Archival Report

Biological Psychiatry

7T Proton Magnetic Resonance Spectroscopy of Gamma-Aminobutyric Acid, Glutamate, and Glutamine Reveals Altered Concentrations in Patients With Schizophrenia and Healthy Siblings

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ABSTRACT

BACKGROUND: The *N*-methyl-D-aspartate receptor hypofunction model of schizophrenia predicts dysfunction in both glutamatergic and gamma-aminobutyric acidergic (GABAergic) transmission. We addressed this hypothesis by measuring GABA, glutamate, glutamine, and the sum of glutamine plus glutamate concentrations in vivo in patients with schizophrenia using proton magnetic resonance spectroscopy at 7T, which allows separation of metabolites that would otherwise overlap at lower field strengths. In addition, we investigated whether altered levels of GABA, glutamate, glutamine, and the sum of glutamine plus glutamate reflect genetic vulnerability to schizophrenia by including healthy first-degree relatives.

METHODS: Proton magnetic resonance spectroscopy at 7T was performed in 21 patients with chronic schizophrenia who were taking medication, 23 healthy first-degree relatives of patients with schizophrenia, and 24 healthy nonrelatives. Glutamate, glutamine, and GABA were measured cortically and subcortically in bilateral basal ganglia and occipital cortex.

RESULTS: Patients with schizophrenia had reduced cortical GABA compared with healthy relatives and the combined sample of healthy relatives and healthy nonrelatives, suggesting that altered GABAergic systems in schizophrenia are associated with either disease state or medication effects. Reduced cortical glutamine relative to healthy control subjects was observed in patients with schizophrenia and the combined sample of healthy relatives and patients with schizophrenia, suggesting that altered glutamatergic metabolite levels are associated with illness liability. No group differences were found in the basal ganglia.

CONCLUSIONS: Taken together, these findings are consistent with alterations in GABAergic and glutamatergic systems in patients with schizophrenia and provide novel insights into these systems in healthy relatives.

Keywords: First-degree relatives, GABA, Glutamate, Glutamine, Magnetic resonance spectroscopy, Schizophrenia

http://dx.doi.org/10.1016/j.biopsych.2016.04.007

N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels that mediate excitatory postsynaptic potentials. Hypofunction of NMDARs is argued to play a key role in the pathogenesis of schizophrenia (1,2). This hypothesis was formulated based on the psychotomimetic effects of NMDAR antagonists (3–6) and has garnered support from drug (7), postmortem (8), and genetic (9) studies. In awake animals, NMDAR blockade leads to an increase in glutamate release (10) and pyramidal neuron spiking (11), potentially via NMDAR hypofunction on fast-spiking gamma-aminobutyric acidergic (GABAergic) interneurons that regulate pyramidal neuron activity (12). NMDAR antagonists preferentially reduce the firing rate of these interneurons, leading to disinhibition of pyramidal neurons (13,14).

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a noninvasive method for measuring metabolite concentrations in living tissue, and GABA, glutamate (Glu), and glutamine (Gln) concentrations can be quantified. Gln is an amino acid synthesized from Glu taken up by astrocytes in the presynaptic terminal. Because Glu and Gln peaks partially overlap, particularly at lower field strengths, the sum of Glu and Gln (Glx) is often reported as a measure of glutamatergic metabolite concentrations. Using MRS, NMDAR antagonists have been shown to increase prefrontal Glu (15) and Gln (16) in humans and to increase the ratio of Gln to Glu (17,18) and decrease GABA (18) in rodents.

Findings from previous ^{1}H-MRS studies in schizophrenia investigating glutamatergic metabolites are mixed, and results vary across illness stage and brain region (19–22). In high-risk patients and antipsychotic-naïve patients with a first episode of schizophrenia, increased Gln in the frontal cortex and

ISSN: 0006-3223

thalamus (23–26), reduced Glu in the thalamus (26–29), and increased striatal Glu and Glx have been reported (30–33). In patients with chronic schizophrenia who are taking medication, glutamatergic metabolites are typically equivalent (34–45) or reduced (46–49) [however, see Chang *et al.* (50)]. In the largest study to date, however, increased Gln and no difference in Glu in frontal cortex in patients with chronic schizophrenia who are taking medication were reported (51). To our knowledge, only two studies have investigated glutamatergic metabolites in unaffected adult siblings of patients with schizophrenia. In one study, no Glu or Glx differences were observed in siblings (52). In the other study, reduced Glu was observed across brain regions in healthy co-twins (48).

Fewer studies have measured GABA in patients with schizophrenia (53), largely because concentrations are small, and the peak overlaps with more dominant metabolites. Using editing techniques, reduced GABA has been observed in patients with chronic schizophrenia who are taking medication (33–35,47,54,55), consistent with NMDAR hypofunction, although increased GABA has been observed in unmedicated patients (56). To our knowledge, no ^{1}H-MRS studies have investigated GABA in healthy relatives of patients with schizophrenia.

Despite general patterns emerging in the levels of glutamatergic and GABAergic metabolites in schizophrenia, there is an enormous amount of variability in findings across studies even after considering clinical factors. Differences in methodology are likely culprits. In particular, at higher magnetic field strengths, better separation between individual metabolites can be achieved, and concentration of these metabolites can be more reliably quantified.

Our aims in the current study were twofold. First, we investigated group differences in Glu, Gln, and GABA at an ultra-high magnetic field strength of 7T. Additionally, we investigated the ratio of Gln to Glu, as this has been found to be increased in patients with schizophrenia (20,51), and the ratio of GABA to Glx (57). Our second aim was to investigate the degree to which potentially altered concentrations of GABA, Glu, and Gln reflect genetic vulnerability to schizophrenia by measuring these metabolites in unaffected siblings of patients with schizophrenia and comparing them with both patients with schizophrenia and healthy nonrelatives.

Data were acquired in the occipital cortex and the basal ganglia. Although the occipital cortex is not traditionally implicated in the pathogenesis of schizophrenia, we measured in the occipital cortex because alterations in early visual processing in patients with schizophrenia (58–62) and healthy first-degree relatives (63) support functional alterations in this region. Moreover, most of the MRS literature from healthy subjects reports on the occipital cortex because of the high signal-to-noise ratio and spectral resolution that can be achieved here. Furthermore, we chose to measure in the basal ganglia given its reported dysfunction in schizophrenia, specifically, altered striatal dopamine transmission (64). Based on evidence for glutamatergic and GABAergic dysfunction in schizophrenia from animal models, postmortem work, and prior ^{1}H-MRS studies, we expected increased Gln and reduced GABA in patients with schizophrenia. We further expected reduced Glu based on previous studies in patients with chronic schizophrenia.

METHODS AND MATERIALS

Participants

Procedures were approved by the Medical Ethical Committee of the University Medical Center Utrecht. All subjects gave written informed consent and were compensated for participation.

The study was completed by 68 participants. There were 21 patients with schizophrenia or schizoaffective disorder (SZP group) recruited from the Genetic Risk and Outcome in Psychosis study (65) and from treatment-seeking patients at a hospital in The Netherlands. Also from the Genetic Risk and Outcome in Psychosis study, 23 healthy siblings of patients with schizophrenia (REL group) were recruited. Through community advertisements, 24 healthy nonrelatives as healthy control subjects (HC group) were recruited. Diagnoses of subjects in the SZP and REL groups were based on DSM-IV criteria and determined by clinicians using the Comprehensive Assessment of Symptoms and History interview (66) or Schedules for Clinical Assessment for Neuropsychiatry version 2.1 (67). All patients were taking antipsychotic medication, and five participants were taking benzodiazepines or mood stabilizers. Chlorpromazine-equivalent antipsychotic dosages were calculated for each patient (68). Subjects in the REL and HC groups were excluded if they had any current DSM-IV-TR Axis I disorder. Subjects in the HC group having a first-degree relative with a DSM-IV-TR Axis I disorder were also excluded. Exclusion criteria for all subjects were history of significant head trauma, history of neurologic illness, or substance abuse or dependence within 6 months before the study. Subjects 18–55 years old were included.

Clinical symptoms were assessed in patients only using the Positive and Negative Syndrome Scale (69). Social and occupational functioning was assessed using the Social Functioning Scale (70). The Dutch equivalent of the National Adult Reading Test (71) was used to estimate premorbid IQ. The Edinburgh Handedness Scale was used to measure handedness (72). Demographic data are presented in Table 1. Groups were matched on sex, handedness, IQ, and smoking status. Subjects in the SZP group were significantly older than subjects in the REL group, so age was taken into account as a covariate in between-group analyses.

Magnetic Resonance Studies

For each participant, one scan session was performed in a 7T whole-body magnetic resonance (MR) scanner (Philips Medical Systems, Cleveland, OH) to acquire ^{1}H-MRS and anatomic images. A second session was performed in a 3T scanner to acquire a high-resolution T1-weighted anatomic image that was later used for segmenting the ^{1}H-MRS voxel into different tissue types. The 3T images generally have more homogeneous image contrast, allowing for superior tissue classification.

Acquisition. In the 7T MR scanner, a birdcage transmit head coil (Nova Medical, Inc., Wilmington, MA) with two independent transmit channels (dual transmit) was used in combination with a 32-channel receive coil (Nova Medical, Inc.). A T1-weighted magnetization prepared rapid acquisition

Table 1. Demographic Data

	HC (n = 24)	REL (n = 23)	SZP (n = 21)	HC vs. REL		HC vs. SZP		REL vs. SZP	
	Mean (SD)	Mean (SD)	Mean (SD)	Statistic	p	Statistic	p	Statistic	p
Age (Years)	33.9 (9.3)	31.2 (5.4)	36.4 (7.3)	t = 1.2	.23	t = 1.0	.32	t = 2.7	.01
Sex (Female/Male)	8/16	8/15	6/15	χ^2 = 0.01	.92	χ^2 = 0.12	.73	χ^2 = 0.20	.66
IQ[a]	100.6 (13.0)	101.2 (14.0)	95.9 (12.7)	t = 0.2	.88	t = 1.2	.24	t = 1.3	.19
Education[b]	7.4 (1.1)	6.4 (1.7)	4.7 (1.8)	t = 2.4	.02	t = 6.1	< .001	t = 3.2	.003
Handedness[c]	0.76 (0.57)	0.87 (0.29)	0.94 (0.19)	t = 0.8	.42	t = 1.4	.18	t = 1.0	.35
Social Functioning	125.8 (6.3)	125.4 (5.3)	113.6 (7.0)	t = 0.2	.82	t = 5.8	< .001	t = 6.3	< .001
Smoking (%)	14.3	30.4	33	χ^2 = 1.6	.2	χ^2 = 1.9	.16	χ^2 = 0.04	.84
Illness Duration (Years)			14.0 (4.2)						
PANSS Positive			11.3 (4.5)						
PANSS Negative			12.7 (6.0)						
PANSS General			25.1 (7.5)						
CPZ Equivalent (mg)			281.4 (241.6)						
Benzodiazepines (No/Yes)			16/5						

CPZ, chlorpromazine; HC, healthy control subjects; PANSS, Positive and Negative Syndrome Scale; REL, healthy relatives; SZP, patients with schizophrenia.

[a]Based on the Nederlandse Leestest voor Volwassenen.

[b]Education category: 0 = <6 years of primary education; 1 = finished 6 years of primary education; 2 = 6 years of primary education and low-level secondary education; 3 = 4 years of low-level secondary education; 4 = 4 years of average-level secondary education; 5 = 5 years of average-level secondary education; 6 = 4 years of secondary vocational training; 7 = 4 years of high-level professional education; 8 = university degree.

[c]Based on the Edinburgh Handedness Inventory; scores range from 0 indicating complete left-handedness to 1 indicating complete right-handedness.

gradient-echo sequence was acquired for voxel placement (174 slices, echo time [TE] = 1.8 ms, repetition time [TR] = 4 ms, flip angle = 7°, field of view = 246 × 246 × 174 mm). A semilocalized by adiabatic selective refocusing sequence (TE = 36 ms, TR = 5000 ms, 32 averages, maximum B1 = 17 μT) (73) was used to measure Glu, Gln, and other major brain metabolites. J-difference spectral editing is necessary to distinguish GABA from other metabolites. GABA-edited ^{1}H-MRS spectra were obtained using a Mescher-Garwood semi-localized by adiabatic selective refocusing sequence (TE = 74 ms, TR = 5000 ms, 64 averages) (74). To eliminate macromolecular contamination in the edited spectrum, refocusing pulses were applied symmetrically around the 1.7-ppm macromolecular resonance. Before MRS acquisition, radio-frequency shimming on the region of interest was used to optimize phase settings of the individual transmit channels. This way a local B1 of 17 μT was reached, minimizing the chemical shift displacement artifacts in the measurement voxel. Second-order B0 shimming was automatically performed before MRS acquisition.

Conventional and GABA-edited spectra were obtained from three voxels (40 × 24 × 25 mm) (Figure 1). Two were placed in left and right basal ganglia and were prescribed to include as much striatum as possible, while avoiding lateral ventricles. The third voxel was positioned in bilateral occipital cortex, centered on the calcarine sulcus. In the 3T Philips Achieva MR

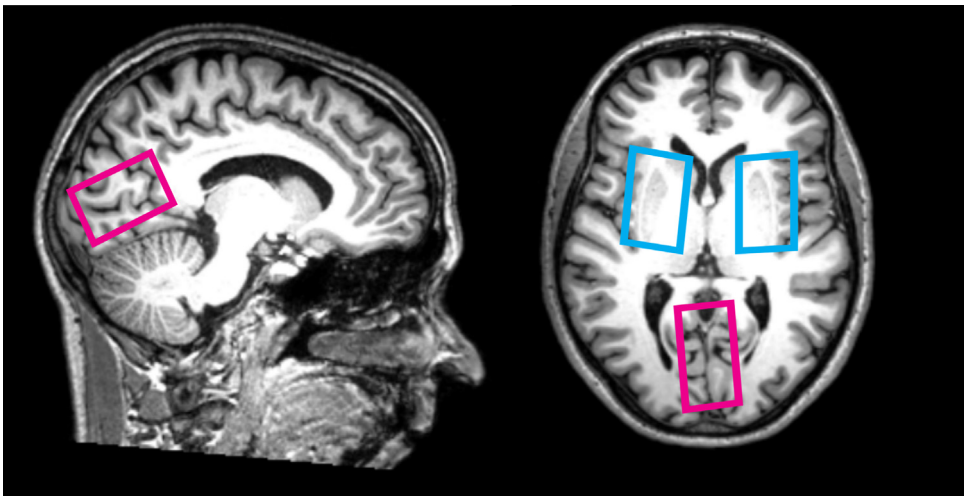

Figure 1. Voxel placement on a T1-weighted anatomic scan of a representative participant. The occipital cortex voxel (magenta) was centered in the left-right direction to include the left and right hemispheres and was positioned symmetrically around the calcarine sulcus. The basal ganglia voxels (cyan) were positioned to include as much striatum as possible, while avoiding the lateral ventricles.

scanner (Philips Medical Systems, Best, The Netherlands), a whole-brain three-dimensional fast field echo T1-weighted scan (200 slices, TR = 10 ms, TE = 4.6 ms, flip angle = 8°, field of view = 240 × 240 × 160 mm, voxel size = 0.75 × 0.8 × 0.75 mm) was acquired using an eight-channel head coil.

MRS Data Analysis. In all MRS data, the 32 receiver coils were combined after amplitude weighting and phasing based on the water reference signal and noise decorrelation based on a noise scan. The water reference was also used for eddy current correction and as an internal standard for quantification. Conventional MR spectra were quantified using an LCModel-based software implemented in MATLAB NMR Wizard (MATLAB; The MathWorks, Inc., Natick, MA) (75), which relies on a priori knowledge of the spectral components of metabolites to fit metabolite profiles. A measured macromolecular profile and the following simulated metabolite profiles were fitted to each spectrum: taurine, *myo*-inositol, glutathione, Gln, Glu, GABA, *N*-acetylaspartylglutamate, *N*-acetylaspartate, phosphocreatine, creatine, phosphoethanolamine, glycerophosphocholine, phosphocholine, choline, aspartate, and acetate. The macromolecular baseline was acquired in the prefrontal cortex and averaged across four healthy individuals who did not participate in the present study, using the same semilocalized by adiabatic selective refocusing sequence with inversion recovery (76). The baseline of the fit was adjusted to incorporate possible lipid and water artifacts. Fitting of GABA-edited spectra was performed by frequency-domain fitting of GABA resonances to Lorentzian lineshapes using in-house MATLAB tools. Creatine resonances were fit to Lorentzian lineshapes in the unedited spectra. See Figure 2 for sample data and fits.

Exclusion Criteria. The following criteria were used to exclude individual metabolites based on spectral quality: 1) rescaled absolute Cramér-Rao lower bound (a goodness-of-fit measure) values >20% (Supplemental Methods and Materials) (21); 2) linewidth >30 Hz. Following exclusion using these criteria, participants with concentrations >3 SDs from the group mean were excluded from further analysis. In addition, there were cases where the measurement voxel was erroneously

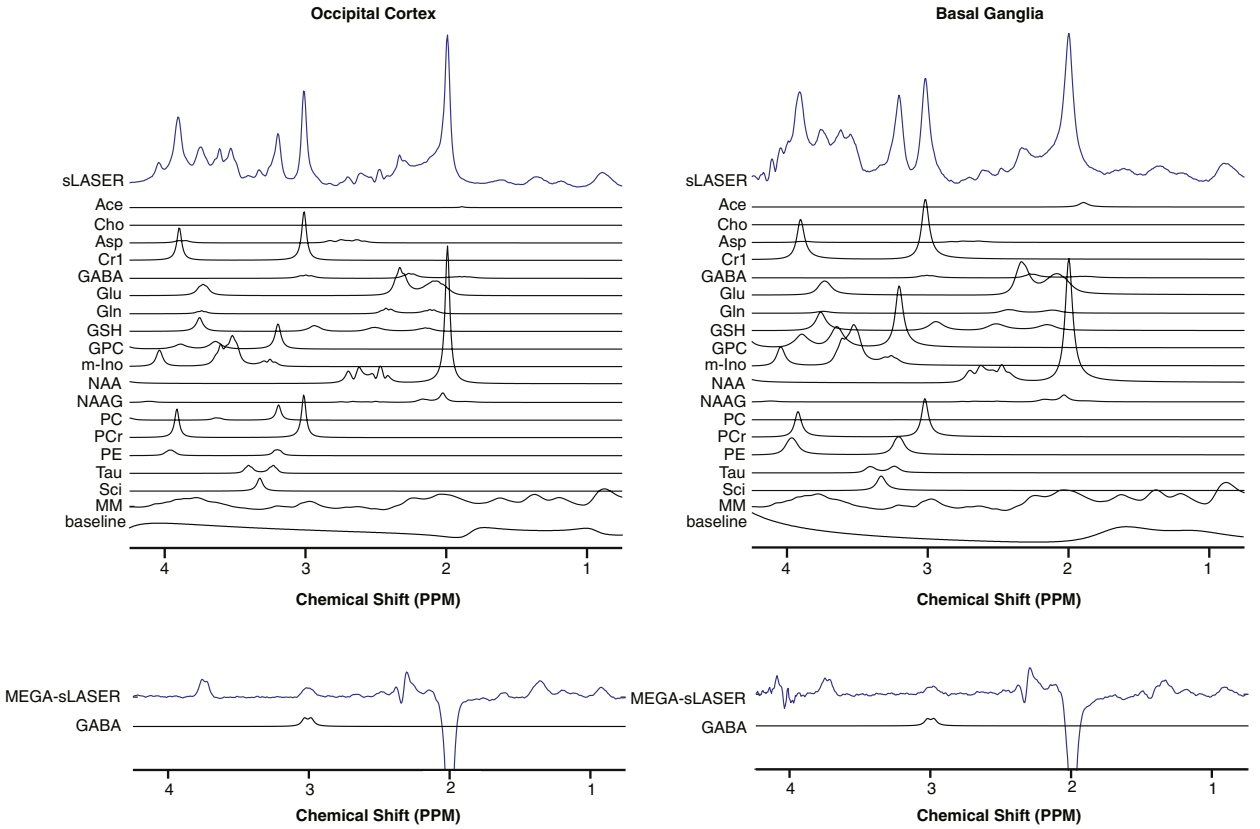

Figure 2. Representative raw data (blue) and spectral fits (black) for the occipital cortex (left) and basal ganglia (right). The baseline includes signal contributions of residual water and lipids. Ace, acetate; Asp, aspartate; Cho, choline; Cr1, creatine; GABA, gamma-aminobutyric acid; Gln, glutamine; Glu, glutamate; GPC, glycerophosphorylcholine; GSH, glutathione; m-Ino, *myo*-inositol; MEGA-sLASER, Mescher-Garwood semilocalized by adiabatic selective refocusing (sequence); MM baseline, macromolecule baseline; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; PC, phosphocholine; PCr, phosphocreatine; PE, phosphoethanolamine; PPM, parts per million; Sci, scyllo-inositol; sLASER, semilocalized by adiabatic selective refocusing (sequence); Tau, taurine.

positioned owing to a bug in the data acquisition software (described in Supplemental Methods and Materials). When possible, we attempted to scan those individuals again on a separate day. For two participants and only in the occipital cortex, the GABA and Glu/Gln/Glx metabolite concentrations are from different sessions as a result of the rescanning, and they were excluded from the analyses of GABA/Glx ratios in the occipital cortex. The number of included subjects from each group, for each metabolite, and from each voxel location is summarized in Supplemental Table S1.

Partial Volume Correction. Fractions of gray matter (both total and basal ganglia only for the subcortical voxel), white matter, and cerebrospinal fluid were obtained via segmentation of anatomic images (see Supplemental Methods and Materials for methods and Supplemental Table S2 for group tissue fractions). The ratios of the area under the metabolite peak to the area under the water peak were then corrected for partial volume effects (Supplemental Methods and Materials).

Statistical Analysis

Our main measures of interest were concentrations (mmol/L) of GABA, Glu, and Gln in the right and left basal ganglia and visual cortex. To investigate group differences in metabolite concentrations, we used separate linear mixed-effects models for each of the voxel locations, with subjects as the random effect and diagnostic group and metabolite (GABA, Glu, Gln) as fixed effects. Linear mixed-effects models accommodate incomplete data sets under missing-at-random assumptions, rather than using listwise deletion of participants that, in our case, were missing usable data for a particular metabolite. These analyses were conducted using the SPSS MIXED procedure (SPSS, Inc, Chicago, IL). Age and proportion of gray matter within the voxel were included as covariates. If there was a significant effect of group or group-by-metabolite interaction, follow-up univariate tests were conducted to investigate the effect of group, controlling for age and gray matter proportion, on each metabolite of interest. If these univariate tests yielded a significant effect of group, pairwise group comparisons were conducted. Additionally, the REL and HC groups were pooled and compared with the SZP group to examine differences in metabolite concentrations that were related to the illness state. The SZP and REL groups were pooled and compared with the HC group to investigate differences related to genetic liability to schizophrenia. The p values for these five post hoc tests were corrected for multiple comparisons using Hommel's approach using SAS PROC MULTTEST.

We were also interested in three additional measures: Glx, and the ratios of both Gln to Glu and GABA to Glx. Glx and the ratios were not included in the main mixed-effects models, as they were derived from the other dependent variables. For each voxel location, analyses of covariance (ANCOVAs) were conducted to test the effect of group on Glx and both the ratio of Gln to Glu and the ratio of GABA to Glx, controlling for age and gray matter proportion. The p values for the effect of group in each of these ANCOVAs were corrected for multiple testing (three metabolite ratios/concentrations in the three measurement voxels) using Hommel's approach. Post hoc tests were evaluated as described earlier.

The relationships between clinical measures (Social Functioning Scale score, Positive and Negative Syndrome Scale positive score, Positive and Negative Syndrome Scale negative score, illness duration), IQ, and metabolite concentrations and ratios were assessed using Spearman's rank correlation coefficient in the SZP group. Medication effects in the SZP group were evaluated by 1) using Spearman's rank correlation coefficients to evaluate the relationship between chlorpromazine equivalent dose and metabolite concentrations and ratios and 2) using Mann-Whitney U tests to evaluate group differences in subjects in the SZP group receiving and not receiving benzodiazepines or other anticonvulsants. Finally, smokers were compared with nonsmokers on metabolite concentrations and ratios within diagnostic groups using Mann-Whitney U tests. This analysis was not performed in the HC group because there were only three smokers.

RESULTS

Group Differences in Metabolite Concentrations and Ratios

Descriptive statistics are presented in Table 2.

Occipital Cortex. The linear mixed-effects model yielded a significant effect of group on metabolite concentrations ($F = 4.12$, $p = .02$) and a statistical trend toward a group-by-metabolite interaction ($F = 2.45$, $p = .056$). Follow-up univariate ANCOVAs were conducted for each metabolite (Figure 3A).

Table 2. Metabolite Concentrations

	HC, Mean (SD)	REL, Mean (SD)	SZP, Mean (SD)
Occipital Cortex			
GABA	2.55 (0.49)	2.56 (0.41)	2.31 (0.55)
Glu	10.14 (1.30)	9.28 (1.09)	9.32 (0.94)
Gln	1.85 (0.55)	1.57 (0.69)	1.78 (0.59)
Glx	12.00 (1.50)	10.84 (1.66)	11.10 (1.17)
Gln/Glu	0.18 (0.05)	0.16 (0.06)	0.19 (0.07)
GABA/Glx	0.21 (0.04)	0.24 (0.05)	0.21 (0.05)
Right Striatum			
GABA	2.58 (0.72)	2.49 (0.62)	2.37 (0.80)
Glu	9.50 (1.59)	9.55 (1.43)	9.37 (1.51)
Gln	3.03 (1.13)	2.48 (1.00)	3.19 (1.24)
Glx	12.54 (2.47)	12.04 (1.93)	12.56 (2.48)
Gln/Glu	0.32 (0.09)	0.26 (0.10)	0.34 (0.11)
GABA/Glx	0.22 (0.08)	0.21 (0.06)	0.20 (0.07)
Left Striatum			
GABA	2.14 (0.67)	1.89 (0.61)	2.25 (0.93)
Glu	7.55 (1.54)	7.43 (1.45)	7.68 (1.30)
Gln	3.50 (1.69)	3.38 (1.45)	2.93 (0.97)
Glx	11.04 (2.21)	10.80 (2.09)	10.62 (1.68)
Gln/Glu	0.50 (0.32)	0.47 (0.26)	0.39 (0.14)
GABA/Glx	0.19 (0.06)	0.19 (0.07)	0.23 (0.08)

GABA, gamma-aminobutyric acid; GABA/Glx, GABA-to-Glx; Gln, glutamine; Glu, glutamate; Gln/Glu, Gln-to-Glu ratio; HC, healthy control subjects; REL, healthy relatives; SZP, patients with schizophrenia.

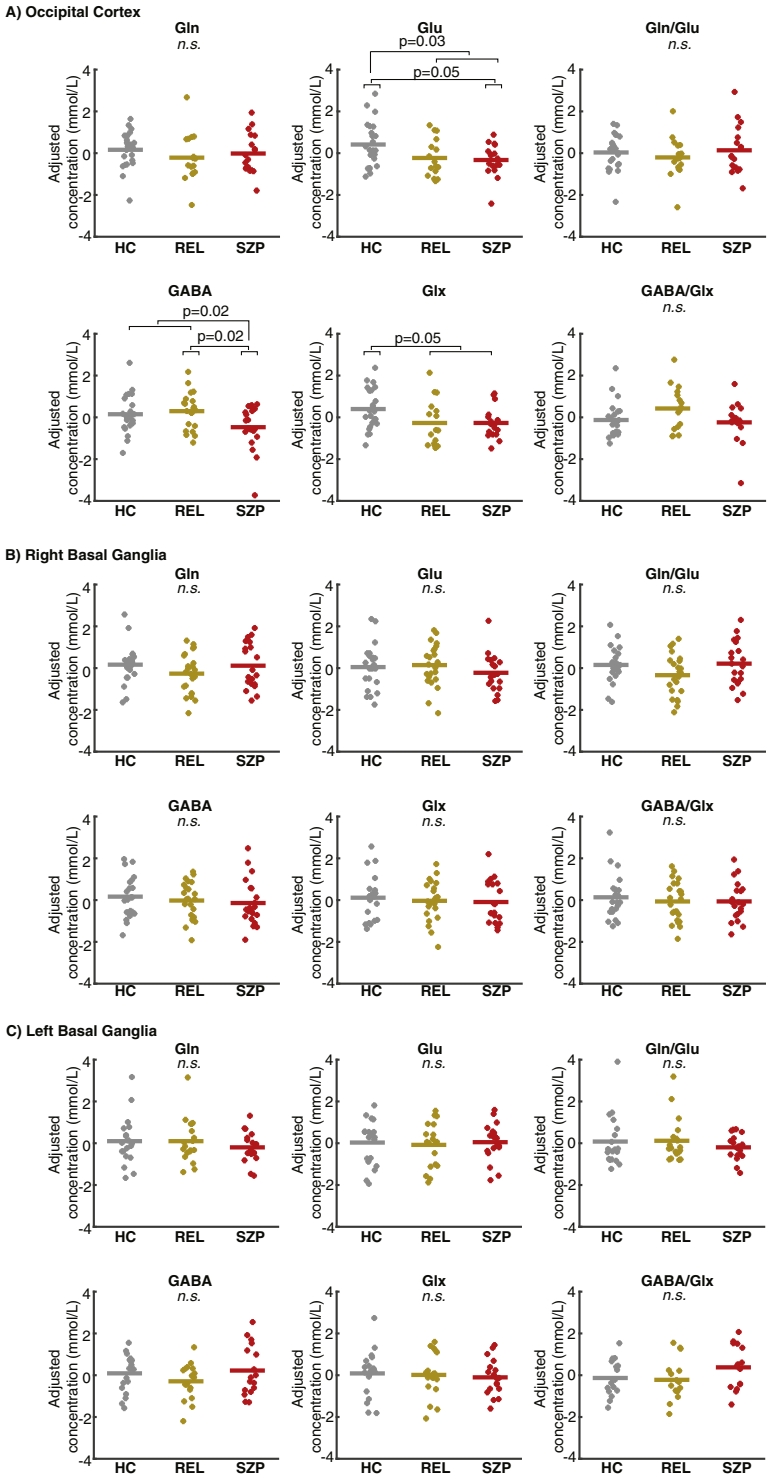

Figure 3. **(A–C)** Glutamine (Gln), glutamate (Glu), Gln-to-Glu ratio (Gln/Glu), gamma-aminobutyric acid (GABA), Gln and Glu (Glx), and GABA-to-Glx (GABA/Glx) ratio for healthy control subjects (HC; gray), healthy relatives (REL; yellow), and patients with schizophrenia (SZP; red). Concentrations and ratios are adjusted for age and proportion of gray matter in the measurement voxel, as the statistical comparisons of group differences were performed controlling for these factors. For each metabolite or metabolite ratio, the adjusted values were obtained by running a regression analysis on the measure of interest, including age and gray matter as predictors. The depicted adjusted values represent the standardized residuals from this regression model. Significant post hoc comparisons are indicated with corrected *p* values. n.s., nonsignificant.

The GABA concentrations differed significantly between groups ($F = 4.23$, $p = .019$). After Hommel correction, differences between the HC and SZP group ($F = 4.65$, $p_{corrected} = .10$) did not reach significance. However, subjects in the SZP group had significantly less GABA than subjects in the REL group ($F = 8.31$, $p_{corrected} = .02$) and the combined healthy group ($F = 8.90$, $p_{corrected} = .016$). There were no differences in GABA between the HC group and the combined liability group ($F = 0.82$, $p_{corrected} = .82$) or between the HC and REL groups ($F = 0.22$, $p_{corrected} = .64$).

The Glu concentrations also differed significantly between groups ($F = 4.03$, $p = .02$). After correction, greater Glu in the HC group than in the REL group ($F = 5.03$, $p_{corrected} = .087$) reached trend level significance, and the HC group had significantly more Glu than the SZP group ($F = 6.84$, $p_{corrected} = .048$). In addition, the HC group had more Glu than the combined liability group ($F = 7.54$, $p_{corrected} = .03$). There was no difference in Glu between the REL and SZP groups ($F = 0.17$, $p_{corrected} = .69$) or between the SZP group and the combined healthy group ($F = 3.27$, $p_{corrected} = .15$). There was no group difference in Gln concentrations ($F = 0.66$, $p = .52$).

In addition, we examined group effects on Glx and the ratio of Gln to Glu using ANCOVAs. After Hommel correction for the number of metabolites and measurement voxels, the effect of group on Glx ($F_{2,52} = 3.36$, $p_{corrected} = .34$) was no longer significant. Pairwise comparisons roughly paralleled our Glu findings, and the difference between the HC and REL groups ($F_{1,52} = 4.41$, $p_{corrected} = .12$) and the HC and SZP groups ($F_{1,52} = 4.80$, $p_{corrected} = .09$) did not reach significance after Hommel correction. However, Glx was significantly reduced in the combined liability group relative to the HC group ($F_{1,52} = 6.70$, $p_{corrected} = .05$). There was no Glx difference between the REL and SZP groups ($F_{1,52} = .002$, $p_{corrected} = .97$) or between the SZP group and the combined healthy group ($F_{1,52} = 1.42$, $p_{corrected} = .48$). There was no group effect on the ratio of Gln to Glu ($F_{2,52} = 0.57$, $p_{corrected} = .83$) or any pairwise group differences (all $p_{corrected} = .82$).

Finally, we measured the ratio of GABA to Glx. After Hommel correction, there was no effect of group ($F_{2,52} = 2.56$, $p_{corrected} = .61$). Pairwise comparisons indicated that although none of the group differences reached Hommel-corrected statistical significance (all $p > .17$), at an uncorrected p value of .05, the REL group showed a higher ratio of GABA to Glx compared with the SZP group ($F_{1,50} = 4.43$, $p = .04$) and, at a trend level, than the HC group ($F_{1,50} = 3.48$, $p = .07$).

Basal Ganglia. Linear mixed-effects models did not yield a significant effect of group or group-by-metabolite interaction on concentrations in either the right (group [$F = 0.37$, $p = .70$], group-by-metabolite [$F = 2.08$, $p = .09$]) (Figure 3B) or the left (group [$F = 0.28$, $p = .76$], group-by-metabolite [$F = 0.75$, $p = .56$]) (Figure 3C) basal ganglia. There was no effect of group for Glx (right [$F_{2,61} = 0.25$, $p_{corrected} = .78$], left [$F_{2,52} = 0.18$, $p_{corrected} = .83$]), the ratio of Gln to Glu (right [$F_{2,61} = 2.31$, $p_{corrected} = .11$], left [$F_{2,52} = 0.18$, $p_{corrected} = .83$]), or the ratio of GABA to Glx (right [$F_{2,59} = 0.28$, $p_{corrected} = .76$]; left [$F_{2,42} = 1.71$, $p_{corrected} = .19$]) in the basal ganglia.

Spectral Quality

Group differences in spectral quality cannot explain differences in metabolite concentrations (Supplemental Table S2), as there were no group differences in spectral line widths or Cramér-Rao lower bound values of GABA, Glu, or Gln in the occipital cortex voxels, where we observed significant effects.

Clinical, Cognitive, and Medication Correlates

No significant correlations between metabolite concentrations/ratios and clinical or cognitive measures were observed in the SZP group, and no medication effects were observed. Finally, no significant differences in metabolite concentrations or ratios between smokers and nonsmokers were observed in either the SZP group or the REL group.

DISCUSSION

Using ^{1}H-MRS at an ultra-high magnetic field strength, we observed reduced cortical GABA in patients with schizophrenia compared with both healthy relatives and the pooled sample of healthy individuals (relatives and nonrelatives), suggesting that altered GABA concentrations in schizophrenia are associated with either illness state or medication effects. Reduced cortical Glu and Glx were observed when pooling healthy relatives and patients with schizophrenia and comparing them with healthy nonrelatives, and Glu was reduced in patients compared with healthy nonrelatives, suggesting that altered Glu concentrations are associated with illness liability. Taken together, these findings are consistent with prior reports in schizophrenia and provide novel insights into glutamatergic and GABAergic systems in healthy relatives.

The present study overcomes several methodologic limitations highlighted in previous studies. Scanning at higher field strengths allows us to reliably separate spectral components. Additionally, we quantified metabolites relative to an internal water reference, rather than expressing quantities as ratios relative to metabolites that differ across clinical populations (e.g., *N*-acetylaspartate, creatine). Furthermore, we considered group differences in voxel tissue composition as potential sources of variance, which has not been consistently performed in previous studies. Finally, we showed that group differences in spectral quality or fit could not account for our findings.

Nonetheless, these findings are consistent with previous reports of reduced cortical GABA (34,47,55) and Glu (20–22) in patients with chronic schizophrenia who are taking medication and reduced Glu in healthy unaffected relatives (48) at lower magnetic field strengths. However, in contrast to a proton MRS study performed at 3T (51), we did not observe increased Gln or an increased Gln-to-Glu ratio in patients. Field strength and measurement location are potential reasons for these discrepant findings. To our knowledge, there is only one other article reporting GABA and Glu at 7T in patients with schizophrenia. Marsman *et al.* (35) measured GABA and Glu in both medial frontal and parieto-occipital cortex. They also observed reduced cortical GABA in patients with schizophrenia but only in frontal cortex. Furthermore, they did not observe any group differences in Glu. Possible explanations for discrepancies between our findings in the occipital cortex and their findings in parieto-occipital cortex include differences in illness duration, voxel location, fitting routine, and GABA quantification methods. New multivoxel techniques (77,78), in which metabolite concentrations can be mapped across whole-brain volumes, will likely highlight possible region-specific metabolite differences.

We did not observe any group differences in metabolite concentrations or ratios in basal ganglia. There are possible physiologic and methodologic reasons for this. Dopamine modulates the excitability of striatal GABAergic neurons (79). Antipsychotics achieve their therapeutic effects largely via blockade of striatal dopamine receptors and might have

normalized GABAergic and glutamatergic signaling within the striatum. Striatal Glu and Glx levels normalized after antipsychotic treatment in previous studies (30,33). Additionally, because of high iron content in basal ganglia, shimming is more challenging (19). This is reflected in the larger peak linewidths in the basal ganglia versus occipital cortex and may have rendered measurements less sensitive to group differences. Furthermore, although we were largely interested in the striatum, it was unavoidable to include other basal ganglia structures in our measurement voxel. Functional heterogeneity among these subcortical structures might have washed out potential group differences. Finally, because of constraints on voxel placement, we often failed to include the head of the caudate, which has been found to be a specific locus of functional abnormalities in schizophrenia (80).

There are several potential mechanistic interpretations of reduced cortical Glu in patients with schizophrenia and relatives. Reduced concentrations might indicate reduced glutamatergic transmission, contrary to the prediction that NMDAR hypofunction causes downregulation of GABAergic interneurons, resulting in increased glutamatergic neurotransmission. However, MRS is measuring only a steady-state concentration of Glu. Although some work has suggested a link between cortical excitability and measured concentrations of Glu using MRS (81), the inferences we can make about Glu concentrations and neurotransmission are severely limited. Additionally, based on work in animals, the metabolic pool of Glu is likely larger than the transmitter pool (82), and MRS does not distinguish between these two compartments. Nevertheless, the finding of reduced Glu in healthy relatives is intriguing and invites a natural question: why did these individuals not become ill? One explanation is that patients and relatives might be arriving at reduced Glu via different routes (e.g., synthesis, degradation, transport, release) (83,84) or that different pools of Glu are reduced in patients and siblings, which potentially confer different risks for symptom expression. Alternatively, dopamine signaling might not be affected by NMDAR dysregulation in healthy relatives, as it is in patients (85), warding against psychosis and more profound cognitive deficits (1).

Yet another possible answer pertains to GABA concentrations (see Supplemental Discussion for a comment on concentration values). We observed reduced GABA in patients with schizophrenia, consistent with a GABAergic contribution to the pathophysiology of the illness, possibly via the effect of NMDAR hypofunction on inhibitory interneurons (86). Despite showing reduced Glu, healthy relatives had normal GABA concentrations. If, as animal studies would suggest (86), GABA dysfunction is secondary to NMDAR dysfunction, it is possible that certain mechanisms maintain normal GABA concentrations despite NMDAR hypofunction in those genetically at-risk individuals who do not go on to develop the disorder. However, the concentrations and metabolite ratios measured with MRS cannot directly speak to neurotransmission. Postmortem studies could shed light on possible neurobiological mechanisms underlying altered metabolite concentrations and ratios in unaffected relatives.

Because all patients in the present study had long-term antipsychotic use, we must consider potential medication confounds. To the extent that reduced Glu and Glx concentrations are indices of the same biological mechanisms in patients and healthy relatives, we would argue that these reductions cannot be solely accounted for by neuroleptic effects, as they were also observed in unaffected relatives at an equivalent magnitude to patients. However, MRS does not allow us to identify the source of metabolite concentrations, and it remains possible that Glu decreases in patients with schizophrenia are driven by medication. Although previous studies have shown either normal (23–25,33,87) or even elevated (44,56) Glu and Glx in unmedicated patients, it is impossible to dissociate effects of medication and illness stage. Arguing against an effect of medication are findings that cortical Glu and Glx concentrations do not change after antipsychotic administration within patients (33).

The GABA concentrations were not altered in healthy relatives, possibly because medication effects are driving reduced GABA concentrations in patients. However, we did not observe any negative correlations between antipsychotic dose and GABA concentrations. Furthermore, Kelemen *et al.* (54) observed reductions in GABA in the occipital cortex in antipsychotic-naïve patients with a first episode of schizophrenia that did not normalize with antipsychotic treatment [however, see Kegeles *et al.* (56)]. Therefore, we would argue that reduced GABA in visual cortex is related to the disease state, but we certainly cannot rule out medication confounds.

In conclusion, we found decreased Glu and Glx concentrations in the occipital cortex of both patients with schizophrenia and healthy first-degree relatives. These metabolic abnormalities might underlie the altered visual processing (58–63) and occipital gray matter loss (88) observed in patients and first-degree relatives. Decreased occipital GABA concentrations were specific to patients with schizophrenia. These findings shed light on the pathophysiology of schizophrenia and are consistent with alterations in glutamatergic and GABAergic systems. Additionally, findings of spared GABA concentrations in unaffected relatives have therapeutic implications for patients with schizophrenia.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported in part by the Netherlands Organisation for Scientific Research Rubicon grant (to KNT), University of Utrecht short-stay fellowship (to KNT), and University of Utrecht Neuroscience and Cognition grant (to SFWN).

We thank Ilse Thompson and Helene Hopman for recruiting participants and conducting symptom ratings, Dr. Melissa Batson and Lotte Houtepen for consultation about data collection, and Dr. Bill Chopik and Abdhi Sakar for consultation on statistical analyses.

The authors report no biomedical financial interests or potential conflicts of interest.

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Received Jun 3, 2015; revised Apr 8, 2016; accepted Apr 11, 2016.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2016.04.007.

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