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Proton magnetic resonance spectroscopy of the substantia nigra in schizophrenia

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Abstract

Background—Converging evidence in schizophrenia points to disruption of the dopamine and glutamate neurotransmitter systems in the pathophysiology of the disorder. Dopamine is produced in the substantia nigra, but few neuroimaging studies have specifically targeted this structure. In fact, no studies of the substantia nigra in schizophrenia have used proton magnetic resonance spectroscopy (MRS). We sought to demonstrate the feasibility of acquiring single-voxel MRS measurements at 3T from the substantia nigra and to determine which metabolites could be reliably quantified in schizophrenia patients and healthy controls.

Methods—We used a turbo spin echo sequence with magnetization transfer contrast to visualize the substantia nigra and single-voxel proton MRS to quantify levels of *N*-acetylaspartate, glutamate and glutamine (Glx), and choline in the left substantia nigra of 35 people with schizophrenia and 22 healthy controls.

Results—We obtained spectra from the substantia nigra and quantified neurometabolites in both groups. We found no differences in levels of *N*-acetylaspartate/creatine, Glx/creatine, or choline/creatine between the groups. We found a significant correlation between Glx/creatine and overall cognitive performance, measured with the Repeatable Battery for the Assessment of

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CONTRIBUTORS

MAR helped create the model for the MRS processing, analyzed and interpreted the data, and prepared the manuscript. NVK was involved in processing and interpreting MRS data. KBA contributed to the MRS and MTC image acquisition parameters and the MRS data collection. DMW was involved in recruiting participants, collecting data, and interpreting data. JAH determined MRS and MTC image acquisition parameters and created the MRS model for processing. As PI, ACL designed the study and supervised participant recruitment, data collection, analysis, and interpretation.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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Neuropsychological Status (RBANS), in controls but not patients, a difference that was statistically significant.

Conclusions—Our study demonstrates the feasibility of obtaining single-voxel MRS data from the substantia nigra in schizophrenia. Such measurements may prove useful in understanding the biochemistry underlying cellular function in a region implicated in the pathophysiology of schizophrenia.

Keywords

schizophrenia; substantia nigra; magnetic resonance spectroscopy; glutamate; *N*-acetylaspartate; cognition

1. INTRODUCTION

Schizophrenia is a complex, debilitating disorder that remains poorly understood (Keshavan et al., 2008). Evidence shows disruption of dopaminergic neurotransmission in schizophrenia (Laruelle et al., 2003). These disruptions include increased amphetamine-induced dopamine release (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996) and elevated ^{18}F - and ^{11}C -DOPA, a precursor of dopamine, accumulation in the striatum (Dao-Castellana et al., 1997; Hietala et al., 1995; Lindstrom et al., 1999; McGowan et al., 2004; Meyer-Lindenberg et al., 2002; Reith et al., 1994). Furthermore, antipsychotic drugs are dopamine D2 receptor antagonists.

Evidence also points to disruption of glutamatergic neurotransmission (Lahti et al., 1995; Lahti et al., 2001). Glutamate interacts with dopamine in the cortex, striatum, and midbrain. The midbrain's substantia nigra (SN) receives glutamatergic afferents from other regions and is the primary site of dopamine synthesis. Yet, despite evidence of increased dopamine D2 receptors (Kessler et al., 2009) and postmortem abnormalities in the SN (Perez-Costas et al., 2010), we know little about the role of glutamate in SN pathophysiology in schizophrenia.

One promising method for investigating the SN is proton magnetic resonance spectroscopy (^1H -MRS). ^1H -MRS is a non-invasive imaging technique used to detect neurochemical insults that affect fundamental brain function and cognitive processes. Specifically, ^1H -MRS measures neurometabolites that have critical roles in cellular functions, including *N*-acetylaspartate (NAA), glutamate and glutamine, and choline. NAA is an amino acid found in neurons and is considered a marker of neuronal integrity (Moffett et al., 2007). Several schizophrenia studies reported reduced NAA (Steen et al., 2005), including trend-level reductions in the basal ganglia (Kraguljac et al., 2012a). Glutamate is the major excitatory neurotransmitter, and glutamine is synthesized from glutamate in astrocytes and broken down to glutamate in neurons. A recent MRS study of high-risk and antipsychotic-naïve first-episode patients found elevated glutamate in the dorsal striatum (de la Fuente-Sandoval et al., 2011), potentially leading to neurotoxicity (Lahti and Reid, 2011). Others reported elevated glutamate in the prefrontal cortex and thalamus of high-risk, antipsychotic-naïve, and unmedicated patients (Bartha et al., 1997; Kegeles et al., 2012; Theberge et al., 2002; Theberge et al., 2007; Tibbo et al., 2004). Choline is involved in inflammatory processes and membrane turnover (Ross and Sachdev, 2004). Some schizophrenia MRS studies, though not all, reported elevated choline in the basal ganglia (Ando et al., 2002; Bustillo et al., 2001; Bustillo et al., 2002; de la Fuente-Sandoval et al., 2011; Fujimoto et al., 1996; Shioiri et al., 1996).

While some schizophrenia MRS studies indicate abnormal basal ganglia metabolites, none of them specifically examined the SN, possibly because of several technical difficulties. The

location of the SN within the midbrain and its relatively small size make image acquisition challenging. Standard magnetic resonance imaging techniques, such as T1- and T2-weighting, poorly delineate the SN, making it difficult to accurately position the MRS voxel of interest. We identified a sequence with magnetization transfer contrast (MTC) that more accurately delineates the SN. We demonstrated through in vivo imaging and histology of non-human primates that MTC accurately localizes the SN (Bolding et al., 2013).

In the present study, we sought to demonstrate feasibility of acquiring ^1H -MRS measurements at 3T from the SN and to determine which metabolites were quantifiable in healthy controls and patients with schizophrenia. We used MTC images to visualize the SN and facilitate positioning of the MRS voxel. Since we were interested in quantifying glutamate and glutamine (Glx), we optimized MRS acquisition for detecting Glx (Schubert et al., 2004).

2. METHODS AND MATERIALS

2.1 Participants

Thirty-five stable medicated patients with schizophrenia and schizoaffective disorder (SZ) and 22 healthy controls (HC) participated in this study (Table 1). SZ were recruited from the psychiatry clinics at the University of Alabama at Birmingham and HC through advertisement in the university's newspaper. Exclusion criteria were major medical conditions, substance abuse within 6 months of imaging, neurologic disorders, previous serious head injury with a loss of consciousness for more than 2 minutes, and pregnancy. General cognitive function was characterized by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Symptom severity was assessed using the Brief Psychiatric Rating Scale (BPRS). Diagnoses were established through review of medical records and the Diagnostic Interview for Genetic Studies. All participants gave written informed consent. Before signing consent, SZ were evaluated for their ability to provide consent by completing a questionnaire probing their understanding of the study. The Institutional Review Board of the University of Alabama at Birmingham approved this study.

2.2 MR Imaging

Imaging was performed on a 3T head-only scanner (Siemens Allegra, Erlangen, Germany) with a circularly polarized transmit/receive head coil. To visualize the SN, images were acquired using a turbo spin echo sequence with magnetization transfer contrast (MTC; TR/TE=2900/11 msec, flip angle 150°, 2 averages, 256×256 matrix, 256×256 mm field of view, 4 mm slice thickness).

The MRS voxel (13×13×13 mm) was positioned around the left SN, which was identified as a hyperintense region on the MTC images (Figure 1A). Voxel size was chosen to encompass the SN, which extends rostrocaudally 12±2 mm with a volume of 759±36 mm³ (Hardman et al., 2002; Öz et al., 2006). Following manual shimming, water-suppressed spectra were collected with the point-resolved spectroscopy sequence [PRESS; TR/TE=2000/80 msec (Schubert et al., 2004), 1200 Hz spectral bandwidth, 1024 points, 640 averages, 21 min 20 sec scanning time].

2.3 MRS Processing

MRS data were processed in jMRUI (version 3.0) (Naressi et al., 2001) as described previously (Reid et al., 2010). The residual water peak was removed using the Hankel-Lanczos singular values decomposition filter (Pijnappel et al., 1992). Spectra were quantified in the time domain by the AMARES algorithm (advanced method for accurate,

robust, and efficient spectral fitting) (Vanhamme et al., 1997). The model consisted of peaks for NAA, choline (Cho), creatine (Cr), and three peaks for glutamate and glutamine (Glx). Prior knowledge for Glx was derived from a phantom solution of glutamate. If the algorithm failed to fit a peak (e.g., Glx), it was removed from the model, and the remaining peaks were quantified. NAA, Glx, and Cho were quantified with respect to Cr. Cramer-Rao lower bounds (CRLB) were used as a measure of uncertainty of the fitting procedure. Ratios with CRLB greater than 30% were excluded from further analyses. Signal-to-noise ratio (SNR) was calculated as the NAA signal divided by the standard deviation of the residual signal (the difference between the original and fitted spectrum). To assess reproducibility, 1 HC was scanned on 5 days.

Some Glx/Cr data were excluded from analysis due to CRLB greater than 30% (9 SZ and 6 HC) and failure to fit the Glx peak (2 SZ and 1 HC). One SZ was identified as an extreme outlier by boxplot in SPSS. Therefore, analyses involving Glx included 23 SZ and 15 HC, while analyses of NAA and Cho included the full group of 35 SZ and 22 HC.

2.4 Statistical Analyses

Statistical analyses were performed in SPSS (version 12). Demographics and RBANS were compared using *t*-tests and χ^2 -tests. MRS ratios were compared using ANCOVA, covarying for age and smoking status (Ende et al., 2000; Gallinat et al., 2007; Haga et al., 2009; Marsman et al., 2013). In exploratory analyses, Pearson's correlation coefficients were used to evaluate the relationship between metabolites (Glx/Cr, NAA/Cr) and clinical measures (RBANS, BPRS). Post-hoc group comparisons of NAA/Cho, Glx/Cho, and Glx/NAA were also performed using ANCOVA. Statistical significance was $p < 0.05$.

3. RESULTS

3.1 Participants

Age, sex, and parental occupation were not significantly different between the groups. SZ had significantly more smokers than HC. SZ scored significantly lower on all domains of the RBANS (Table 1).

3.2 ¹H-MRS

Figure 1B is a sample SN spectrum and fit from jMRUI. For the 1 HC scanned across 5 days, the coefficients of variation for NAA/Cr, Glx/Cr, and Cho/Cr were 10%, 18%, and 10%, respectively. Metabolite ratios were not significantly different between the groups, although the Glx/NAA difference was near significant (Table 2). The relative differences for NAA/Cr, Glx/Cr, and Glx/NAA were 2.1%, 14.0%, and 19.4%, respectively (Figure 2). CRLBs were not significantly different between the groups (all $p > .88$). Creatine, which was used as a reference, was not significantly different between the groups ($p = 0.87$).

Glx/Cr was correlated with the RBANS total score in HC [$r(13) = 0.55$, $p = 0.03$] but not in SZ [$r(20) = -0.10$, $p = 0.66$]. The difference in correlation coefficients was significant [$Z = 1.95$, one-tailed $p = 0.03$] (Figure 3). NAA/Cr positively correlated with the BPRS negative subscale [$r(33) = 0.37$, $p = 0.03$] but did not withstand correction for multiple comparisons ($p = 0.05/6 = 0.008$, 6 comparisons: Glx/Cr and NAA/Cr correlated with BPRS total, positive, and negative).

4. DISCUSSION

In this study, we sought to obtain ¹H-MRS measurements from the SN in schizophrenia patients and healthy controls to demonstrate feasibility of using MRS to explore the pathophysiology of schizophrenia. Only two previous studies, both in Parkinson's disease,

have used MRS to explore metabolites in the SN (O'Neill et al., 2002; Öz et al., 2006). To our knowledge, no published MRS studies of schizophrenia report measurements from the SN. We acquired spectra from the SN and quantified metabolite ratios in schizophrenia patients and controls. We found that Glx/Cr positively correlated with the RBANS total score, a global measure of cognitive function, in the controls but not schizophrenia patients. The difference in these correlations was statistically significant.

The paucity of MRS studies of the SN is likely due to its small size and sensitive location as a deep subcortical structure in the midbrain. O'Neill et al. (2002) used proton-density and T2-weighted images to position a bilateral voxel in the SN region and acquired data using a STEAM sequence at 1.5T. Öz et al. (2006) also used T2-weighted images to position their voxel but acquired data from the unilateral SN with a short-echo STEAM sequence at 4T. Like Öz et al., we chose a unilateral SN voxel to increase anatomical precision. Importantly, we believe we better visualized the SN by using the MTC images, which we showed to more accurately delineate the SN (Bolding et al., 2013). This improved delineation ensured that we obtained MRS measurements from a region encompassing the SN. However, partial volume effects are a common problem in MRS studies. While our voxel was approximately 35% SN by volume (Hardman et al., 2002) and contained portions of the red nucleus and cerebral peduncles, this situation was unavoidable due to the SN's small size and unique shape.

We used a PRESS sequence with 80 msec echo time to optimize detection of glutamate and reduce contamination from macromolecules and NAA (Schubert et al., 2004). With this sequence we already obtained reliable measurements from the anterior cingulate cortex and hippocampus in large groups of patients and controls (Kraguljac et al., 2012b). In this study, our SN spectral quality was comparable to Öz et al. (2006) and O'Neill et al. (2002) based on linewidths, SNRs, and CRLBs. Unlike them, however, our spectra had little contribution from background signals and a better-resolved Glx peak because of our acquisition parameters. Further, our patients demonstrated spectral quality similar to our controls: the CRLBs for NAA and choline were 12% and 8%, respectively, whereas CRLB was 25% for Glx. Öz et al. (2006) used a cut-off CRLB of 50% to define reliable data with a median CRLB < 30% for glutamate, while O'Neill et al. (2002) excluded data with unrecognizable metabolite peaks and spectral linewidths less than 2 Hz or greater than 10 Hz. Based on these studies and the difficulty in acquiring MRS from the midbrain, we defined an exclusion criterion of CRLB < 30% as acceptable for our feasibility study. The CRLBs for NAA and choline were within the commonly accepted range of less than 20%. Since they were lower than the group standard deviations, we believe overall variation could result from interindividual physiological differences rather than estimation uncertainty in the fitting procedure.

We observed that the RBANS total score, a global measure of cognitive function, positively correlated with Glx/Cr in controls but not patients. The difference between these correlations was statistically significant. In view of the key role played by glutamatergic transmission in learning and memory function (Bliss and Collingridge, 1993), the relationship seen in healthy controls has face validity. For example, studies pharmacologically manipulating the glutamatergic system in healthy people have shown impaired cognitive performance (Krystal et al., 1994; Parwani et al., 2005) and altered functional imaging responses (van Wagoningen et al., 2010), highlighting the key role of glutamate in normal cognitive processes. Further supporting these findings, a recent study at 7T identified a similar correlation between cognitive function and glutamate in the posterior cingulate of Huntington's Disease (HD) mutation carriers (Unschuld et al., 2012), which was found despite decreased glutamate. Speculatively, the increased Glx/Cr seen in our patients could have led to impaired cognition, possibly through an excitotoxic mechanism (Olney et al.,

1999). Our finding contrasts with a report showing a positive correlation between cognition and Glx in parietal gray matter in schizophrenia patients but not controls (Bustillo et al., 2011). This inconsistency could be driven by differences in regions of interest, populations, or spectroscopy acquisition and analysis techniques. Clearly the field needs more work in this area, as understanding the pathophysiology of cognitive dysfunction is of critical importance in schizophrenia.

We did not find differences in Glx/Cr between patients and controls. One possibility is that no true difference exists. Another possibility is a small effect size, which could result from sampling a smaller voxel in a region farther removed from the head coil, thus lowering the signal-to-noise ratio. Since power could have been reduced by using a CRLB cut-off of 30%, we did a post-hoc calculation with a cut-off of 25% and found similar results [SZ: $n=14$, HC: $n=8$; $F(1,18)=1.94$, $p=0.18$; 12% relative increase].

Similarly, we did not find a difference in NAA/Cr between the groups. Our recent meta-analysis found only a trend towards reduced NAA in the basal ganglia (Kraguljac et al., 2012a), with most studies reporting negative findings (Bertolino et al., 1996; Block et al., 2000; Bustillo et al., 2008; Callicott et al., 2000; Ende et al., 2003; Fannon et al., 2003; Heimberg et al., 1998; Tayoshi et al., 2009; Yamasue et al., 2003). Overall decrease in NAA in schizophrenia was estimated to be about 5% (Steen et al., 2005), so our sample sizes may have been too low to detect changes in this range. Alternatively, given the number of negative findings in the basal ganglia, no NAA abnormality may be present in this midbrain region. Therefore, our preliminary study needs replication with larger samples. We can potentially reduce critical confounds of illness chronicity and treatment by enrolling newly diagnosed and medication-free patients.

Choline is an indicator of cellular membrane or myelin breakdown and cellular turnover (Bracken et al., 2011; Govindaraju et al., 2000; Ross and Sachdev, 2004). Findings in the basal ganglia in schizophrenia have been mixed, with some studies showing increases (Ando et al., 2002; Bustillo et al., 2001; Bustillo et al., 2002; de la Fuente-Sandoval et al., 2011; Fujimoto et al., 1996; Shioiri et al., 1996) and others showing no difference (Bertolino et al., 1996; Block et al., 2000; Heimberg et al., 1998; Yamasue et al., 2003). Our results indicated no abnormalities in Cho/Cr, which is consistent with meta-analytic findings in the basal ganglia (Kraguljac et al., 2012a). The reason for these different findings is unclear. A focus on choline in future studies would be helpful, especially since choline is sometimes used as an internal reference when quantifying metabolites.

We acknowledge several limitations in our study. We excluded many participants from Glx analyses because of poor signal quality. Since glutamate is of significant interest, we are working to improve its detection by acquiring data at higher field strength (7T) to achieve better spectral resolution. We quantified metabolite ratios using creatine as an internal reference because we did not collect unsuppressed water spectra or scan an external phantom. Since creatine abnormalities may be present in schizophrenia (Öngür et al., 2009), our study should be repeated using absolute quantitation. We did not segment the tissue within the MRS voxel because of the limitations of our acquisition protocol. Future studies would benefit from acquiring 3D images with magnetization transfer contrast for the purpose of segmentation (Helms et al., 2009). We used numerous averages to increase the signal-to-noise ratio, leading to a long scan time. Our participants tolerated the long scan, but it may not be ideal for all clinical populations. We included both schizophrenia and schizoaffective patients in this study, which may have introduced a confounding factor. In addition, our patients were medicated. Several studies reported elevated glutamate, glutamine, or Glx in antipsychotic-naïve, minimally treated, first-episode, and unmedicated patients (Bustillo et al., 2010; de la Fuente-Sandoval et al., 2011; Kegeles et al., 2012;

Theberge et al., 2002), while studies of chronic and medicated patients found the same or reduced levels (Lutkenhoff et al., 2010; Reid et al., 2010; Theberge et al., 2003; Wood et al., 2007). Furthermore, Egerton et al. (2012) recently reported elevated glutamate levels in the anterior cingulate cortex of symptomatic compared to stable first-episode patients. In light of their finding, we grouped our patients into symptomatic ($n=16$) and stable ($n=19$) using similar criteria (Andreasen et al., 2005). Post-hoc subgroup comparisons showed no significant differences between the groups (data not shown). Nevertheless, diagnosis, illness stage, and clinical status are important factors to consider in future studies as they may account for some variability in findings. Additional longitudinal studies will also be critical for determining the effects of antipsychotic medications on MRS measurements.

In summary, we conducted a preliminary study to acquire MR spectroscopy from the SN of schizophrenia patients. Our spectral quality was comparable to previously published studies of Parkinson's disease. We showed that Glx/Cr correlated with cognition in controls but not schizophrenia patients. Since this study is the first of its kind in schizophrenia, future studies will be needed to replicate our findings. If technical difficulties can be overcome in the SN, the MRS technique will be an important tool for future studies of psychiatric disorders, including neurometabolite abnormalities underlying cognitive dysfunction in schizophrenia.

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Figure 1.

(A) MRS voxel position in the left substantia nigra as viewed on a magnetization transfer contrast (MTC) axial image. Inset shows the midbrain without the MRS voxel. (B) Sample spectrum (black) from the left substantia nigra with jMRUI AMARES fitting (red). Exponential line broadening of 7 Hz was used for display purposes only.

Figure 2.

Metabolite levels in the left substantia nigra in patients with schizophrenia and healthy controls. Horizontal lines indicate group means. (A) *N*-acetylaspartate/creatine (NAA/Cr) (schizophrenia: $n = 35$; control: $n = 22$). (B) Glutamate + glutamine/creatine (Glx/Cr) (schizophrenia: $n = 23$; control: $n = 15$). (C) glutamate + glutamine/*N*-acetylaspartate (Glx/NAA) (schizophrenia: $n = 23$; control: $n = 15$).

Figure 3. Correlation between total score on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and glutamate + glutamine/creatine (Glx/Cr). (A) Control ($r = 0.55$, $p = 0.03$). (B) Schizophrenia ($r = -0.10$, $p = 0.66$).

Table 1Demographics ^{a, b}

Measure	SZ (n = 35)	HC (n = 22)	t/ χ^2	p-value
Age, years	37.9 (12.0)	37.9 (12.4)	0.02	0.99
Sex, M/F	26/9	13/9	1.44	0.23
Parental occupation ^c	7.6 (5.1)	7.2 (4.8)	0.29	0.77
Smoker/Non-smoker ^d	24/10	9/13	4.86	0.03
RBANS ^e				
Total index	74.1 (11.4)	94.1 (11.4)	6.32	< 0.001
Immediate memory	78.7 (14.7)	96.9 (10.8)	4.96	< 0.001
Visuospatial	77.2 (17.6)	93.3 (16.3)	3.38	0.001
Language	87.3 (11.3)	97.3 (15.0)	2.78	0.007
Attention	84.0 (15.4)	98.5 (15.8)	3.34	0.002
Delayed memory	71.6 (19.3)	93.9 (12.5)	4.77	< 0.001
BPRS				
Total	30.4 (8.4)	—	—	—
Positive ^f	5.7 (3.7)	—	—	—
Negative ^g	4.6 (2.2)	—	—	—
Illness duration, years	17.2 (11.2)	—	—	—
Diagnosis				
Schizophrenia/Schizoaffective	25/10	—	—	—
Medication, 1 st /2 nd generation	2/33	—	—	—
Aripiprazole	1	—	—	—
Clozapine	1	—	—	—
Clozapine & ziprasidone	1	—	—	—
Olanzapine	3	—	—	—
Olanzapine & paliperidone	1	—	—	—
Olanzapine & ziprasidone	1	—	—	—
Paliperidone	1	—	—	—
Quetiapine	1	—	—	—
Risperidone	21	—	—	—
Risperidone & prolixin	1	—	—	—
Risperidone & quetiapine	1	—	—	—
Prolixin	1	—	—	—
Trifluoperazine	1	—	—	—

^a Abbreviations: BPRS, Brief Psychiatric Rating Scale; HC, healthy control; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; SZ, schizophrenia

^b Mean (SD) unless indicated otherwise.

^c Parental occupation determined from Diagnostic Interview for Genetic Studies (1–18 scale). Lower numerical value corresponds to higher socioeconomic status. Information not available for 4 SZ and 3 HC.

^dSmoking status not available for 1 SZ.

^eRBANS not available for 3 SZ.

^fBPRS Positive: conceptual disorganization, hallucinatory behavior, and unusual thought content

^gBPRS Negative: emotional withdrawal, motor retardation, and blunted affect

Table 2

Comparison of metabolite ratios in the left substantia nigra using ANCOVA with age and smoking status as covariates ^{a, b}

Metabolite	SZ (n = 35) Mean (SD)	HC (n = 22) Mean (SD)	F-statistic	p-value
NAA/Cr	1.87 (0.36)	1.91 (0.31)	$F(1,52) = 0.89$	0.35
CRLB, %	11.6 (4.6)	11.8 (4.4)		
Glx/Cr ^c	0.65 (0.20)	0.57 (0.24)	$F(1,33) = 1.84$	0.19
CRLB, %	24.4 (2.9)	24.6 (3.3)		
Cho/Cr	1.05 (0.25)	1.05 (0.25)	$F(1,52) = 0.21$	0.65
CRLB, %	7.9 (2.9)	8.0 (3.1)		
NAA/Cho	1.81 (0.33)	1.86 (0.29)	$F(1,52) = 0.11$	0.74
Glx/Cho ^c	0.66 (0.28)	0.60 (0.27)	$F(1,33) = 1.73$	0.20
Glx/NAA ^c	0.37 (0.14)	0.31 (0.12)	$F(1,33) = 4.034$	0.05
Linewidth, Hz	9.86 (1.50)	10.11 (1.75)	$F(1,55) = 0.31$	0.58
SNR	7.84 (1.21)	8.10 (1.23)	$F(1,55) = 0.64$	0.43

^a Abbreviations: Cho, choline; Cr, creatine; CRLB, Cramer-Rao lower bounds; Glx, glutamate and glutamine; HC, healthy control; Hz, hertz; NAA, N-acetylaspartate; SNR, signal-to-noise ratio; SZ, schizophrenia

^b Smoking status not available for 1 SZ.

^c Analyses involving Glx include 23 SZ and 15 HC. See Methods for reasons for exclusion.