

ONLINE FIRST

Elevated Prefrontal Cortex γ -Aminobutyric Acid and Glutamate-Glutamine Levels in Schizophrenia Measured In Vivo With Proton Magnetic Resonance Spectroscopy

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Context: Postmortem studies have found evidence of γ -aminobutyric acid (GABA) deficits in fast-spiking, parvalbumin-positive interneurons in the prefrontal cortex in schizophrenia. Magnetic resonance spectroscopy studies in unmedicated patients have reported glutamine or glutamate-glutamine (Glx) elevations in this region. Abnormalities in these transmitters are thought to play a role in cognitive impairments in the illness.

Objective: To measure GABA and Glx levels in vivo in 2 prefrontal brain regions in unmedicated and medicated patients with schizophrenia and healthy controls.

Design: Case-control study.

Setting: Inpatient psychiatric research unit and associated outpatient clinic.

Participants: Sixteen unmedicated patients with schizophrenia, 16 medicated patients, and 22 healthy controls matched for age, sex, ethnicity, parental socioeconomic status, and cigarette smoking.

Methods: Proton magnetic resonance spectroscopy with a 3-T system and the J-edited spin-echo difference method. The GABA and Glx levels were measured in the dorsolateral and medial prefrontal cortex and normalized to the simultaneously acquired water signal. Working memory performance was assessed in all subjects.

Main Outcome Measures: The GABA and Glx concentrations determined by proton magnetic resonance spectroscopy.

Results: In the medial prefrontal cortex region, 30% elevations were found in GABA ($P = .02$) and Glx ($P = .03$) levels in unmedicated patients compared with controls. There were no alterations in the medicated patients or in either group in the dorsolateral prefrontal cortex. Both regions showed correlations between GABA and Glx levels in patients and controls. No correlations with working memory performance were found.

Conclusions: To our knowledge, this study presents the first GABA concentration measurements in unmedicated patients with schizophrenia, who showed elevations in both GABA and Glx levels in the medial prefrontal cortex but not the dorsolateral prefrontal cortex. Medicated patients did not show these elevations, suggesting possible normalization of levels with antipsychotic medication. The Glx elevations agree with prior magnetic resonance spectroscopy literature, but GABA elevations were unexpected and suggest possible involvement of classes of interneurons not found to show impairments in postmortem studies.

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THE CRITICAL ROLE OF γ -AMINObutyric acid (GABA) in coordinating pyramidal cell activity has focused interest on GABA and glutamate as factors in information processing deficits in schizophrenia. Postmortem studies^{1,2} have found evidence of GABA deficits in fast-spiking, parvalbumin-positive cortical interneurons in schizophrenia that are essential for synchronizing neuronal function,² raising the possibility of cortical GABA deficits in the

illness. However, a number of recent proton magnetic resonance spectroscopy (¹H MRS) studies investigating frontal or occipital cortex or striatal GABA in vivo in patients treated or mostly treated with antipsychotic medications have yielded mixed results, with 2 studies reporting GABA reductions,^{3,4} 1 finding no changes,⁵ and another reporting elevations.⁶ In vivo ¹H MRS studies investigating the glutamate system in medicated patients with schizophrenia have yielded similarly variable results.⁷⁻¹³

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Table 1. Demographic and Clinical Variables

Characteristic	Control Subjects	Unmedicated Patients	Medicated Patients
Sample size	22	16	16
Age, y, mean (SD)	33 (8)	32 (11)	32 (10)
Sex, No.			
F	8	5	5
M	14	11	11
Ethnicity, No. ^a			
African American	8	5	5
Asian	1	2	2
White	7	6	6
Hispanic	6	3	3
Diagnosis, No.			
Schizophrenia		12	10
Schizoaffective disorder		4	6
Schizophrenia subtypes ^b			
Paranoid		4	5
Undifferentiated		7	3
Disorganized		1	1
Residual		0	1
Smoker, No.			
Yes	1	4	1
No	21	12	15
Parental SES, mean (SD) ^c	42 (11)	44 (14)	43 (17)
Subject SES, mean (SD) ^c	30 (13)	21 (6)	30 (10)
Antipsychotic free, No.		7	
Antipsychotic naive, No.		9	
Antipsychotic-free interval, mo, mean (SD) [range]		21 (17) [14 d to 4 y] ^d	
Total PANSS score, mean (SD)		71 (15)	57 (8)
Duration of illness, y, mean (SD)		7 (7)	9 (8)

Abbreviations: PANSS, Positive and Negative Syndrome Scale³³; SES, socioeconomic status.

^aSelf-reported.

^bUnmedicated, n = 12; medicated, n = 10.

^cHollingshead Index.³²

^dFor the 7 previously medicated patients.

To our knowledge, no study to date has investigated GABA in unmedicated patients with schizophrenia. Proton MRS studies of the glutamate system in medication-naive patients with first-episode schizophrenia¹⁴⁻¹⁶ or unmedicated subjects at high risk for schizophrenia¹⁷ have generally found elevations of combined glutamate-glutamine (Glx) or glutamine levels in the medial prefrontal cortex (MPFC). While some studies in unmedicated subjects have found no changes,^{7,18,19} 2 of these negative studies reported data from the dorsolateral prefrontal cortex (DLPFC),^{7,18} suggesting that the MPFC region may more consistently show abnormal elevations of Glx or glutamine levels.

The *N*-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia²⁰⁻²³ has been invoked to provide a mechanism for glutamate system elevations in terms of pyramidal cell disinhibition by acute impairment of fast-spiking GABA interneuron function.²⁴ Rapid elevation of glutamate levels in the extracellular space in the prefrontal cortex in rodents is a replicated acute neurochemical effect of *N*-methyl-D-aspartate receptor antagonists.^{25,26} The MRS findings of elevated Glx levels in the MPFC in schizophrenia potentially extend the face va-

lidity of the *N*-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia from symptom induction to neurochemical alterations. Whether chronically elevated Glx levels in schizophrenia might be attributable to *N*-methyl-D-aspartate receptor hypofunction, and in turn to chronic disinhibition by impaired GABA interneuron function, is not known. We sought in this study to begin to address this question by evaluating concurrent Glx and GABA function in the illness.

In this study, we report what we believe are the first measurements of GABA levels in unmedicated patients with schizophrenia. We used an optimized ¹H MRS spectral editing technique²⁷⁻²⁹ to measure levels of GABA and Glx in 2 frontal cortical regions in groups of patients taking and not taking antipsychotic medications to assess the dependence of these neurochemicals on brain region and medication status. We selected the DLPFC and MPFC for this investigation because they have been widely implicated in schizophrenia, including by the postmortem^{1,2} and MRS studies¹⁴⁻¹⁷ mentioned. Based on these prior studies, we hypothesized that GABA levels are decreased and Glx levels are increased in unmedicated patients. We compared the measured levels with working memory performance and clinical status.

METHODS

SUBJECTS

Fifty-four subjects participated in this study including 32 patients who met *DSM-IV* criteria for schizophrenia or schizoaffective disorder as assessed with a structured interview (Diagnostic Interview for Genetic Studies³⁰). The patients were divided equally into an unmedicated group of 16 patients free of antipsychotic medication treatment for a minimum of 14 days prior to the scan and a medicated group of 16 patients in treatment with a clinically determined fixed dose of second-generation antipsychotic medication for at least 4 weeks. Of the 16 unmedicated patients, 9 were antipsychotic medication naive. Twenty-two psychiatrically healthy subjects assessed with the *Structured Clinical Interview for DSM-IV Axis I Disorders*³¹ participated as a control group. Patients were included who were of any ethnicity, male or female, aged 18 to 60 years, and met the additional criteria of (1) no other *DSM-IV* Axis I diagnosis; (2) no lifetime history of alcohol or substance abuse or dependence; (3) no concomitant or past severe medical conditions, including head trauma; (4) no pregnancy, (5) no metallic or other material in the body that would preclude safe exposure to the magnetic resonance imaging (MRI); and (6) ability to provide informed consent. Inclusion criteria for the control group were absence of past or present Axis I psychiatric diagnosis, including substance abuse, plus criteria 3 through 6 as mentioned earlier for the patients. Groups were matched for age, sex, ethnicity, nicotine smoking, and parental socioeconomic level,³² and all subjects were right handed. Severity of clinical symptoms was measured with the Positive and Negative Syndrome Scale (PANSS)³³ and working memory performance was assessed with the *N*-back test.³⁴ Clinical and demographic characteristics of the subjects are further detailed in **Table 1**.

The study was approved by the institutional review boards of the New York State Psychiatric Institute and Columbia University Medical Center. All subjects provided written informed consent. Patients were recruited after voluntary admission to a research ward (Schizophrenia Research Unit, New York State Psychiatric Institute) or the affiliated research clinic (Schizophre-

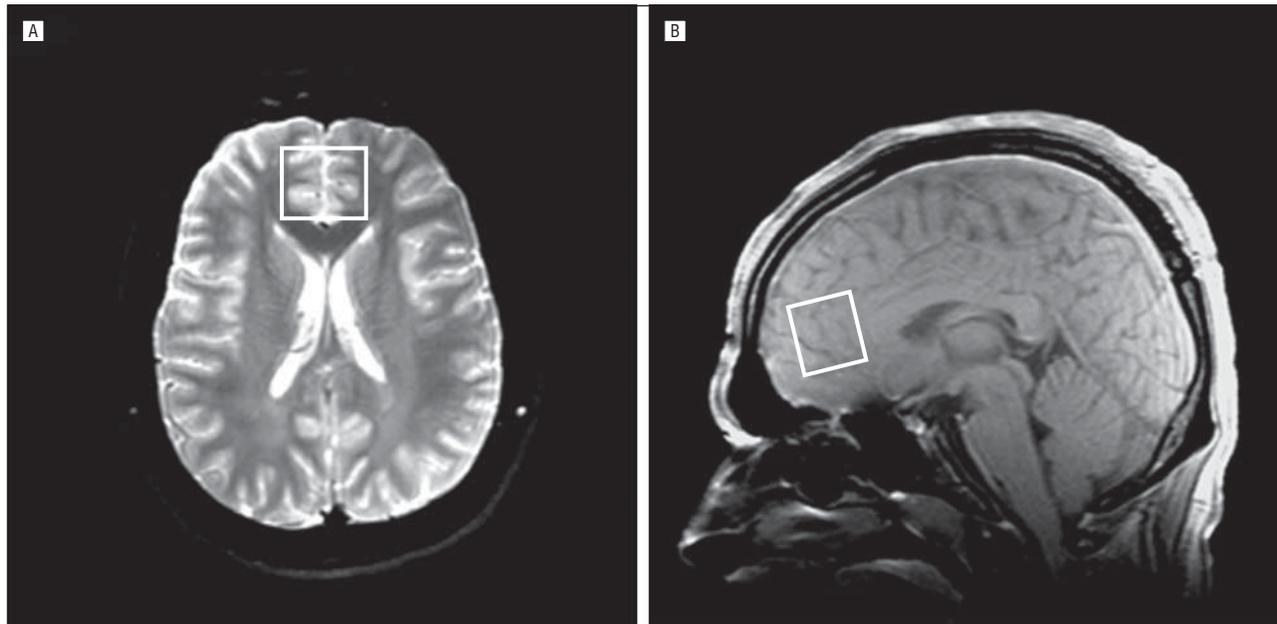


Figure 1. Axial (A) and midsagittal (B) images showing the medial prefrontal cortex voxel size and location. The voxel is located anterior to the genu of the corpus callosum, oriented along the anterior-posterior commissure line and centered on the interhemispheric fissure. Its dimensions are 2.5 cm (anterior-posterior) \times 3 cm (left-right) \times 2.5 cm (superior-inferior), and volume is 18.8 cm³.

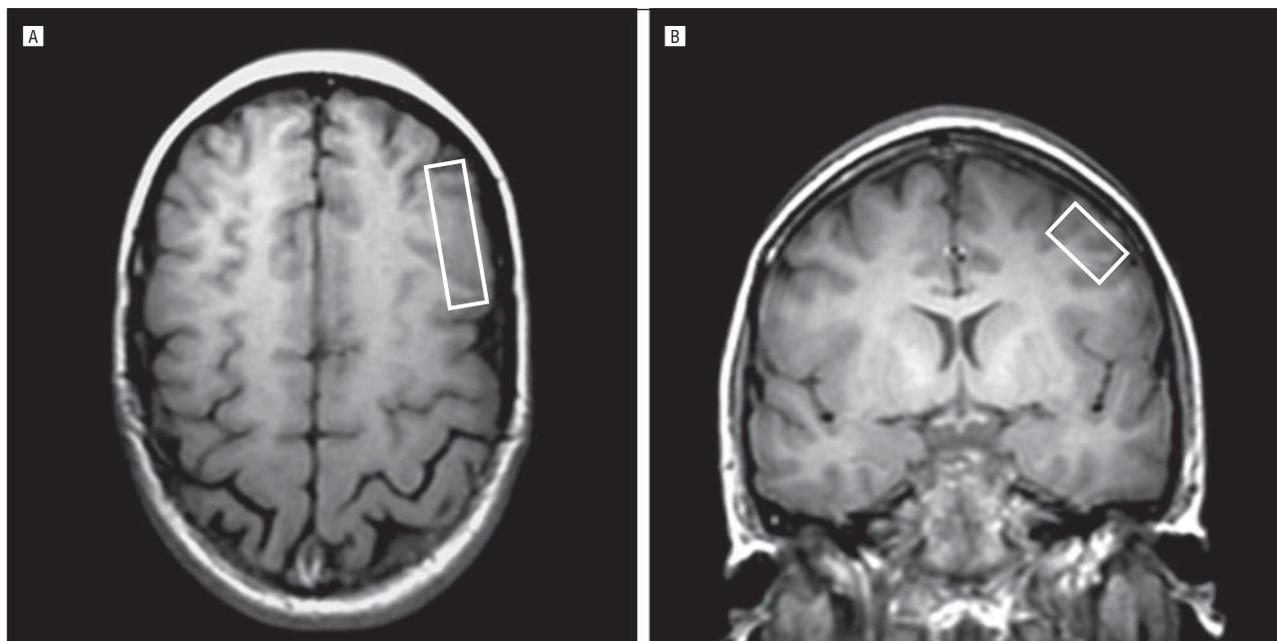


Figure 2. Oblique axial (A) and coronal (B) images showing the dorsolateral prefrontal cortex voxel size and location. The voxel is angled to lie in the left middle frontal gyrus parallel to the brain surface. Its dimensions are 4.8 cm (anterior-posterior) \times 1 cm (perpendicular to brain surface) \times 2 cm (superior-inferior), and volume is 9.6 cm³.

nia Recovery Clinic). Capacity to provide informed consent was evaluated by a psychiatrist not associated with the study. Assent from involved family members was also obtained.

NEUROIMAGING DATA ACQUISITION METHODS

All neuroimaging studies were conducted on a research-dedicated General Electric 3.0-T EXCITE magnetic resonance system at the New York State Psychiatric Institute.

STRUCTURAL MRI

A series of high-resolution scans, consisting of standardized axial, coronal, and sagittal T1-, T2-, and spin density-weighted scans were acquired that were appropriately obliqued for prescribing the ¹H MRS voxels. In addition, a T1-weighted spoiled gradient-recalled echo volumetric scan (repetition time = 30 milliseconds, echo time = 8 milliseconds, flip angle = 45°, field of view = 24 cm, 256 \times 256 matrix, 124 coronal slices, and a slice thickness of 1.0 mm) was acquired for brain tissue segmentation.

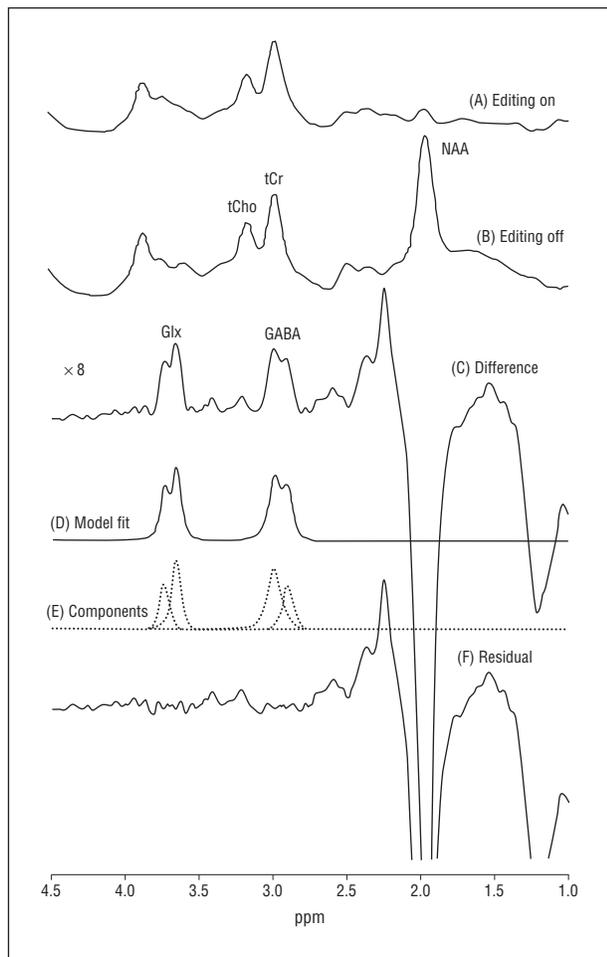


Figure 3. Point-resolved spectroscopy proton magnetic resonance spectroscopy spectra with the editing radiofrequency pulse on (A) and off (B). With the editing pulse off, a standard point-resolved spectroscopy spectrum is obtained, allowing quantification of the *N*-acetylaspartate (NAA), total creatine (tCr), and total choline (tCho) peaks. Spectrum C is the difference of the spectra A minus B (magnified 8-fold) yielding the detected γ -aminobutyric acid (GABA) and glutamate-glutamine (Glx) peaks, and spectrum D shows the best-fit model spectra. The component peaks of the model fit are shown in spectrum E and the residuals, in spectrum F. The component peaks permit quantification of the areas under the peaks and thus the concentrations of GABA and Glx. The data were acquired using an echo time of 68 milliseconds and a repetition time of 1500 milliseconds and 256 interleaved excitations (total, 512) with the editing pulse on or off.

GABA AND Glx MRS

Measurements were made in the MPFC (containing portions of Brodmann areas 24, 32, and 10 including the pregenual anterior cingulate cortex) (**Figure 1**) and the left DLPFC (Brodmann areas 9 and 46) (**Figure 2**). Voxels were reproducibly placed by using internal landmarks.³⁵ The MPFC voxel was located anterior to the genu of the corpus callosum, oriented along the anterior-posterior commissure line and centered on the interhemispheric fissure, with dimensions of 2.5 cm \times 3 cm \times 2.5 cm (volume, 18.8 cm³; 13-minute acquisition) (Figure 1). The DLPFC voxel was placed in the left middle frontal gyrus angled parallel to the brain surface with dimensions of 1 cm \times 2 cm \times 4.8 cm (volume, 9.6 cm³; 26-minute acquisition) (Figure 2).

The MRS data were acquired using a standard J-editing difference method²⁷ as modified by Sailasuta et al²⁸ that has recently been described in detail.³⁶ The acquisition used a single-voxel point-resolved spectroscopy localized J-editing difference method with a receive-only 8-channel phased-array head coil by

applying a frequency-selective inversion pulse that avoids excitation of the GABA C-3 peak at 1.9 ppm on alternate scans, with an echo time of 68 milliseconds and a repetition time of 1500 milliseconds. The data were acquired using 256 interleaved excitations (total, 512) with the editing pulse on or off in the MPFC and 512 interleaved excitations (total, 1024) in the DLPFC. The 8-channel phased-array coil data were combined into a single regular time-domain free-induction decay signal, using the unsuppressed voxel tissue water signal from each receiver coil element to derive the required relative phased-array coil sensitivities. The total scan time to set up and then acquire the 2 MRS and the volumetric MRI scans was about 1 hour.

¹H MRS DATA PROCESSING AND QUANTIFICATION

The areas of the individual spectral peaks, which are proportional to their respective concentrations, were obtained by frequency-domain fitting of each resonance to a Gauss-Lorentz (ie, pseudo-Voigt) line-shape function using the Levenberg-Marquardt nonlinear least-squares algorithm written in IDL (Research Systems Inc) (**Figure 3**). The levels of GABA and Glx in the edited spectra were then expressed as ratios of their peak areas relative to that of the simultaneously acquired unsuppressed water signal (W) from each voxel, a method that yielded a high test-retest reliability,³⁵ as were those of total creatine (tCr), total choline, and *N*-acetylaspartate (NAA) in the unedited spectra. In a secondary analysis, ratios to tCr, a frequently used normalization in clinical MRS studies, were examined. These analyses were performed by a rater blinded to diagnosis and treatment status.

ASSESSMENT OF VOXEL TISSUE HETEROGENEITY

Differences in GABA concentration have been reported between gray matter and white matter,^{37,38} while cerebrospinal fluid (CSF) concentrations are negligible in comparison with gray matter and white matter.³⁹ We therefore implemented volumetric MRI-based tissue segmentation to correct the ¹H MRS-derived levels of these neurotransmitters for brain matter heterogeneity in our relatively large voxels. Segmentation and classification were based on the signal-intensity histogram obtained using the commercial software MEDx (Medical Numerics) from each subject's volumetric (spoiled gradient-recalled echo) MRI. From the histogram, a segmentation mask of each voxel was generated and the proportions of gray matter, white matter, and CSF for the voxel were computed. If the proportions of tissue types differed significantly between the diagnostic groups, voxel tissue type was entered as a covariate in the statistical analysis.

STATISTICAL ANALYSIS

Group means are indicated as means and standard deviations. Linear mixed-effects models for each of the 2 regions (DLPFC and MPFC) with subjects as the random effect and group (unmedicated patients, medicated patients, and healthy controls) and neurochemicals (GABA/W and Glx/W) as fixed effects were used for omnibus testing. Significant results were followed with post hoc 2-tailed independent-samples *t* tests. In secondary analyses, we used the Pearson product-moment correlation coefficient to examine the relationship between the main outcome measures (GABA/W and Glx/W) across subjects and between these measures and age, tissue composition measures, and clinical variables. Group differences in the secondary ratio analysis performed with the tCr normalization were tested with *t* tests. Analysis of covariance with tissue composition fraction as a covariate was used to confirm that group differences

Table 2. MPFC Metabolite and Tissue Composition Results

	Mean (SD)			P Value		
	U (n=16)	M (n=16)	HC (n=22)	U, HC	M, HC	U, M
MRS metabolite						
GABA/W	2.69 (1.19) × 10 ⁻³	2.14 (0.78) × 10 ⁻³	2.02 (0.30) × 10 ⁻³	.02 ^a	.51	.14
Glx/W	2.07 (0.94) × 10 ⁻³	1.64 (0.47) × 10 ⁻³	1.60 (0.25) × 10 ⁻³	.03 ^a	.70	.11
Tissue composition measure						
Internal water	8.71 (2.62) × 10 ¹¹	9.11 (2.09) × 10 ¹¹	9.17 (2.49) × 10 ¹¹	.58	.94	.64
CSF fraction	0.103 (0.041)	0.106 (0.021)	0.093 (0.018)	.32	.06	.83
GM fraction	0.511 (0.023)	0.499 (0.053)	0.512 (0.023)	.97	.31	.38
WM fraction	0.385 (0.041)	0.395 (0.044)	0.395 (0.024)	.36	.95	.50

Abbreviations: CSF, cerebrospinal fluid; GABA, γ -aminobutyric acid; Glx, glutamate-glutamine; GM, gray matter; HC, healthy controls; M, medicated patients; MPFC, medial prefrontal cortex; MRS, magnetic resonance spectroscopy; U, unmedicated patients; W, water signal; WM, white matter.

^aSignificant by post hoc independent-samples 2-tailed *t* test.

in metabolites were unaffected by this potential confound. A probability value of .05 was selected as the significance level.

RESULTS

GABA AND GLX LEVELS

In the MPFC voxel, the linear mixed model was significant for the main effect of group ($F=3.67$; $P=.03$), with unmedicated patients showing higher neurochemical levels compared with controls ($t=2.54$; $P=.01$) and trend higher levels compared with medicated patients ($t=1.93$; $P=.06$). Post hoc *t* tests showed both GABA/W ($t=2.52$; $P=.02$) and Glx/W ($t=2.27$; $P=.03$) levels were elevated in the unmedicated patients relative to controls, while GABA/W and Glx/W levels in medicated patients were not different from controls (**Table 2** and **Figure 4**). Trend-level elevations in both neurochemicals were seen in the unmedicated compared with the medicated patients (Table 2). Tissue composition comparisons showed no significant group differences in this region (Table 2).

In the DLPFC, the linear mixed model showed no main group effect ($F=0.55$; $P=.58$), and GABA and Glx levels did not differ from controls in either patient group or between medicated and unmedicated patients (**Table 3** and **Figure 4**). In this region, tissue segmentation again showed no differences in tissue types between the groups (Table 3).

Levels of GABA and Glx were not different between women and men in the patients or controls.

There was a strong positive correlation between GABA and Glx levels across all subjects in the MPFC ($F=155.9$; $R=0.87$; $P<.001$) (**Figure 5**) as well as in the DLPFC ($F=7.2$; $R=0.35$; $P=.01$), which did not differ between patients and controls. No age dependence of either GABA or Glx levels was found in any group or the full sample in either brain region.

In the secondary ratio analysis, tCr/W ratios showed no significant differences in comparisons of the patient groups with controls or with each other in the MPFC region, but there were trend or significant reductions in both patient groups relative to controls in the DLPFC⁴⁰ (**Table 4**). The GABA and Glx ratios to tCr replicated our main findings based on voxel tissue water normalization (Table 4). In the MPFC, the GABA/tCr ratio was

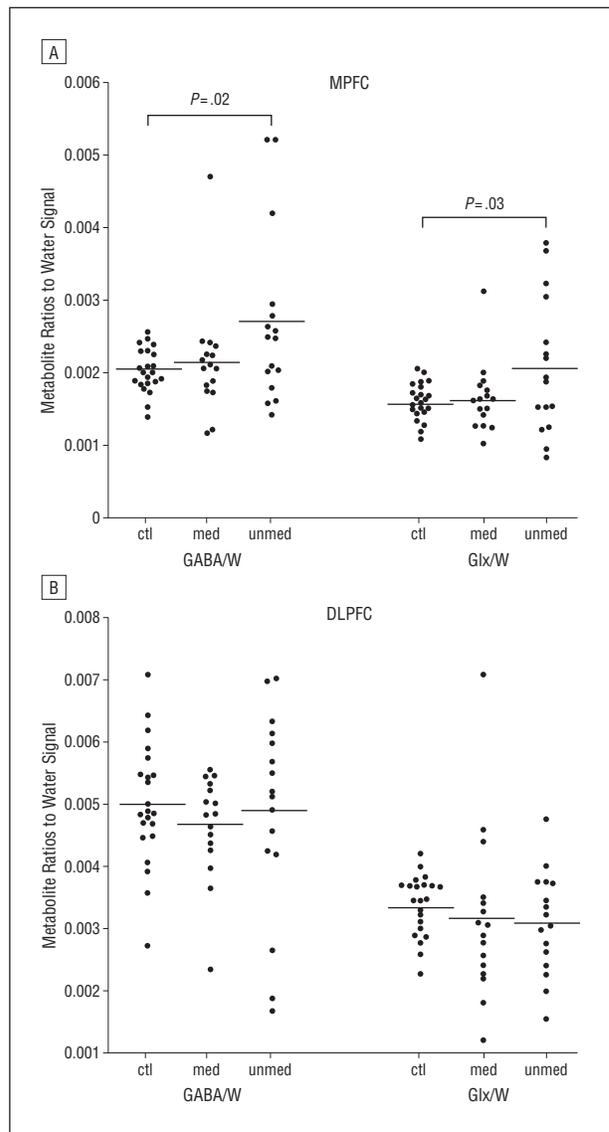


Figure 4. Metabolite ratios to voxel internal water signal showing means (horizontal lines) for 2 brain regions, the medial prefrontal cortex (MPFC) (A) and the left dorsolateral prefrontal cortex (DLPFC) (B). ctl Indicates healthy control group; GABA, γ -aminobutyric acid; Glx, glutamate-glutamine; med, medicated patient group; unmed, unmedicated patient group; and W, water signal.

Table 3. DLPFC Metabolite and Tissue Composition Results

	Mean (SD)			P Value		
	U (n=16)	M (n=16)	HC (n=22)	U, HC	M, HC	U, M
MRS metabolite						
GABA/W	4.88 (1.64) × 10 ⁻³	4.65 (0.83) × 10 ⁻³	5.00 (0.98) × 10 ⁻³	.78	.26	.63
Glx/W	3.09 (0.83) × 10 ⁻³	3.15 (1.36) × 10 ⁻³	3.37 (0.49) × 10 ⁻³	.20	.48	.89
Tissue composition measure						
Internal water	7.66 (1.56) × 10 ¹¹	6.79 (1.36) × 10 ¹¹	7.18 (1.13) × 10 ¹¹	.28	.34	.10
CSF fraction	0.161 (0.085)	0.182 (0.084)	0.150 (0.084)	.70	.26	.49
GM fraction	0.509 (0.062)	0.501 (0.054)	0.521 (0.058)	.54	.29	.69
WM fraction	0.330 (0.084)	0.317 (0.067)	0.329 (0.067)	.96	.61	.64

Abbreviations: CSF, cerebrospinal fluid; DLPFC, dorsolateral prefrontal cortex; GABA, γ -aminobutyric acid; Glx, glutamate-glutamine; GM, gray matter; HC, healthy controls; M, medicated patients; MRS, magnetic resonance spectroscopy; U, unmedicated patients; W, water signal; WM, white matter.

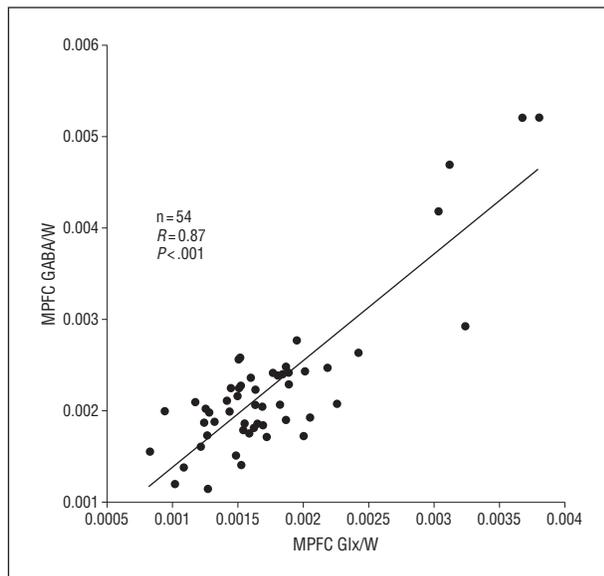


Figure 5. Scatterplot with linear regression fit for the relationship between γ -aminobutyric acid ratio to water signal (GABA/W) and glutamate-glutamine ratio to water signal (Glx/W) in the medial prefrontal cortex (MPFC) region across all study subjects.

significantly higher in unmedicated than medicated patients ($P=.04$), a comparison that showed trend-level differences in the same direction for GABA/W (Table 2).

The GABA and Glx elevations were not confounded by cigarette smoking. The MPFC GABA and Glx levels were nonsignificantly lower among the unmedicated patients who were smokers. Similarly, benzodiazepine use did not contribute to GABA or Glx elevations in the unmedicated patients. No subjects were taking benzodiazepines at scan time. However, 3 patients in the unmedicated patient group received an oral dose as needed of lorazepam (1 mg/d) at least 24 hours prior to the scan. These 3 patients exhibited numerically lower MPFC GABA and Glx levels than the rest of the unmedicated group. Further, the significant elevation in MPFC GABA and Glx levels was present in the remaining 13 patients untreated with antipsychotic medications ($P=.02$ and $P=.03$, respectively) compared with controls. Also, within the medicated patient group, 3 of 16 were taking an anticonvulsant medication at the time of

study (lithium, topiramate, and valproate). The 3 patients taking anticonvulsants showed no elevation of GABA level in either brain region compared with the rest of the medicated patients.

EFFECTS OF TISSUE HETEROGENEITY

Tissue composition did not differ by group in either region (Table 2 and Table 3). However, there was a trend-level higher CSF fraction in the medicated patients compared with controls in the MPFC region (Table 2). Analysis of covariance with CSF fraction as a covariate replicated the finding of absence of group differences between the medicated patients and controls for both GABA and Glx levels in this region (for Glx levels, $F=3.41$; $P=.07$ for CSF fraction and $F=0.921$; $P=.34$ for group; for GABA levels, $F=9.98$; $P=.003$ for CSF fraction and $F=2.86$; $P=.10$ for group).

Gray matter fraction showed no significant correlation with GABA or Glx level in the DLPFC or with Glx level in the MPFC but did correlate significantly with GABA level in the MPFC ($F=5.93$; $R=0.32$; $P=.02$, all subjects). Analysis of covariance with gray matter fraction as a covariate showed gray matter fraction to be a significant covariate for GABA level in the MPFC ($F=6.15$; $P=.02$), which did not affect the main group effect ($F=3.61$; $P=.03$) for GABA level in unmedicated patients compared with controls. Gray matter fraction was not a significant covariate for Glx level in the MPFC or for either measure in the DLPFC.

OTHER METABOLITES

We compared other major metabolites in the unedited proton spectrum across the groups. We examined total choline/W, tCr/W, and NAA/W in both regions. Unpaired t tests showed NAA/W was lower in unmedicated patients ($[3.27 \pm 1.02] \times 10^{-2}$) compared with controls ($[3.89 \pm 0.38] \times 10^{-2}$; $P=.01$) only in the DLPFC, with no significant differences in any of the other metabolites in either region. There were marked positive correlations of GABA levels with NAA in both patient groups in both regions, and Glx level was correlated with NAA in unmedicated patients in both regions. Healthy controls did not show these correlations.

Table 4. Metabolite Ratios to Total Creatine

MRS Metabolite	Mean (SD)			P Value		
	U (n=16)	M (n=16)	HC (n=22)	U, HC	M, HC	U, M
MPFC						
tCr/W	1.28 (0.42) × 10 ⁻²	1.24 (0.27) × 10 ⁻²	1.26 (0.16) × 10 ⁻²	.84	.79	.76
GABA/tCr	2.01 (0.42) × 10 ⁻¹	1.72 (0.35) × 10 ⁻¹	1.63 (0.33) × 10 ⁻¹	.005 ^a	.46	.04 ^a
Glx/tCr	1.53 (0.37) × 10 ⁻¹	1.34 (0.32) × 10 ⁻¹	1.29 (0.23) × 10 ⁻¹	.02 ^a	.59	.14
DLPFC						
tCr/W	2.03 (0.58) × 10 ⁻²	1.97 (0.43) × 10 ⁻²	2.29 (0.34) × 10 ⁻²	.08	.01 ^a	.75
GABA/tCr	2.43 (0.75) × 10 ⁻¹	2.46 (0.70) × 10 ⁻¹	2.23 (0.56) × 10 ⁻¹	.35	.27	.92
Glx/tCr	1.61 (0.54) × 10 ⁻¹	1.63 (0.72) × 10 ⁻¹	1.50 (0.32) × 10 ⁻¹	.42	.46	.94

Abbreviations: DLPFC, dorsolateral prefrontal cortex; GABA, γ-aminobutyric acid; Glx, glutamate-glutamine; HC, healthy controls; M, medicated patients; MPFC, medial prefrontal cortex; MRS, magnetic resonance spectroscopy; tCr, total creatine; U, unmedicated patients; W, water signal.

^aSignificant by independent-samples 2-tailed *t* test.

CLINICAL MEASURES

N-Back

Participants performed the N-back working memory test on the day of scan. Medication status did not affect performance assessed with the 2-back adjusted hit rate (medicated compared with unmedicated patients, *P* = .72). Patients as a whole tended to perform at a lower level than controls (*P* = .06), and unmedicated patients showed significantly lower performance (*P* = .048). Working memory performance did not correlate with GABA or Glx levels in either region.

Positive and Negative Syndrome Scale

Total mean (SD) PANSS scores were 71 (15) for the unmedicated patients and 57 (8) for the medicated patients (*P* = .01). The PANSS ratings showed significant correlations only for the positive symptom subscale in the MPFC for the pooled patient sample (for GABA level, *F* = 6.26; *R* = 0.46; *P* = .02; and for Glx level, *F* = 5.59; *R* = 0.44; *P* = .03) but not in the DLPFC. These did not survive correction for the 6 comparisons (PANSS total and positive and negative symptom subscales in 2 regions) for each neurochemical.

EFFECTS OF PREVIOUS ANTIPSYCHOTIC TREATMENT

Nine of the 16 unmedicated patients were naive to antipsychotic medication treatment (mean [SD] age, 29 [10] years), and the remaining 7 had received previous antipsychotics (mean [SD] age, 36 [11] years). Of these 7, one had not taken medications for 14 days; another, for 28 days; and the rest, for 10 months to 4 years at the time of scan. There were no differences in GABA or Glx levels between the antipsychotic-naive and previously treated patients in either region (in the MPFC, *P* = .25 for GABA level and *P* = .29 for Glx level, and in the DLPFC, *P* = .38 for GABA level and *P* = .29 for Glx level).

COMMENT

This study presents what are, to our knowledge, the first MRS measurements of GABA levels in vivo in unmedicated patients with schizophrenia. The main new finding reported herein is elevation of MPFC GABA levels in unmedicated patients compared with controls. We also found elevated Glx levels in unmedicated patients in the same region, in agreement with several reports in patients with first-episode schizophrenia or unmedicated patients or at-risk subjects.¹⁴⁻¹⁷

MPFC GABA ELEVATIONS

The finding of significant cross-sectional elevations of MPFC GABA levels in unmedicated patients with schizophrenia compared with controls suggests the possibility of their connection to Glx elevations found in this and other recent studies.¹⁴⁻¹⁷ A putative mechanism that implicates GABA dysfunction in glutamate elevations in schizophrenia involves deficient function (including deficits in the GAD67 enzyme that converts glutamate to GABA) of fast-spiking GABA interneurons, based on postmortem evidence.² This interneuron functional deficit disinhibits pyramidal cell firing, suggesting a concurrent GABA deficit and glutamate excess in the illness.^{24,41} Within this framework, our finding of net GABA elevations in unmedicated patients with schizophrenia might be reconciled with postmortem evidence of GABA deficits as a compensation by, or glutamatergic stimulation of, other classes of interneurons with an unimpaired GAD67 enzyme. While a number of studies of GAD67 messenger RNA or protein have reported evidence of deficits in schizophrenia,⁴²⁻⁴⁴ at least 2 studies^{44,45} did not find evidence of GAD65 protein alterations. Volk et al⁴³ found GAD67 messenger RNA deficits in only a subset of interneurons, leaving the possibility that the remaining unimpaired interneurons might be stimulated by elevated glutamate levels to release excess GABA.

Another potential site of excess GABA is presynaptic accumulation in interneurons, which would be sup-

ported by data indicating abnormal GABA transporter or vesicular GABA transporter function in schizophrenia.

GABA AND Glx ELEVATIONS AND COGNITION

Neuronal synchrony is essential for normal cognitive function,^{46,47} and fast-spiking GABAergic interneurons play a critical role in maintaining synchronous control of pyramidal cell firing.⁴⁸ Some studies have reported elevations in gamma-band power of the electroencephalogram in schizophrenia at baseline,^{49,50} and recent studies in rodents⁵¹ and healthy humans⁵² have found elevations of gamma-band power under acute *N*-methyl-D-aspartate receptor hypofunction induced by ketamine administration. In light of these reports, the elevations in GABA and Glx levels in unmedicated patients with schizophrenia found herein might suggest corresponding abnormally elevated gamma-band power, potentially correlating with impaired measures of cognition. However, to our knowledge, to date, no concurrent measurements of GABA, glutamate, gamma-band power, and cognition have been reported in schizophrenia.

REGIONAL EFFECTS

The regional selectivity of our findings may have specificity to schizophrenia. In major depression, MPFC GABA levels were found to be unchanged and MPFC Glx levels were reduced, while both GABA and Glx levels were reduced in a more dorsal voxel.⁵³ In obsessive-compulsive disorder, reductions in MPFC GABA levels without Glx alterations have been found (H. B. Simpson, MD, PhD, unpublished data, 2011). Schizophrenia appears to be unique in exhibiting elevations in both GABA and Glx levels in the prefrontal cortex in the regionally selective pattern reported herein.

The MPFC plays a key role in executive control, conflict monitoring, and error monitoring,^{54,55} functions in which deficits are found in schizophrenia.⁵⁶⁻⁵⁸ These impairments in MPFC function in schizophrenia may be related to the neurochemical abnormalities reported herein and to circuitry abnormalities in the illness. The MPFC sends significant projections to the dorsal caudate nucleus,⁵⁹ the striatal subregion found to exhibit glutamate level elevations in subjects at risk for schizophrenia⁶⁰ as well as the most pronounced dopaminergic hyperfunction in the illness.⁶¹ The striatal projections from the DLPFC partially overlap with those from the MPFC,⁵⁹ and the lack of full overlap may contribute to the dissimilar neurochemistry of these 2 regions in schizophrenia reported herein.

ABNORMALITIES IN OTHER METABOLITES

Our finding of decreased NAA in the DLPFC is consistent with prior studies and with a review and meta-analysis that found similar deficits.⁶² While these deficits might reflect generalized neuronal dysfunction in view of the role of NAA as a putative marker of neuronal functional integrity, the positive correlations with GABA and Glx suggest some degree of specificity to the amino acid transmitter systems.⁶³

EFFECTS OF ANTIPSYCHOTIC MEDICATIONS

In contrast with the unmedicated patients, this study did not find significant MPFC GABA and Glx elevations in patients taking antipsychotic medications, suggesting a “normalization” of these transmitter levels with treatment. Our cross-sectional data cannot distinguish possible cohort effects from effects of the medications. While a recent medication study reported GABA levels in a within-subject design,⁶⁴ which could separate the cohort and medication effects, the patients in that study were minimally medicated rather than medication free at baseline,³ potentially contributing to the finding of unchanged GABA levels following treatment. The mechanism by which treatment might lower MPFC GABA and Glx levels is unclear, and available evidence is mixed. Since local and systemic administration of dopamine agonists increases prefrontal extracellular GABA levels,⁶⁵ and D₂ antagonists decrease these levels,⁶⁶ it is plausible that antipsychotic medications may lower GABA levels through the action of dopamine at D₂-like receptors. However, an *ex vivo* rodent proton nuclear magnetic resonance spectroscopy study of multiple antipsychotic medications found glutamate- but not GABA-lowering effects in the frontal cortex.⁶⁷ An MRS study in cocaine-dependent humans reported GABA increases as a consequence of treatment with pramipexole, a dopamine D_{2/3} receptor agonist medication.⁶⁸ In bipolar disorder, MRS has shown lower GABA and glutamate levels in patients taking antipsychotic medications compared with patients not taking antipsychotics,⁶⁹ although another study of this disorder found no effects on Glx levels from these medications.⁷⁰ The variable MRS findings in medicated patients reported for both GABA³⁻⁶ and the glutamate system,⁷⁻¹³ and the suggestion from our data that these medications may lower GABA and Glx levels, emphasize the need for a within-subject medication vs no medication study design for more definitive assessment of the effect of treatment on these neurochemicals. Such a design would also address the question of a possible role of GABA or Glx changes in clinical improvement with antipsychotic medication.

Prior antipsychotic medication treatment had minimal effects on GABA and Glx levels, since there were no differences between the previously treated patients and the medication-naïve patients in the unmedicated group.

CLINICAL CORRELATIONS

Cognitive deficits represent a significant impairment in schizophrenia.⁷¹⁻⁷⁴ If MPFC GABA elevation is interpreted as a compensation for elevated glutamate, these data seem to indicate an ineffectual compensation. The comparisons with N-back data in the subjects studied herein showed a lack of benefit of higher baseline GABA levels both relative to controls and within the patient sample. The correlations with PANSS positive symptoms rather than cognitive performance were not expected. While neuroreceptor imaging has been used to evaluate dopamine function in relation to positive symptoms,^{75,76} to our knowledge, no study to date has jointly evaluated dopamine, GABA, and glutamate in relation to this class of symptoms.

STRENGTHS AND LIMITATIONS

The interpretation of GABA and Glx measurements detected by *in vivo* MRS has some limitations. Magnetic resonance spectroscopy combines the signal from all tissue compartments, and extracellular GABA or Glx levels cannot be distinguished from intrasynaptic or vesicular compartments. The measured totals over all brain compartments are mainly intracellular.^{77,78} On the other hand, intracellular and extracellular GABA levels tend to be related,⁷⁹ raising the possibility that elevated MRS levels of GABA in the MPFC may indicate net increased GABA transmission. As with GABA, extracellular MRS measurements of Glx cannot be distinguished from intrasynaptic or vesicular compartments, and the measured totals over all brain compartments are mainly intracellular.^{77,80} Glutamate-glutamine itself is a mixture of glutamate and glutamine that we infer is mainly glutamate from knowledge of relative abundance of these 2 neurochemicals.⁸¹

Some limitations of this study are inherent in all MRS *in vivo* measurements. The voxels are relatively large, and the low brain concentration of GABA (of the order of millimolar) necessitates larger voxels than for more abundant metabolites. A technical strength of our study that tends to compensate for low signal from GABA is the use of an 8-channel phased-array head coil, enhancing the signal to noise ratio by more than a factor of 2.⁸² This permitted a volume-for-acquisition-time trade-off that allowed us to acquire 2 voxels smaller than used in many GABA studies, particularly the smaller DLPFC voxel, in a 1-hour scan session. The GABA MRS peak is an admixture of mobile macromolecule signal with that from GABA itself,⁸³ and we previously assessed the macromolecular contribution to the total peak in healthy volunteers under our experimental conditions.⁸⁴ However, it is not known whether this contribution might differ in schizophrenia. Evaluation of GABA and Glx in 2 distinct regions within subjects in the same scan with the finding of selective alterations in only 1 region is another strength of the study. The contrast between the regions lessens the possibility that the selective neurochemical elevations we reported resulted from systemic effects such as residual medications. Potential confounds of cigarette smoking and recent benzodiazepine use in the unmedicated patients did not contribute to MPFC GABA or Glx elevations, since any bias they introduced was opposite to the main study findings. Replication of the main study findings in a secondary ratio analysis with normalization to tCr supports robustness and absence of methodological bias in the measurements.

CONCLUSIONS

The novel and unexpected finding reported herein is elevation of baseline GABA levels in the MPFC in unmedicated patients with schizophrenia. Replication and further characterization of elevated MPFC GABA and Glx levels will be needed to assess their significance as potential targets for therapeutic intervention. Currently available MRS technology^{85,86} can provide multivoxel assessment of the anatomic extent of glutamate abnormalities

in schizophrenia that might facilitate characterization of the involved circuitry. If concurrent elevations in baseline GABA and Glx levels are found to resolve with antipsychotic medication or other treatment, such as GABA_A^{87,88} or GABA_B⁸⁹ receptor modulators, these measures might provide a framework for evaluation of possible cognitive or symptomatic benefit of interventions that normalize them.

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