The glutamate system is complex; it includes multiple modulating cotransmitters, interacting receptors, and multisystem synapses. The regional characteristics of neuronal architecture, local circuitry, and signaling mechanisms suggest that important aspects of glutamate metabolism are modulated at a local level.1 Imbalances in glutamatergic neurotransmission and metabolism are proposed as key pathomechanisms in schizophrenia.1,2 N-methyl-D-aspartate receptor hypofunction on γ-aminobutyric acid neurons could result in disinhibition of postsynaptic glutamate neurons, excessive glutamate release, and neurotoxicity. Although excitotoxicity may not be the cause of schizophrenia, it could contribute to the course of the disorder (see Konradi and Heckers3).

Decreased hippocampal volume is one of the most robust findings in schizophrenia. It is present in first-episode patients and appears to modestly progress in chronic disease.4 Although no change in the number of hippocampal neurons is found,5,6 alterations in the packing density of neurons,7,8 decreased cross-sectional area of neuronal cell bodies,9,10 altered pyramidal cell shapes,11 and decreased dendritic density12 are reported. Olney and Farber13 suggested that alterations in hippocampal glutamatergic neurotransmission potentially account for histological abnormalities and are the basis for the structural deficits observed in imaging studies.

Proton magnetic resonance spectroscopy (1H-MRS) allows for in vivo measurements of neurometabolites, such as glutamate and N-acetylaspartate (NAA), a putative marker of neuronal integrity.14,15 Proton magnetic resonance spectroscopy studies of the hippocampus in patients with schizophrenia have documented reductions in levels of NAA.16,17 Glutamate measurements have been less common because of difficulties in quantifying this metabolite at low magnetic fields, and results have varied.18-21 In healthy participants, studies have reported on a positive correlation between glutamate and NAA.22,23 which is perhaps not surprising given that...
glutamate and NAA metabolisms are linked via the glutamate/glutamine cycle and tricarboxylic acid cycle.\textsuperscript{14} We previously compared hippocampus metabolites with anterior cingulate cortex metabolites in a large group of medicated patients with schizophrenia and healthy controls. We replicated the finding of positive correlation between glutamate and glutamine (Glx) and creatine (Cr) (i.e., the Glx/Cr ratio) and between NAA and Cr (the NAA/Cr ratio) in healthy controls. In schizophrenia, this correlation was absent only in the hippocampus.\textsuperscript{24} We postulated the ratio between Glx/Cr and NAA/Cr in the hippocampus to be a potential biomarker for schizophrenia that captures the pathophysiology of schizophrenia better than either neurometabolite alone.\textsuperscript{24}

The goal of our study was to examine volumetric and neurometabolite alterations in the hippocampus in patients with schizophrenia. We performed structural magnetic resonance imaging and \textsuperscript{1}H-MRS for 27 unmedicated patients with schizophrenia and 27 healthy controls. Based on our own data\textsuperscript{24} and the existing literature,\textsuperscript{4} we tested the following hypotheses: (1) hippocampal volumetric deficits are present in patients with schizophrenia, (2) hippocampal Glx/Cr is altered in patients with schizophrenia, (3) the correlation between Glx/Cr and NAA/Cr is absent in patients with schizophrenia, and (4) Glx/Cr and Glx/Cr:NAA/Cr correlate with decreased hippocampal volumetric measures in patients with schizophrenia.

### Methods

#### Participants

Patients with schizophrenia were recruited from the emergency department and outpatient psychiatry clinics at the University of Alabama at Birmingham. Healthy controls, matched on age, sex, and parental occupation, were recruited through advertisements. Approval by the local institutional review board was obtained. Each patient with schizophrenia completed an Evaluation to Sign Consent form before written informed consent was obtained.\textsuperscript{25}

Diagnoses were established by a review of the medical records and by the consensus of 2 clinicians, and then confirmed with the Diagnostic Interview for Genetic Studies.\textsuperscript{26} Exclusion criteria were major medical/neurologic conditions, substance abuse within 6 months prior to enrollment, pregnancy, and head injury with loss of consciousness. Healthy controls could not have a first-degree relative with an Axis I disorder.

In this analysis, we included 27 patients with schizophrenia who were unmedicated for at least 2 weeks prior to enrollment and 27 healthy controls. All participants were recruited for a prospective study exploring imaging markers of treatment response to risperidone. The neurometabolite measurements of some of the healthy controls have been reported earlier.\textsuperscript{24,27}

#### Clinical Assessment

The Brief Psychiatric Rating Scale (BPRS)\textsuperscript{29} and its positive and negative subscales were used to assess symptom severity. Cognitive function was characterized using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS).\textsuperscript{29}

#### Image Acquisition

Imaging was performed on a 3-T head-only scanner (Magnetom Allegra; Siemens Medical Solutions), equipped with a circularly polarized transmit/receive head coil. A high-resolution structural scan was acquired using the 3-dimensional T1-weighted magnetization-prepared rapid acquisition gradient-echo sequence (repetition time [TR]/echo time [TE]/inversion time [TI], 2300/3.93/1100 milliseconds; flip angle, 12\textdegree; 256 × 256 matrix; and 1-mm isotropic voxels).

#### Voxel-Based Morphometry With DARTEL

Data were processed using Statistical Parametric Mapping 8 (SPM8; Welcome Trust Centre for Neuroimaging) in MATLAB version 7.11.0 (Mathworks). Each participant’s T1-weighted magnetization-prepared rapid acquisition gradient-echo image was segmented into gray matter (GM), white matter (WM), cerebrospinal fluid (CSF), skull, and scalp using the “new segment” routine in SPM8. The DARTEL (diffeomorphic anatomical registration through exponentiated Lie algebra) toolbox for SPM8 was used to improve registration of magnetic resonance imaging scans (www.fil.ion.ucl.ac.uk/~john/misc/VBMclass10.pdf).\textsuperscript{30} This method uses a subject-specific template that we created by averaging images from 39 patients with schizophrenia and 39 healthy controls. The resulting flow fields created by DARTEL were used to generate native space and spatially normalized (in Montreal Neurological Institute space), modulated (to adjust for volume changes during normalization), resliced (1.5-mm isotropic voxels), and smoothed (6-mm full-width at half maximum) GM and WM images. Using native-space masks, we calculated the total GM, WM, and CSF volumes and the total intracranial volume (which is the sum of the GM, WM, and CSF volumes) by use of a script adapted from John's SPM Gems (http://www-personal.umich.edu/~nichols/JohnsGems.html) in MATLAB. To localize areas of volumetric deficits, the results were visually inspected by 2 raters (N.V.K. and D.M.W.) and identified by consensus based on the atlas of hippocampal anatomy by Duvernoy.\textsuperscript{31}

#### Proton Magnetic Resonance Spectroscopy

A series of sagittal, coronal, and axial T1-weighted anatomical scans (gradient-recalled echo sequence; TR/TE = 250/3.48 milliseconds; flip angle, 70\textdegree; 5-mm slice thickness; 1.5-mm gap; and 512 × 512 matrix) were acquired for spectroscopic voxel placement. Data were collected from a voxel in the hippocampus (2.7 × 1.5 × 1 cm) (Figure 1). To control for head tilt, slices were realigned to midline anatomical landmarks in transverse and coronal orthogonal planes. To facilitate prescription of hippocampal volume, axial magnetic resonance images were obtained in an orientation tilted along the long axis of the hippocampus, as viewed in the sagittal images (Figure 1). The voxel was placed in the left hippocampus such that the amount of GM was maximized while avoiding major vessels. Manual shimming was performed to optimize field homoge-
neity across the voxel. Chemical shift selective pulses were used to suppress the water signal. Water-suppressed spectra were acquired using the point-resolved spectroscopy sequence (with TR/TE = 2000/80 milliseconds to optimize the Glx signal and minimize macromolecule contribution, a spectral bandwidth of 1200 Hz, 1024 points, and 640 averages [21 minutes, 20 seconds]). To limit the effects of nicotine intoxication or withdrawal, patients were allowed, but not encouraged, to smoke up to 1 hour prior to image acquisition.

1H-MRS Data Processing

We determined the volume of GM, WM, and CSF in the 1H-MRS voxels by segmenting participants’ structural scans in SPM8. The 1H-MRS voxel images, created from the 1H-MRS raw data headers, were used to mask each of the tissue classes, and the volumes were calculated in MATLAB. Voxel segmentation was not successful for 2 patients with schizophrenia. The 1H-MRS data were analyzed in jMRUI version 3.0. The residual water peak was removed using the Hankel-Lanczos singular values decomposition filter. Spectra were quantified in the time domain using the AMARES (advanced method for accurate, robust, and efficient spectral fitting) algorithm. Prior knowledge derived from in vitro and in vivo metabolite spectra was included in the model. A phantom solution of 20mM glutamate in buffer (30mM sodium hydrogen carbonate and 30mM sodium carbonate; pH, 7.1) was imaged using the parameters from the in vivo study. The resulting spectrum was quantified and used to fit the in vivo data (eFigure 1 in Supplement). The AMARES prior knowledge model consisted of peaks for NAA, choline (Cho), Cr, and the C4 resonance of glutamate (Glu). Glutamate was modeled as triplet (large peak with 2 small outer wings). The amplitudes of NAA, Cho, Cr, and the center Glu peak were estimated by use of the algorithm. The amplitudes of the Glu outer wings were set at a fixed ratio relative to the center peak based on the Glu phantom spectrum. The relative phases of NAA, Cho, Cr, and the center Glu peak were fixed at 0, and the phases of the Glu outer wings were fixed at the phase determined from the Glu phantom spectrum. The line width of NAA was estimated by the algorithm, and the line widths of the remaining peaks were set to be equal to that of NAA. The frequencies of NAA, Cho, Cr, and the center Glu peak were estimated by use of AMARES. The Glu outer wing frequencies were set at fixed shifts relative to the center peak based on the Glu phantom spectrum. All peak shapes were fixed at Lorentzian. Cramér-Rao lower bounds, used as an estimate of uncertainty, were calculated for each peak. The exclusion criteria were (1) a line width of the magnitude signal during manual shimming of greater than 25 Hz at full-width at half maximum; (2) a Cramér-Rao lower bound of greater than 25%, and (3) failure of the fitting algorithm. No spectra were excluded based on these criteria, and NAA, Cho, and Glx were quantified with respect to Cr. As previously reported, the coefficients of variability of the MRS measurements for 1 healthy control were 2.87% (NAA/Cr), 11.93% (Glx/Cr), and 2.15% (Cho/Cr).

Statistical Analyses

Statistical analyses were performed using SPSS 12.0 for Microsoft Windows (SPSS Inc). For analyses of group differences in demographic and clinical variables, independent t tests and χ2 analyses were performed. Differences in metabolites were investigated with multivariate analyses of covariance, using disease state as the between-group factor and age, smoking status, and voxel GM and WM content as covariates. Planned contrasts were created to assess group differences in individual metabolites. P < .05 was considered statistically significant.
Statistical parametric maps of GM content within the hippocampus mask for patients with schizophrenia were compared with those for healthy controls using an independent-sample t test. To evaluate correlations of GM content within the hippocampus mask and neurometabolites, we performed regression analyses, corrected for age, smoking status (packs per day), and total intracranial volume in each group separately. Using the results from this statistical parametric map (P < .001), we extracted the first eigenvector from the residual of GM, controlling for age, smoking status (packs per day), and total intracranial volume for each individual. The eigenvectors were used to correlate with Glx/Cr and to create a scatterplot, allowing for inspection of variance between these variables. In an exploratory analysis, we performed regression for both RBANS and BPRS values on hippocampal volumes and neurometabolites. All statistical parametric mapping analyses were controlled for multiple comparisons by implementing a small-volume correction, confined to the left hippocampus from the Automated Anatomical Labeling atlas implemented in the Wake Forest University PickAtlas software version 2.33,34 and corrected for multiple comparisons using a familywise error (FWE)–corrected P < .05.

Results

Demographics

Groups did not differ with regard to sex, age, or parental occupation. More patients with schizophrenia than healthy controls were smokers. Healthy controls scored significantly higher on than RBANS than did patients with schizophrenia. Of the 27 patients with schizophrenia, 16 had previously been treated with antipsychotics. The remaining 11 patients had no prior exposure to antipsychotics; 5 were first-episode patients, and the others had not received prior treatment for a prolonged period after illness onset (eTable 1 in Supplement).

Hippocampal Volumetric Changes and Neurometabolite Alterations

Analyses revealed 1 cluster of decreased hippocampal voxel-based morphometry (VBM) measure in patients with schizophrenia (t = 4.47; P = .03; cluster extent [i.e., number of voxels] kE = 46; Montreal Neurological Institute coordinates: x = −18, y = −30, z = −9). The area of maximum deficit was mapped to the dentate gyrus extending posterolaterally to the cornu ammonis (CA) and parahippocampal gyrus (Figure 2A).

There were no group differences in GM, WM, or CSF content in the MRS voxel (GM: F52 = 1.350, P = .25; WM: F52 = 0.489, P = .49; CSF: F52 = 1.232, P = .27). The mean (SD) signal to noise ratio of spectra did not differ between groups: 11.77 (1.08) for healthy controls and 11.39 (1.77) for patients with schizophrenia (t = 0.966, P = .34). However, the mean (SD) full-width at half maximum was higher for patients with schizophrenia, 8.86 (1.99) Hz, than for healthy controls, 7.93 (1.16) Hz (t = −2.13, P = .04).

The results of overall multivariate analyses of covariance for neurometabolites were significant at the trend level (Wilks λ = 0.853; F = 2.351; P = .09). Planned contrasts showed no group differences in NAA/Cr, Glx/Cr was elevated in patients with schizophrenia (P = .02) (Figure 3; eFigure 2 in Supplement), and Glx/Cr:NAA/Cr was increased (P < .01) (Table; eTable 2 in Supplement). We identified correlations between Glx/Cr and NAA/Cr in healthy controls (r = 0.39, P = .04) but not in patients with schizophrenia (r = −0.19, P = .21) (Figure 4). Illness duration did not correlate with neurometabolite levels (NAA/Cr: r = 0.38, P = .14; Glx/Cr: r = −0.34, P = .17; Cho/Cr: r = 0.46, P = .09) or hippocampal VBM measure (P > .05). In previously medicated patients, the interval since last antipsychotic treatment did not correlate with neurometabolites (NAA/Cr: r = 0.33, P = .17; Glx/Cr: r = −0.12, P = .38; Cho/Cr: r = 0.26, P = .24). There was no difference in hippocampal VBM measure (P > .05) or neurometabolites between previously medicated and medication-naïve patients or between first-episode and chronic patients (eTable 3 in Supplement).

Correlations Between Hippocampal VBM Measures and Neurometabolites

We found 2 clusters of negative correlation between hippocampal VBM measure and Glx/Cr in patients with schizophrenia (t = 4.81; FWE-corrected P = .02; kE = 110; Montreal Neurological Institute coordinates: x = −26, y = −32, z = −4 vs t = 4.96; FWE-corrected P = .04; kE = 110; Montreal Neurological Institute coordinates: x = −36, y = −15, z = 16) but not in healthy controls (Figure 2B and C); NAA/Cr did not correlate with the hippocampal VBM measure in patients with schizophrenia or healthy controls (P > .05). Unexpectedly, we did not find correlations between Glx/Cr:NAA/Cr and the hippocampal VBM measure in either group.

Correlations Between Neuroimaging and Clinical Findings

Neither the BPRS total nor the positive and negative subscales correlated with VBM measures in patients with schizophrenia (FWE-corrected P > .05). We did not find correlations between VBM and RBANS total score in either group (FWE-corrected P > .05).

We did not find correlations in patients with schizophrenia between Glx/Cr and BPRS (BPRS total score: r = 0.18, P = .46; BPRS positive subscales: r = −0.08, P = .73; BPRS negative subscales: r = 0.25, P = .29) or NAA/Cr and BPRS (BPRS total score: r = 0.25, P = .29; BPRS positive subscales: r = 0.05, P = .82; BPRS negative subscales: r = −0.3; P = .90). The RBANS total scores did not correlate with Glx/Cr (healthy controls: r = 0.29; P = .19; patients with schizophrenia: r = 0.35, P = .15) or NAA/Cr (healthy controls: r = −0.23, P = .57; patients with schizophrenia: r = −0.03, P = .90).

Discussion

The findings of our study include (1) GM volumetric deficits in the hippocampus, (2) increased Glx/Cr but no difference in NAA/Cr, (3) loss of correlation between Glx/Cr and NAA/Cr, and (4) negative correlations between Glx/Cr and hippocampal VBM measure in unmedicated patients with schizophrenia. To our knowledge, we are the first to report increased Glx/Cr and a
possible link between hippocampal glutamate abnormalities and volumetric deficits in a relatively large group of unmedicated patients.

A decreased hippocampal volume is present in first-episode patients and appears to modestly progress in chronic disease. Voxel-based morphometry studies showed GM deficits in the medial temporal lobe in more than 50% of studies, with total hippocampal volume deficits of 8%. Although these findings are widely replicated, regional specificity has been less explored. The volumetric deficits in our study were most prominent in the dentate gyrus with posterolateral extensions to the CA and parahippocampal gyrus. Similar to our study, the studies by Narr et al reported pronounced posterior hippocampal volume reductions in 2 groups of patients with schizophrenia and their unaffected twins. In first-episode patients, Narr et al reported volume loss in the anterior, but not the posterior, hippocampus, as well as perihippocampal CSF increases surrounding anterolateral and midbody hippocampal regions. Anterior volume reductions without posterior hippocampal volume loss in male patients with chronic schizophrenia have also been reported.

We found increased hippocampal Glx/Cr in unmedicated patients with schizophrenia but no alterations in NAA/Cr. Only one study explored NAA and Glx in the medial temporal lobe in a heterogeneous group of 34 patients with first-episode psychosis that included affective psychoses (some medicated patients and some medication-naïve patients). Wood et al reported no differences in NAA or Glx levels among these patients when compared with healthy controls. Although the unaltered NAA levels in the study by Wood et al agreed with our results, the differences in the findings for Glx...
between their study and ours may be due to differences in patient populations or sample size.

Examining Glx in other areas of the brain, Kegeles et al. reported elevated Glx levels in the medial prefrontal cortex in 16 unmedicated patients with schizophrenia, and de la Fuente-Sandoval et al. reported the same in the dorsal caudate of 18 antipsychotic-naïve patients. However, Tibbo et al. did not find alterations in the glutamate levels of unmedicated patients with first-episode psychosis.

Few studies reported hippocampal glutamate measurements in medicated patients. Van Elst et al. found higher glutamate levels in 21 medicated, acutely psychotic, hospitalized patients. Lutkenhoff et al. failed to identify such differences in 9 patients with schizophrenia or their unaffected co-twins, and Olbrich et al. did not detect a change in glutamate levels in 9 medicated, first-episode patients. Similarly, we did not find elevated Glx/Cr in 46 stable, medicated patients with schizophrenia.

Taken together, elevated levels of glutamate in patients with schizophrenia may be present in acute psychosis but can possibly be corrected if successfully treated with antipsychotics, thus underscoring the importance of continuity of treatment. Increases in hippocampal regional cerebral blood flow, hypothesized to be secondary to elevated synaptic glutamate levels, have been reported to be associated with psychosis, indirectly suggesting a link between abnormal glutamatergic neurotransmission and clinical presentation. Assuming elevated Glx/Cr to be excitotoxic, it is unclear why progressive hippocampal volume loss is not more pronounced over the course of the illness or to what extent antipsychotic medications may counteract the progression of volume loss. Our assumptions are rather speculative and need more definite, prospective, longitudinal structural and neurometabolite assessments over the course of antipsychotic treatment before conclusions can be drawn.

Some previous studies have identified decreased hippocampal NAA levels in patients with schizophrenia, but some have not. A recent meta-analysis showed significantly decreased levels of NAA. Our lack of detecting abnormalities in NAA may be due to lack of power.

Correlations between glutamate and NAA have consistently been reported in healthy individuals. In our previous study, Glx/Cr and NAA/Cr were correlated in healthy controls. In addition, we replicated our finding of a loss of correlation between Glx/Cr and NAA/Cr in the hippocampus of patients with schizophrenia, this time in unmedicated patients. Correlation coefficients between metabolites were similar in both our present study and our previous study. Correlation in medicated patients is important because it shows that it is not purely a phenomenon related to antipsychotic medications. It also confirms that it is not merely a reflection of illness severity because we found it in both stable and acutely ill patients. In an attempt to quantify the extent of loss of correlation, we calculated Glx/Cr:NAA/Cr to explore their potential as a biomarker. Group differences were more robust than with either neurometabolite alone, indicating Glx/Cr:NAA/Cr may be a more sensitive marker differentiating between groups than either Glx/Cr or NAA/Cr alone. In a similar approach, Savic et al. reported that, in comparison with NAA/Cr, Glx/NAA was superior in correctly identifying epileptic regions in neocortical epilepsy. Although Savic et al. did not explicitly explore correlations between Glx/Cr and NAA/Cr, their findings suggest that a loss of correlation between metabolites could

### Table. Data on Hippocampal Neurometabolites

<table>
<thead>
<tr>
<th>Neurometabolites</th>
<th>Healthy Controls (n = 27)</th>
<th>Patients With Schizophrenia (n = 27)</th>
<th>Difference, %</th>
<th>F Value*</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glx/Cr</td>
<td>Mean (SD) Mean CRLB (SD)</td>
<td>Mean (SD) Mean CRLB (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy Controls</td>
<td>0.60 (0.08) 0.09 (0.01)</td>
<td>0.66 (0.11) 0.11 (0.03)</td>
<td>10</td>
<td>5.643</td>
<td>.02</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.28 (0.10) 0.04 (0.01)</td>
<td>1.23 (0.10) 0.04 (0.01)</td>
<td>−4</td>
<td>1.573</td>
<td>.22</td>
</tr>
<tr>
<td>Cho/Cr</td>
<td>0.91 (0.08) 0.03 (0.01)</td>
<td>0.88 (0.11) 0.03 (0.01)</td>
<td>−3</td>
<td>1.171</td>
<td>.29</td>
</tr>
<tr>
<td>Glx/Cr:NAA/Cr</td>
<td>0.479 (0.06) 0.543 (0.11)</td>
<td>12</td>
<td>7.443</td>
<td>&lt;.01</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Cho, choline; Cr, creatine; CRLB, Cramér-Rao lower bound; Glx, glutamate and glutamine; NAA, N-acetylaspartate.

* Multivariate analysis of covariance, within-group factor metabolite level, between-group factor disease state, age, smoking status (packs per day), and voxel gray matter and white matter content as covariates.
be present in neocortical epilepsy, implying that it may not be specific for schizophrenia. Further studies will be helpful to clarify illness specificity.

Olney and Farber\textsuperscript{13} suggested that subtle alterations in hippocampal glutamatergic neurotransmission potentially account for histological abnormalities that cause observed structural deficits. We found a negative correlation between Glx/Cr and hippocampal VBM measure in patients with schizophrenia, suggesting that glutamatergic excitotoxicity may be associated with hippocampal volumetric deficits. Interestingly, though, VBM measures that negatively correlate with Glx/Cr were more extensive than VBM measures that were significantly decreased in patients with schizophrenia when compared with healthy controls. Few studies combined structural magnetic resonance imaging and $^1$H-MRS to explore connections between hippocampal volume and neurometabolites. With NAA being an assumed marker of neuronal integrity, a positive correlation between hippocampal volume and NAA levels would be expected. We did not find correlations between NAA/Cr and hippocampal VBM measure in patients with schizophrenia or healthy controls. Like us, most investigators have failed to identify a correlation between NAA and hippocampal volume, suggesting that volume deficits and NAA reductions are independent surrogate markers in schizophrenia.\textsuperscript{50-53}

Klär et al\textsuperscript{46} support this assumption by reporting negative correlations between hippocampal volume and NAA. They state that their finding is not congruent with the view of NAA as a marker of neuronal integrity, and they hypothesize that NAA may mediate the synthesis pathways of glutamatergic neurotransmission via N-acetylaspartate-glutamate.\textsuperscript{46} Contrary to our hypothesis, we did not find a correlation between Glx/Cr:NAA/Cr and the hippocampal VBM measure; Glx/Cr: NAA/Cr may measure subtle alterations in glutamatergic neurotransmission that do not lead to structural deficits in the hippocampus.

Our results must be interpreted in the context of several limitations. Association does not necessarily imply a causative relationship. Also, we planned to match participants on sex, age, and smoking status, factors that are reported to influence hippocampal neurometabolites.\textsuperscript{54,55} Matching on smoking status was incomplete; therefore, we used smoking status as a covariate in our analyses.

To investigate volumetric alterations, we chose a VBM approach using the DARTEL algorithm, which is more sensitive and more able to identify more localized morphologic alterations than conventional VBM.\textsuperscript{56} Although this method does not allow for the calculation of absolute volumes in native space, it was shown to be of equal sensitivity in detecting group differences in the hippocampus when compared with region-of-interest analyses with manual tracings for patients with schizophrenia, depression, or Alzheimer dementia.\textsuperscript{56-59} In addition, it has the advantage that it is not operator dependent and is less time intensive than manual tracings. Areas of volumetric deficits were identified by visual inspection, and given the spatial resolution, we did not narrow findings to CA subfields.

We acknowledge that this method is not ideal. However, we believe that it is informative nonetheless, especially given that the dentate gyrus has been implicated as a key hippocampal structure affected by altered glutamatergic neurotransmission in schizophrenia.\textsuperscript{1} To investigate neurometabolites, we used $^1$H-MRS. Test-retest reliability in healthy controls was comparable to previous reports,\textsuperscript{60} but we were unable to estimate measures in unmedicated patients with schizophrenia because of the need to medicate them as soon as possible.

**Figure 4. Partial Correlations Between Glx/Cr and NAA/Cr in the Left Hippocampus**

For each group, the solid line represents the linear regression fit across all participants, and the dashed lines represent the 95% confidence bands for the regression line. Data were controlled for age, smoking status (packs per day), and voxel gray and white matter content (healthy controls: $r = 0.39, P = .04$; patients with schizophrenia: $r = -0.19, P = .21$). Cr indicates creatine; Glx, glutamate and glutamine; and NAA, N-acetylaspartate.
We recognize that the remaining uncertainty about the retest reliability in our sample is a potential confounding factor, as is the higher full-width at half maximum in patients with schizophrenia. Furthermore, we calculated neurometabolites normalized to Cr; data acquisition parameters did not allow for the calculation of absolute metabolite levels. Few studies reported alterations in Cr levels in schizophrenia, which thus questions the validity of referencing neurometabolites to Cr.1,6,9,22

In a meta-analytic approach, we did not detect significant Cr abnormalities across studies in the hippocampus in patients with schizophrenia, although heterogeneity between studies was significant.48 Although we cannot rule out that Cr may be altered in this sample, we conclude that our findings more likely reflect Glx abnormalities than Cr abnormalities. Furthermore, the MRS voxel was not exclusively confined to the hippocampus; metabolites from adjacent structures included in the voxel may have affected the findings. Also, the Glx peak does not reflect synaptic glutamate alone but is a combination of neuronal, glial, and synaptic glutamate, glutamine, and γ-aminobutyric acid in the voxel. It is therefore not possible to equate Glx with glutamatergic neurotransmission.

A technical strength of our study that partially compensates for this is the use of an echo time of 80 milliseconds, which appears to optimize the glutamal signal by diminishing the macromolecule contribution and reducing the glutamine contribution, but elevations in the level of glutamine cannot be ruled out,60 especially since our phantom did not include glutamine.

Future studies explicitly investigating individual glutamate and glutamine peaks at high field strength are needed to clarify how much of the Glx abnormalities reported are contributable to glutamine abnormalities. In summary, our findings show that hippocampal volumetric deficits correlate with elevated Glx/Cr in unmedicated patients with schizophrenia, supporting the theory that an altered hippocampal glutamate level potentially accounts for the structural deficits in the hippocampus observed in neuroimaging studies.

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Study concept and design: White, Lahti.

Acquisition of data: All authors.

Analysis and interpretation of data: All authors.

Drafting of the manuscript: Kraguljac, White, Lahti.

Critical revision of the manuscript for important intellectual content: White, Reid.

Statistical analysis: Kraguljac, White, Reid.

Obtained funding: Lahti.

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Study supervision: White.

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Hippocampal Glutamate and Volumetric Deficits


