


## Original Investigation

# Glutamate Levels in the Associative Striatum Before and After 4 Weeks of Antipsychotic Treatment in First-Episode Psychosis

## A Longitudinal Proton Magnetic Resonance Spectroscopy Study

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**IMPORTANCE** Increased glutamate levels in the right associative striatum have been described in patients during a first episode of psychosis. Whether this increase would persist after effective antipsychotic treatment is unknown.

**OBJECTIVES** To compare the glutamate levels in antipsychotic-naive patients with first-episode psychosis in the right associative striatum and right cerebellar cortex using proton magnetic resonance spectroscopy before and 4 weeks after antipsychotic treatment and to compare these results with normative data from sex-matched healthy control subjects.

**DESIGN, SETTING, AND PARTICIPANTS** Before-after trial in an inpatient psychiatric research unit among 24 antipsychotic-naive patients with first-episode psychosis and 18 healthy controls matched for age, sex, handedness, and cigarette smoking.

**INTERVENTIONS** Participants underwent 2 proton magnetic resonance spectroscopy studies: patients were imaged at baseline and after 4 weeks of antipsychotic treatment, while controls were imaged at baseline and at 4 weeks after the baseline measurement. Patients were treated with oral risperidone (open label) for 4 weeks with dosages that were titrated on the basis of clinical judgment.

**MAIN OUTCOMES AND MEASURES** Glutamate levels were estimated using LCModel (version 6.2-1T) and were corrected for the cerebrospinal fluid proportion within the voxel.

**RESULTS** Patients with first-episode psychosis had higher levels of glutamate in the associative striatum and the cerebellum during the antipsychotic-naive condition compared with controls. After clinically effective antipsychotic treatment, glutamate levels significantly decreased in the associative striatum, with no significant change in the cerebellum. No differences in glutamate levels were observed between groups at 4 weeks.

**CONCLUSIONS AND RELEVANCE** Increased glutamate levels observed at baseline in patients with first-episode psychosis normalized after 4 weeks of clinically effective antipsychotic treatment. These results provide support for the hypothesis that improvement in clinical symptoms might be related to a decrease in glutamate levels.

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The current hypothesis for the origin of schizophrenia proposes that the disorder stems from neurodevelopmental deficits that result in a disturbance of glutamatergic neurotransmission, especially for *N*-methyl-D-aspartate receptor-mediated signaling.<sup>1</sup> The deteriorating course of the disease may be partially explained by cortical neuronal toxic effects secondary to enhanced glutamatergic exposure,<sup>2</sup> and dopaminergic dysregulation may be the final common pathway that results from altered glutamatergic neurotransmission.<sup>3</sup>

Neuroimaging studies<sup>4-8</sup> using proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) have shown increased levels of the metabolites glutamate, glutamine, glutamate + glutamine (Glx), and glutamine/glutamate in patients with first-episode psychosis (FEP) who are antipsychotic-naïve or who have been minimally exposed to antipsychotic medication. On the other hand, studies<sup>9-12</sup> in medicated patients have demonstrated the same levels or decreased levels of these metabolites compared with control subjects.

Recently, our group reported a cross-sectional study<sup>13</sup> in antipsychotic-naïve patients with FEP that showed increased glutamate levels in the right associative striatum (ie, precommissural dorsal caudate), a region characterized by a high density of dopamine receptors and dopamine afferents. Conversely, glutamate levels were similar in patients with FEP and controls in the cerebellum, a brain region with a negligible quantity of dopamine receptors and an absence of dopamine afferents, suggesting that the glutamate level anomalies may be restricted to dopamine-rich regions of the brain.

The objectives of this study were to compare the striatal and cerebellar glutamate levels in the right associative striatum and the right cerebellar cortex in antipsychotic-naïve patients with FEP before and 4 weeks after antipsychotic treatment and to compare these results with normative data from age- and sex-matched healthy control subjects.

## Methods

### Participants

The study was approved by the ethics and scientific committees of the Instituto Nacional de Neurología y Neurocirugía. Participants were included in the study after successful completion of an informed consent procedure in which written consent was obtained from both parents for individuals younger than 18 years.

Twenty-eight patients were recruited from the Emergency Department, the Neuropsychiatry Department, and the Adolescent Program of Neuropsychiatric and Imaging Study at the Instituto Nacional de Neurología y Neurocirugía during their first nonaffective psychosis episode. For inclusion in the study, individuals were assessed using the Structured Clinical Interview for *DSM-IV*. Patients were deemed eligible if they were antipsychotic naïve and had less than 2 years of psychotic symptoms. Exclusion criteria included high risk for suicide, psychomotor agitation, comorbidity with other Axis I disorders, concomitant medical or neurological illness, and current substance abuse or a history of substance depen-

dence (excluding nicotine). Twenty-four of 28 patients initially recruited completed the study. Two patients did not respond to treatment, the third patient demonstrated excessive movement during the baseline <sup>1</sup>H-MRS, and the fourth patient withdrew consent. Only patients who responded to treatment were scheduled for follow-up <sup>1</sup>H-MRS.

Eighteen right-handed, age- and sex-matched healthy control subjects were also recruited. The controls were assessed in the same manner as the patients, and any individual with a history of psychiatric illness or a family history of schizophrenia was excluded. All participants (patients and controls) were screened for drugs of abuse (cannabis, cocaine, heroin, opioids, and benzodiazepines) at the time of study inclusion and 1 hour before <sup>1</sup>H-MRS.

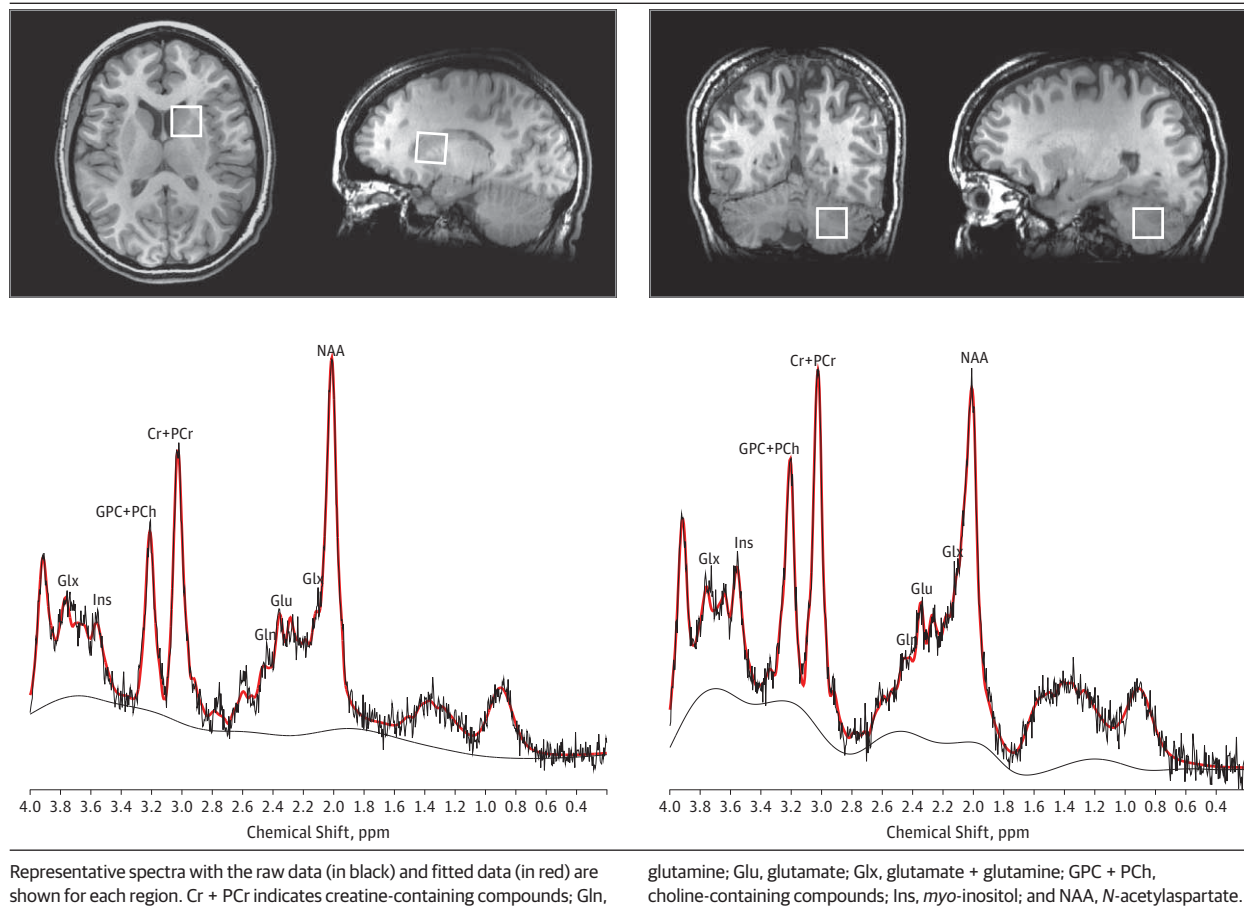
After baseline <sup>1</sup>H-MRS, patients began treatment with oral risperidone (open label) that was titrated over 4 weeks using a flexible dosing schedule starting at 2 mg/d up to a maximum of 5 mg/d. The risperidone dosage was titrated according to clinical judgment and treatment response. Treatment response was defined clinically as a reduction of at least 30% on the total score of the Positive and Negative Syndrome Scale (PANSS) after 4 weeks of treatment. Patients were hospitalized at the Neuropsychiatry Department of the Instituto Nacional de Neurología y Neurocirugía for the duration of the study. The use of other concomitant medications (eg, benzodiazepines, mood stabilizers, and antidepressants) was not allowed for the duration of the study. Participants underwent two <sup>1</sup>H-MRS studies: patients were imaged at baseline (antipsychotic free) and after 4 weeks of antipsychotic treatment, and controls were imaged at baseline and at 4 weeks after the baseline measurement.

### Magnetic Resonance Studies

Proton magnetic resonance spectroscopy was performed in a 3-T whole-body imaging system (Signa Excite HDxt; GE Healthcare) with a high-resolution 8-channel head coil at the Neuroimaging Department of the Instituto Nacional de Neurología y Neurocirugía. The participant's head was positioned along the canthomeatal line and immobilized by a forehead strap. Participants were initially imaged with a T1-weighted spoiled gradient-echo 3-dimensional axial acquisition (SPGR) oriented above and parallel to the anterior commissure-posterior commissure line (echo time, 5.7 milliseconds; repetition time, 13.4 milliseconds; inversion time, 450 milliseconds; flip angle, 20°; field of view, 25.6 cm; 256 × 256-pixel matrix; and section thickness, 1.2 mm). These T1-weighted SPGR images were reformatted to sagittal and coronal views and were used for optimal <sup>1</sup>H-MRS voxel placement.

The <sup>1</sup>H-MRS spectra were obtained using point-resolved spectroscopy centered on the right dorsal caudate nucleus and on the right cerebellar cortex (echo time, 35 milliseconds; repetition time, 2000 milliseconds; spectral width, 5000 Hz; 4096 data points used; and 128 water-suppressed averages and 16 water-unsuppressed averages) in volume elements (voxels) of 8 mL (2 × 2 × 2 cm). The lower end of the dorsal caudate voxel (ie, associative striatum) was located 3 mm dorsal to the anterior commissure to include the maximum amount of gray matter (GM) and with a dorsal extension (thickness) of 2 cm.

Figure 1. Voxel Placement in 2 Regions of Interest: the Right Associative Striatum and the Right Cerebellar Cortex



The cerebellar voxel was located in the cerebellar cortex below the inferior cerebellar peduncle avoiding the midline (Figure 1). During acquisition,  $^1\text{H}$ -MRS spectra were shimmed to achieve full-width at half maximum (FWHM) of 12 Hz or less (measured on the unsuppressed water signal from the voxel). Spectra with larger FWHM were excluded from further analyses.

#### $^1\text{H}$ -MRS Data Analysis

Water-suppressed spectra were analyzed using LCModel (version 6.2-1T).<sup>14</sup> Spectra were normalized to the unsuppressed water signal, yielding quantification of glutamate, creatine (Cr), phosphocreatine, *myo*-inositol (Ins), phosphocholine (PCh), *N*-acetylaspartate (NAA), and glycerophosphocholine (GPC). Spectroscopic values are expressed in institutional units.

The analysis used a standard basis set of metabolites, including Cr, Ins, NAA, PCh, GPC, taurine, L-lactate, aspartate, L-alanine, glucose, glutamate, glutamine, glutathione, *scyllo*-inositol, phosphocreatine, guanidinoacetate,  $\gamma$ -aminobutyric acid, Cr methylene group, and *N*-acetylaspartylglutamate acid, as well as lipids (Lip) and macromolecules (MMs) (Lip09, Lip13a, Lip13b, Lip20, MM09, MM12, MM14, MM17, and MM20). This basis set, which was included in LCModel, was acquired with the same sequence parameters used in our study. All metabolites with a Cramer-Rao lower bound (CRLB) ex-

ceeding 20% as reported by LCModel were considered poor quality and were excluded from further analyses.<sup>15</sup>

The SPGR images used for localization of the voxels were subsequently segmented into GM, white matter, and cerebrospinal fluid using a computer program (Statistical Parametric Mapping 8; Wellcome Department of Imaging Neurosciences, University College London). To calculate the percentages of GM, white matter, and cerebrospinal fluid content within a voxel, the size and location of each area were extracted from the spectra file headers using in-house software that allowed for the correction of the cerebrospinal fluid fraction of the spectroscopic values.<sup>13</sup>

For the images at 4 weeks, the same acquisition parameters were used. The images from the baseline image session, corresponding to the axial, sagittal, and coronal projections of the spectroscopic voxels, were saved, including the location of the  $^1\text{H}$ -MRS voxels. These baseline images and the sulcal-gyral pattern assisted with relocalization of the  $^1\text{H}$ -MRS voxels in the images at 4 weeks.<sup>16</sup>

#### Statistical Analysis

The results are presented as means (SDs). Demographic and clinical characteristics and  $^1\text{H}$ -MRS metabolites were compared between FEP and control groups. Analyses of variance with repeated measures ( $2$  [diagnostic groups]  $\times$   $2$  [re-

gions]  $\times$  2 [times]) were conducted to investigate glutamate levels, the metabolite of interest. Independent-sample *t* tests and paired-sample *t* tests were used for comparisons within groups for the rest of the metabolites. Frequency data were analyzed using  $\chi^2$  test or Fisher exact test. The percentages of GM in the  $^1\text{H-MRS}$  voxels of the associative striatum and cerebellum were included as covariates. The statistical comparisons were performed with a significance level set at  $P < .05$ .

Partial Spearman rank correlations controlling for the effect of GM were performed to examine the relationship between clinical scale scores and glutamate levels for each region. The statistical threshold was established at  $P \leq .016$  ( $P = 0.5 \div 3$  to control for multiple comparisons) to control for multiple comparisons for the PANSS subscales in the FEP group.

Outlier participants were detected using Cook distance criteria.<sup>17</sup> Outliers were defined based on the absolute change in glutamate levels between baseline and 4 weeks. This method identified 2 outliers in the FEP group. The results are presented with inclusion and exclusion of the outliers.

Test-retest reliability for the control group was calculated as follows: (glutamate 1 level minus glutamate 2 level) divided by (glutamate 1 level plus glutamate 2 level divided by 2) times 100 for absolute (unsigned value) percentage difference and (glutamate 1 level minus glutamate 2 level) divided by glutamate 2 level times 100 for absolute percentage error. Coefficient of variation was defined as follows: the SD of glutamate 1 level and glutamate 2 level divided by the mean of glutamate 1 level and glutamate 2 level. The values for the percentage difference, percentage error, and coefficient of variation are presented as an individual's mean (SD). Intraclass correlation coefficients were estimated using a 2-way random-effects model (average measure).

## Results

### Demographic and Clinical Characteristics

The DSM-IV diagnoses of the patients with FEP included in the study were as follows: schizophrenia ( $n = 9$ ), brief psychotic disorder ( $n = 8$ ), and schizophreniform disorder ( $n = 7$ ). The educational level was higher in the control group compared with the FEP group ( $t_{40} = 4.57, P < .001$ ). The FEP group had a mean (SD) duration of untreated psychosis of 20.33 (25.84) weeks (range, 1-104 weeks). The FEP and control groups were similar in age, sex, handedness, and cannabis or tobacco use (Table 1). The mean (SD) PANSS total scores for the FEP group were 94.5 (13.6) at baseline and 58.7 (9.6) after 4 weeks of antipsychotic treatment ( $t_{23} = 12.35, P < .001$ ; mean [SD] decrease, 39% [9%]). The mean (SD) daily dose of risperidone used during the study was 3.42 (1.14) mg.

### Glutamate Levels

Analyses were performed with inclusion of the full sample (24 patients and 18 controls) and with exclusion of the 2 outliers as identified by Cook distance criteria. The time  $\times$  group  $\times$  region (multivariate) interaction was not significant within the full sample ( $F = 2.45, P = .10$ ), but this interaction was significant

**Table 1. Demographic and Clinical Characteristics of the Study Participants at Baseline**

Variable	FEP Group	Control Group
Age, mean (SD) [range], y	26.58 (8.49) [18-47]	24.56 (5.07) [18-35]
Educational level, mean (SD), y	10.96 (3.44)	15.61 (2.99) <sup>a</sup>
Sex, No.		
Male	13	8
Female	11	10
Handedness, No.		
Right	24	18
Left	0	0
Ever used, No./total No.		
Tobacco	8/24	2/18
Cannabis	3/24	0/18
Duration of untreated psychosis, mean (SD), wk	20.33 (25.84)	NA
PANSS subscale score		
Positive	23.33 (4.99)	NA
Negative	24.08 (6.03)	NA
General psychopathology	47.92 (8.75)	NA

Abbreviations: FEP, first-episode psychosis; NA, not applicable; PANSS, Positive and Negative Syndrome Scale.

<sup>a</sup>  $P < .05$ .

after exclusion of the 2 outliers ( $F = 3.41, P = .04$ ). Univariate analysis showed that the time  $\times$  group interaction was significant for the associative striatum but not for the cerebellum within the entire sample ( $F = 4.73, P = .04$  vs  $F = 0.22, P = .64$ ) and after exclusion of the 2 outliers ( $F = 6.83, P = .01$  vs  $F = 1.04, P = .31$ ).

Post hoc pairwise comparisons revealed that glutamate levels in the associative striatum were higher in patients compared with controls ( $t_{40} = 2.61, P = .01$ ). This difference was maintained after exclusion of the 2 outliers ( $t_{38} = 2.89, P = .008$ ). The FEP group had a decrease in their glutamate levels in the associative striatum after 4 weeks of antipsychotic treatment ( $t_{23} = 2.21, P = .04$ ). This finding remained significant after exclusion of the 2 outliers ( $t_{21} = 3.00, P = .007$ ). After treatment, patients did not differ from controls at 4 weeks ( $t_{40} = 1.36, P = .18$ ). This finding remained nonsignificant after exclusion of the 2 outliers ( $t_{38} = 1.15, P = .25$ ) (Figure 2).

Pairwise analysis also revealed differences in glutamate levels in the cerebellum between patients and controls at baseline ( $t_{39} = 2.05, P = .047$ ) and no difference at 4 weeks ( $t_{40} = 0.89, P = .37$ ). After 4 weeks of antipsychotic treatment, patients ( $t_{22} = 1.00, P = .32$ ) and controls ( $t_{17} = 1.70, P = .11$ ) showed no significant glutamate level changes in the cerebellum.

Test-retest mean (SD) reliability for glutamate levels between baseline and 4 weeks in 18 controls showed an absolute percentage difference of 5.35% (4.77%), an absolute percentage error of 5.32% (4.75%), and a coefficient of variation of 0.038 (0.034) for the associative striatum, as well as an absolute percentage difference of 13.72% (10.64%), an absolute percentage error of 14.66% (11.83%), and a coefficient of variation of 0.097 (0.075) for the cerebellum. The intraclass correlation coefficients were 0.857 (95% CI, 0.619-0.947,  $df = 17$ ,

$P < .001$ ) for the associative striatum and 0.637 (95% CI, 0.031-0.864,  $df = 17$ ,  $P = .02$ ) for the cerebellum. Test-retest reliability measures for all the metabolites are given in the eTable in the Supplement.

**Other Metabolites**

At baseline, patients showed higher Glx levels in the associative striatum compared with controls ( $t_{40} = 2.03$ ,  $P = .048$ ). After 4 weeks of antipsychotic treatment, patients demonstrated no difference in Glx levels in the associative striatum compared with controls ( $t_{40} = 1.27$ ,  $P = .14$ ). The Glx levels in the cerebellum did not differ between patients and controls at baseline ( $t_{39} = 0.14$ ,  $P = .88$ ) or at 4 weeks ( $t_{39} = 0.38$ ,  $P = .28$ ). Glutamine was not analyzed because of poor spectra fitting in both regions. The NAA levels in the associative striatum showed a trend-level difference between patients and con-

trols at baseline ( $t_{40} = 1.97$ ,  $P = .06$ ) and no difference at 4 weeks ( $t_{40} = 0.80$ ,  $P = .49$ ). Higher NAA levels in patients vs controls were shown in the cerebellum at baseline ( $t_{40} = 2.24$ ,  $P = .03$ ), as well as a trend after antipsychotic treatment ( $t_{40} = 1.74$ ,  $P = .08$ ).

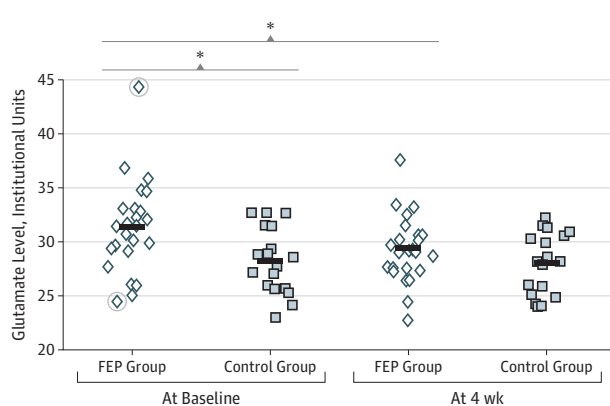
The GPC + PCh levels in the associative striatum were higher in the FEP group compared with the control group at baseline ( $t_{40} = 3.33$ ,  $P = .002$ ) and after antipsychotic treatment ( $t_{40} = 2.64$ ,  $P = .01$ ). Trend-level elevations of GPC + PCh levels in the cerebellum were observed for the FEP group compared with the control group at baseline ( $t_{40} = 2.01$ ,  $P = .05$ ) and after 4 weeks ( $t_{40} = 1.76$ ,  $P = .09$ ). Higher Ins levels were also found in the associative striatum of patients compared with controls at baseline ( $t_{40} = 3.85$ ,  $P < .001$ ) and after antipsychotic treatment ( $t_{40} = 2.88$ ,  $P = .006$ ). No differences in Ins levels were found in the cerebellum at baseline ( $t_{40} = 0.63$ ,  $P = .52$ ) or after 4 weeks of antipsychotic treatment ( $t_{40} = 0.40$ ,  $P = .68$ ). No differences were observed in Cr + phosphocreatine levels between the FEP and control groups in any region or under any conditions.

A comparison between images at baseline and 4 weeks showed no difference in the control group and demonstrated trends in the associative striatum for Glx levels ( $t_{23} = 1.77$ ,  $P = .09$ ) and Ins levels ( $t_{23} = 1.85$ ,  $P = .08$ ) in the FEP group. These results are summarized in Table 2.

**Relationships With Clinical Measures**

Exploratory partial correlations between changes in metabolites and changes in clinical measures (PANSS total score and positive, negative, and general psychopathology subscales) were performed with inclusion of the proportion of GM as a covariate. This exploratory analysis revealed significant correlations ( $P < .016$ ) between glutamate level ( $r = -0.531$ ,  $df = 19$ ,  $P = .013$ ) and Glx level ( $r = -0.585$ ,  $df = 19$ ,  $P = .005$ ) changes in the associative striatum and the score change on the PANSS general psychopathology subscale. In addition, a correlation trend was found between glutamate level in the associative

**Figure 2. Glutamate Levels in the Right Associative Striatum of Patients With First-Episode Psychosis (FEP) and Healthy Control Subjects at Baseline and at 4 Weeks**



Horizontal bars represent the mean for the groups. \* $P < .05$  after the removal of 2 outlying participants indicated within the circles.

**Table 2. Each Metabolite in 2 Regions of Interest at Baseline and at 4 Weeks in the FEP Group and the Control Group**

Variable	Mean (SD)											
	At Baseline						At 4 wk					
	Glu	Glx	NAA	GPC + PCh	Ins	Cr + PCr	Glu	Glx	NAA	GPC + PCh	Ins	Cr + PCr
<b>Right associative striatum</b>												
FEP group	31.34 (4.29) <sup>a</sup>	34.93 (5.53) <sup>a</sup>	24.20 (3.16)	5.13 (0.70) <sup>a</sup>	11.20 (1.73) <sup>a</sup>	18.49 (2.21)	29.25 (3.08)	33.03 (3.41)	23.33 (2.97)	5.05 (0.59) <sup>a</sup>	10.45 (1.39) <sup>a</sup>	17.95 (1.86)
Control group	28.23 (3.03)	31.94 (3.25)	22.43 (2.46)	4.51 (0.37)	9.32 (1.31)	17.73 (1.16)	27.97 (2.85)	31.29 (4.22)	22.68 (3.22)	4.55 (0.59)	9.16 (1.48)	17.47 (1.72)
<b>Right cerebellar cortex</b>												
FEP group	24.46 (3.57) <sup>a,b</sup>	26.63 (4.48) <sup>b</sup>	19.49 (3.05) <sup>a</sup>	5.41 (0.95)	14.38 (2.68)	19.54 (2.73)	25.19 (4.73)	28.98 (5.68) <sup>a</sup>	19.96 (4.24) <sup>b</sup>	5.58 (1.14)	15.07 (3.54)	20.32 (3.89)
Control group	22.24 (3.24)	26.42 (4.51)	17.13 (3.78)	4.75 (1.13)	13.84 (2.71)	19.05 (3.78)	23.88 (4.58)	27.20 (4.55)	17.70 (4.02)	4.93 (1.20)	14.64 (3.10)	19.95 (3.97)

Abbreviations: Cr + PCr, creatine-containing compounds; FEP, first-episode psychosis; Glu, glutamate; Glx, glutamate + glutamine; GPC + PCh, choline-containing compounds; Ins, *myo*-inositol; NAA, *N*-acetylaspartate.

<sup>a</sup>  $P < .05$ .

<sup>b</sup> One spectrum was rejected because of a Cramer-Rao lower bound exceeding 20%.

**Table 3. Cramer-Rao Lower Bound for Each Metabolite in 2 Regions of Interest at Baseline and at 4 Weeks in the FEP Group and the Control Group**

Variable	Mean (SD)											
	At Baseline						At 4 wk					
	Glu	Glx	NAA	GPC + PCh	Ins	Cr + PCr	Glu	Glx	NAA	GPC + PCh	Ins	Cr + PCr
Right associative striatum												
FEP group	6.67 (0.92)	8.38 (1.28)	3.38 (1.01)	3.58 (0.72)	8.33 (1.34)	3.00 (0.51)	7.17 (2.33)	8.79 (2.00)	4.08 (1.25)	4.00 (0.93)	9.58 (2.52)	3.29 (0.95)
Control group	6.83 (1.04)	8.33 (1.64)	4.22 (1.86)	4.00 (0.90)	9.61 (2.06) <sup>a</sup>	3.28 (0.57)	6.56 (0.86)	8.17 (1.76)	3.33 (1.19)	3.72 (0.46)	9.78 (2.98)	2.94 (0.42)
Right cerebellar cortex												
FEP group	7.00 (1.13) <sup>b</sup>	8.58 (1.81) <sup>b</sup>	4.88 (1.30)	3.42 (1.25)	6.38 (2.31)	2.58 (1.32)	7.42 (1.71)	8.88 (1.96) <sup>b</sup>	4.88 (1.03)	3.33 (0.56)	6.29 (1.23)	2.58 (0.72)
Control group	7.61 (1.50)	9.39 (1.85)	4.72 (1.32)	3.44 (0.51)	6.39 (0.98)	2.39 (0.50)	7.11 (1.08)	8.33 (1.32)	4.39 (0.85)	3.33 (0.48)	5.89 (1.08)	2.33 (0.48)

Abbreviations: Cr + PCr, creatine-containing compounds; FEP, first-episode psychosis; Glu, glutamate; Glx, glutamate + glutamine; GPC + PCh, choline-containing compounds; Ins, *myo*-inositol; NAA, *N*-acetylaspartate.

<sup>b</sup>One spectrum was rejected because of a Cramer-Rao lower bound exceeding 20%.

<sup>a</sup>*P* < .05.

**Table 4. The FWHM and SNR in 2 Regions of Interest at Baseline and at 4 Weeks in the FEP Group and the Control Group**

Variable	Mean (SD)		<i>P</i> Value
	FEP Group	Control Group	
Right associative striatum at baseline			
FWHM, ppm	0.07 (0.01)	0.08 (0.01)	.03
SNR	15.46 (2.39)	14.78 (2.88)	.41
Right associative striatum at 4 wk			
FWHM, ppm	0.08 (0.01)	0.08 (0.01)	.38
SNR	13.83 (4.38)	15.78 (2.26)	.10
Right cerebellar cortex at baseline			
FWHM, ppm	0.07 (0.01)	0.07 (0.01)	.51
SNR	17.69 (2.60)	17.00 (3.06)	.44
Right cerebellar cortex at 4 wk			
FWHM, ppm	0.07 (0.01)	0.06 (0.01)	.29
SNR	16.54 (3.20)	18.55 (2.72)	.04

Abbreviations: FEP, first-episode psychosis; FWHM, full-width at half maximum; SNR, signal-to-noise ratio.

striatum at 4 weeks and the PANSS positive subscale score at 4 weeks (*r* = 0.451, *df* = 19, *P* = .04).

### CRLB, FWHM, and Signal-to-Noise Ratios

The CRLB values for each metabolite in the associative striatum and the cerebellum are given in **Table 3**. Controls had a higher Ins CRLB compared with patients in the associative striatum at baseline (*t*<sub>40</sub> = 1.28, *P* = .02) but not after 4 weeks (*t*<sub>40</sub> = 0.22, *P* = .82). No differences in CRLBs were observed in the associative striatum for other metabolites at baseline or after 4 weeks. No differences in CRLBs were observed in the cerebellum for any condition. The FWHM values and signal-to-noise ratios are given in **Table 4**. Patients had lower FWHM than controls in the caudate at baseline (*t*<sub>40</sub> = 2.24, *P* = .03). Patients also had lower signal-to-noise ratios compared with controls in the cerebellum at 4 weeks (*t*<sub>40</sub> = 2.14, *P* = .04).

### Effects of Tissue Heterogeneity

The percentages of GM in the associative striatum did not differ between patients and controls at baseline (*t*<sub>40</sub> = 0.53, *P* = .59) and at 4 weeks (*t*<sub>40</sub> = 1.59, *P* = .11). The percentages of GM in the cerebellum were lower in the FEP group compared with the control group at baseline (*t*<sub>40</sub> = 5.56, *P* < .001) and at 4 weeks (*t*<sub>40</sub> = 4.65, *P* < .001). In addition to these differences in the percentages of GM, the results of the metabolite comparisons did not change regardless of whether the percentages of GM were included as a covariate (data not shown).

## Discussion

To our knowledge, this is the first study to report using a within-participant design that clinically effective antipsychotic treat-

ment normalizes glutamate levels in antipsychotic-naive patients with FEP. In the present study, we found that patients with FEP had increased levels of glutamate in both of the studied regions, the associative striatum and the cerebellum. The results are in agreement with a recent study<sup>18</sup> finding that patients who had FEP and were clinically stable had lower glutamate levels compared with patients who were still symptomatic. Moreover, patients who are antipsychotic naive or minimally exposed to antipsychotic medication<sup>4-8</sup> or are experiencing psychotic-state exacerbations<sup>19,20</sup> have shown elevations in glutamate compounds, while studies<sup>9-12</sup> in medicated patients have demonstrated the same levels or decreased levels of these metabolites compared with control subjects, suggesting that an improvement in clinical symptoms might relate to decreases in glutamate levels. Moreover, a recent study<sup>21</sup> from our group found that antipsychotic-naive individuals who are initially at ultrahigh risk for psychosis and who later transitioned to psychosis had higher glutamate levels in the associative striatum but not in the cerebellum compared with those who did not develop psychosis and compared with healthy controls.

The associative striatum, or cognitive striatum,<sup>22</sup> a region thought to be involved in the pathogenesis of schizophrenia,<sup>23,24</sup> includes the rostral and dorsal part of the caudate nuclei or precommissural dorsal section, also known as the neostriatum. This structure is rich in dopamine afferents and dopamine receptor D<sub>2</sub> and is frequently included in the quantification of *in vivo* occupancy studies of antipsychotics.<sup>25,26</sup> In addition, the associative striatum establishes major connections with the medial prefrontal cortex, which has been implicated in the neurocognitive deficits observed in schizophrenia<sup>27</sup> and in individuals at ultrahigh risk for psychosis who progressed to a psychotic disorder.<sup>28</sup>

The associative striatum, especially the precommissural dorsal caudate, has shown the highest dopamine receptor D<sub>2</sub> availability after acute pharmacologically induced dopamine depletion in antipsychotic-free patients with schizophrenia.<sup>24</sup> In the same study, no differences in receptor availability were observed in the other functional subdivisions of the striatum (limbic and sensorimotor striatum), suggesting that schizophrenia is associated with elevated dopamine function in the associative regions of the striatum.

Although our main interest was to explore differences in glutamate levels associated with a dopamine-rich region, we included the cerebellar cortex for comparison because it has a negligible amount of dopamine receptors and has no dopamine afferents.<sup>29</sup> On the other hand, the associative striatum and the cerebellar cortex have abundant glutamatergic cells and cortical afferents from the frontal cortex.<sup>30</sup> In this sense, one of the differences between the associative striatum and the cerebellum is the dopaminergic afferents, which are restricted to the associative striatum. Although it is tempting to speculate that differences observed in glutamate levels in the associative striatum and not in the cerebellum may be related to dopaminergic tone, our study was not designed to address this question and would require direct quantification of dopamine neurotransmission.

Contrary to a previous report by our group,<sup>13</sup> the present study found increased glutamate levels in the cerebellum during the baseline study but not after antipsychotic treatment. While the mean (SD) glutamate level in the associative striatum decreased from baseline to posttreatment (from 31.34 [4.29] to 29.25 [3.08] institutional units), the mean (SD) level in the cerebellum slightly increased (from 24.46 [3.57] to 25.19 [4.73] institutional units). The Glx levels in the cerebellum did not differ between patients with FEP and control subjects at baseline or at 4 weeks. Nevertheless, studies with larger samples are needed to confirm these results.

Preclinical investigations have shown that an elevation in striatal endogenous glutamate induces an increase in striatal dopamine release.<sup>31</sup> The administration of the glutamate *N*-methyl-*D*-aspartate antagonist ketamine to human control subjects has been shown to enhance amphetamine-induced dopamine release<sup>32</sup> and to decrease striatal <sup>11</sup>C-raclopride binding, reflecting an increase in striatal synaptic dopamine even in the absence of an amphetamine challenge.<sup>33</sup> Other studies<sup>34,35</sup> in humans have documented the interaction between glutamate and dopamine. The conclusion of these studies is that glutamatergic antagonism is associated with an increase in dopamine release in the cerebral cortex and the striatum. In addition, *N*-methyl-*D*-aspartate receptor blockade in healthy humans has been shown to increase glutamine<sup>36</sup> and glutamate<sup>37</sup> levels in the anterior cingulate as measured by <sup>1</sup>H-MRS.

#### Abnormalities in Metabolites Other Than Glutamate

In this study, we report a trend elevation in NAA levels in the associative striatum and higher NAA levels in the cerebellum of patients having FEP compared with control subjects at baseline. Although we replicated these findings from a previous study by our group,<sup>13</sup> they are inconsistent with the results of a meta-analysis<sup>38</sup> and of a study<sup>16</sup> in patients with early schizophrenia. The reasons for these discrepant findings are unclear but may be related to spectroscopic acquisition, differences in patients' clinical status, previous exposure to antipsychotic medication, and the use of ratios (NAA to Cr or PCh) instead of absolute metabolite levels. *N*-acetylaspartate, one of the prominent peaks shown consistently in <sup>1</sup>H-MRS, is present almost exclusively in neurons and is thought to be a marker of neuronal functional integrity<sup>39</sup> and of axonal mitochondrial metabolism.<sup>40</sup> Higher NAA levels may be driven by increased axonal mitochondrial metabolism to maintain axonal conduction.<sup>41</sup>

Elevated levels of choline-containing compounds (represented collectively as GPC + PCh) have been interpreted as being supportive of the membrane hypothesis of schizophrenia,<sup>42</sup> suggesting that phospholipid disturbances and increased myelin degradation support a generalized membrane disorder in patients with schizophrenia.<sup>43</sup> Our results of elevated levels of GPC + PCh in the associative striatum of patients having FEP are in agreement with previous evidence in patients with schizophrenia.<sup>44</sup> Glycerophosphocholine is one of the main products of membrane phospholipid breakdown. The increase in levels of GPC + PCh in the associative striatum observed in the FEP group suggests greater membrane

turnover, which may result from changes in membrane mass or from proliferation of dendrites and synaptic connections in the associative striatum.<sup>45</sup> We also found higher Ins levels in the associative striatum of patients with FEP at baseline and after treatment. These results are in agreement with previous findings in patients with FEP<sup>46</sup> and in treated patients with schizophrenia.<sup>43</sup> This increase in Ins levels could be related to astrocyte activation.<sup>47</sup>

Although we found no difference in the percentages of GM within the associative striatum voxel between patients having FEP and control subjects at baseline or at retest, we observed a decrease in the percentages of the cerebellar GM voxel in the FEP group compared with the control group at both imaging sessions. This finding is consistent with 2 studies<sup>13,48</sup> in antipsychotic-naïve patients having FEP.

### Study Limitations

The limitations of this study need to be considered. First, we did not include cognitive evaluations. Therefore, we could not address the possible effect of cognition on glutamate levels. Second, we did not measure serum levels of risperidone in the FEP group to confirm treatment compliance; nevertheless, all patients studied were initially seen with a significant reduction of the PANSS total score as a primary measure of treatment efficacy, and patients were admitted to the hospital for supervised administration of the antipsychotic. Third, the groups could not be matched for education; educational levels were higher in the control group than in the FEP group. Parental education and socioeconomic status data were not obtained, but these would be better variables to use for matching given that the patients were young at their diagnosis of FEP and became ill before reaching their full academic potential.

Fourth, direct measurement of glutamate neurotransmission is not possible with <sup>1</sup>H-MRS. This technique measures both metabolic and vesicular glutamate. Fifth, Glx contamination of the NAA peak has been observed at short echo times, including the one used in this study (echo time, 35 milliseconds).<sup>49</sup> Therefore, this potential contamination could be reflected by an artificial increase in NAA when glutamate is increased. Sixth, MMs were not measured, and they have a potential influence on Glx and glutamine quantification. Macromolecule contribution was modeled using the default LCModel function. Seventh, we only studied the right hemisphere for both regions to reduce imaging time in individuals with active psychosis. Nevertheless, during the course of a previous pilot study,<sup>50</sup> our group studied both right and left associative striatum in patients with active psychotic symptoms without observing differences between the right and left sides in terms of glutamate quantification. Moreover, <sup>1</sup>H-MRS studies that have assessed both hemispheres have failed to demonstrate distinct glutamatergic laterality in FEP,<sup>46</sup> in individuals at high risk,<sup>51</sup> in childhood-onset schizophrenia,<sup>52</sup> and in patients with schizophrenia.<sup>11,53</sup> However, we acknowledge that bilateral <sup>1</sup>H-MRS examinations of the associative striatum are necessary to generalize these findings.

In conclusion, our results indicate initially increased glutamate levels in the associative striatum and in the cerebellum in antipsychotic-naïve patients with FEP. After clinically effective antipsychotic treatment, glutamate levels significantly decreased in the associative striatum (Cohen *d* effect size, 0.47), with minor increases in the cerebellum (Cohen *d* effect size, -0.13). Overall, the findings suggest that glutamate levels in the associative striatum normalized after effective antipsychotic treatment during the first episode of psychosis.

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