was the first to obtain spectroscopic measures of GABA in prefrontal ROIs of currently depressed patients. Previous studies assessing GABA MRS measures in MDD limited their measurements to the occipital lobe due to the surface coil used.^{6,7} The GABA-editing sequence applied herein, which enabled GABA concentration assays in prefrontal ROIs, depended on a spectroscopic editing technique that used a series of RF pulses to change the signal of GABA-4 (3.0 ppm) resonances through J-coupling with GABA-3 (1.9 ppm) resonances.³⁴ These RF pulses were calibrated to optimize the performance of the editing sequence. The excellent RF field homogeneity rendered by transmit-receive head coils minimized signal loss due to the cumulative effects of RF field inhomogeneity on the edited GABA signal.^{43,44} The high magnet field (3 T) and the homogeneous RF field used in the present study mitigate the inherent low sensitivity of the transmit-receive head coils. The homogeneous RF field profile allowed the acquisition of GABA measurements in the prefrontal cortex.⁴³

The present study thus extended the findings of Sanacora et al^{16,7,18} of reduced occipital GABA levels in MDD^{6,7}

to also involve the DM/DA-PF areas. The present findings that prefrontal Glx concentrations are abnormally decreased in MDD replicated and extended the results of previous MRS studies in MDD that reported reductions in Glx levels in the anterior cingulate gyrus,^{9,10} dorsolateral prefrontal brain regions,¹² and amygdala/anterior hippocampus¹¹ of adults with MDD relative to controls. To our knowledge, this study is the first to show that this abnormality is not due to drug effects because a limitation of the previous studies in depressed adults had been that the patients studied either were currently receiving psychotropic medications or that the medication washout period had not been long enough to avoid confounding effects of psychotropic drugs on the spectroscopic measures.

The present MRS results also extended the anatomical locations where the Glx concentration is abnormal in MDD to include the medial and dorsal anterolateral brain regions situated anterior to the anterior cingulate gyrus. Although the DM/DA-PF and VM-PF ROIs assessed included portions of the anterior cingulate gyrus, they also extended 1 to 2 cm anterior to this gyrus to include the adjacent areas corresponding to Brodmann area 32 and the medial prefrontal and frontal polar cortices corresponding to Brodmann areas 9 and 10.³³ The relatively low spatial resolution of the present data precluded the ability to resolve differences in the MRS data across these distinct anatomical areas (a technical limitation of the MRS approach we applied for assessing prefrontal GABA concentrations was that the voxel size of the ROI needed to be relatively large to provide a sufficient signal-to-noise ratio). Nevertheless, because the magnitude of the differences found between depressive patients and controls in the DM/DA-PF and the VM-PF regions in the present study (13.9% and 10.5%, respectively) was comparable with that reported in previous studies of the anterior cingulate gyrus in MDD (eg, 10.4% in the study by Auer et al⁹), the differences extant in the prefrontal regions located anterior to the anterior cingulate gyrus would seem to have been at least as prominent as those found in the anterior cingulate gyrus alone.⁹ Another technical limitation of the MRS approach for assessing prefrontal GABA concentrations was the relatively long time needed to image each voxel (27 minutes, in addition to setup time). As a result, only 2 ROIs were assessed. Because Glx can be reliably determined in a voxel as small as $0.75 \times 0.75 \times 0.75 \text{ cm}^3$ at 3 T using short-echo time single-voxel proton MRS and linear combination model analysis, future MRS studies have the potential to measure Glx across these distinct anatomical areas in the same subject sample estimating the relative effect sizes of the Glx abnormalities in MDD across the various medial prefrontal gyri.

A third limitation that affected the specificity of these findings involved normalization of the GABA spectra by the creatine levels, raising the possibility that the differences found in MDD may have reflected elevations in creatine concentrations rather than reductions in GABA concentrations. This possibility seemed unlikely because none of the state-of-the-art MRS studies using absolute quantification reported a change in creatine levels in MDD (see the "Discussion" section in the study by Coupland et al⁴⁵).

It is noteworthy that 1 study⁴⁶ measured an increase in creatine of 16% to 22% in MDD, although this study has not been replicated by others. The overall creatine concentration reported⁴⁶ is significantly lower than most other literature values. Another study found an association between creatine levels and mood ratings in severely depressed patients.¹² The effect size for the creatine/NAA ratio in the present study was $d=0.25$, which is close to a medium effect, and the sample may have been too small to be sensitive enough to detect a significant difference in creatine levels between the diagnostic groups.

The clinical specificity of the GABA and Glx measures also requires further investigation. In a previous study⁸ that applied the same MRS methods for assessing GABA and Glx concentrations we found no significant differences in the DM/DA-PF and VM-PF ROIs between patients with fully remitted MDD vs controls. When these negative results are considered together with the significant differences reported herein between currently depressed patients with MDD and controls, they suggest that the Glx and GABA abnormalities reflect either mood state-dependent neurochemical correlates or subtype-dependent pathophysiologic differences between the independent MDD samples assessed in our 2 studies. Previous studies showing a negative correlation between Glx and depression severity¹² and an increase in Glx and GABA concentrations after successful electroconvulsive therapy^{10,18} would seem to support the former interpretation. In the present study, however, depression severity was not correlated significantly with the Glx or GABA measures, consistent with the reports of other studies.^{6,7,9} Moreover, potentially relevant clinical distinctions, including age at MDD onset, number of episodes, and capacity to remain in full remission when not taking medication, were observed between individuals with remitted MDD assessed in a previous study⁸ and the currently depressed patients with MDD studied herein (Table 1). Longitudinal MRS studies are needed to address the specificity of the Glx and GABA abnormalities for mood state vs illness subtype in MDD.

The Glx concentration assesses the entire brain pool of glutamate and glutamine in the spectroscopic voxels. The ratio of the intracellular-extracellular concentration of glutamate is 2000 to 5000:1,²⁰ whereas that for glutamine is 30 to 70:1.¹⁹ Differences found in the Glx concentration between depressive patients and controls thus predominantly reflect abnormalities in the intracellular pools of glutamate or glutamine in MDD and do not address whether differences in glutamatergic neurotransmission or in extracellular fluid excitatory amino acid concentrations also exist in MDD. Histopathologic changes in MDD, including reductions in prefrontal glial cell counts, density, and gene products,²²⁻²⁹ may, thus, underlie the reductions found in the Glx concentrations in the present study.

Most of the GABA pool exists in GABAergic neurons.⁴⁷ Reductions in the density of GABAergic neurons have been reported in the anterior cingulate cortex in post-mortem studies of bipolar disorder.⁴ If such abnormalities extend to unipolar depression, they potentially could contribute to the reductions in the regional GABA concentration found herein (Table 3). The GABA concen-

tration also may be dynamically modulated by stress or emotion, however, because it was observed in animals that stress reduced total brain GABA levels.^{48,49} The reduction in GABA concentrations found herein in the depressed sample thus may reflect a neurochemical correlate of their emotional and stressed state. Because extracellular GABA is regulated by intracellular GABA concentration,⁵⁰ a reduced MRS-determined GABA concentration most likely correlates with reduced extracellular GABA, less GABAergic inhibition, and possibly increased glutamatergic excitotoxicity.⁵¹ This mechanism may contribute to the reduction in gray matter volume observed in MDD.²¹

In contrast to a previous study⁷ that found a negative correlation between Glx and GABA, we found a positive association between the 2 major neurotransmitter pools in both of the prefrontal regions examined. Our finding seems to be consistent with the reported positive associations between glutamatergic and GABAergic release and metabolism found in neurophysiologic studies.⁵²

In conclusion, the data reported herein converge with the results of studies using other approaches to implicate the glutamatergic and GABAergic systems in the pathophysiologic mechanisms of MDD.²²⁻²⁹ Further research is needed to determine the anatomical and clinical specificity of the abnormalities in Glx and GABA levels found in the depressed phase of MDD. Longitudinal MRS studies also are needed to evaluate the relationship between the Glx and GABA concentration abnormalities and the presence of depressive symptoms. Finally, studies using other approaches are needed to establish the specific cellular processes that underlie the abnormalities in Glx and GABA levels observed in MDD. The findings from such studies ultimately may prove relevant for guiding the development of more effective therapeutics for MDD because research^{53,54} aimed at elucidating the mechanisms of antidepressant drugs increasingly has demonstrated a role for such treatments in the modulation of glutamatergic and GABAergic function.

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