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Higher Levels of Glutamate in the Associative-Striatum of Subjects with Prodromal Symptoms of Schizophrenia and Patients with First-Episode Psychosis

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The glutamatergic and dopaminergic systems are thought to be involved in the pathophysiology of schizophrenia. Their interaction has been widely documented and may have a role in the neurobiological basis of the disease. The aim of this study was to compare, using proton magnetic resonance spectroscopy (^{1}H-MRS), glutamate levels in the precommissural dorsal-caudate (a dopamine-rich region) and the cerebellar cortex (negligible for dopamine) in the following: (1) 18 antipsychotic-naïve subjects with prodromal symptoms and considered to be at ultra high-risk for schizophrenia (UHR), (2) 18 antipsychotic-naïve first- episode psychosis patients (FEP), and (3) 40 age- and sex- matched healthy controls. All subjects underwent a ^{1}H-MRS study using a 3Tesla scanner. Glutamate levels were quantified and corrected for the proportion of cerebrospinal fluid and percentage of gray matter in the voxel. The UHR and FEP groups showed higher levels of glutamate than controls, without differences between UHR and FEP. In the cerebellum, no differences were seen between the three groups. The higher glutamate level in the precommissural dorsal-caudate and not in the cerebellum of UHR and FEP suggests that a high glutamate level (a) precedes the onset of schizophrenia, and (b) is present in a dopamine-rich region previously implicated in the pathophysiology of schizophrenia.

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INTRODUCTION

Schizophrenia is a chronic mental illness characterized by psychotic or positive symptoms (hallucinations and delusions), and negative and cognitive symptoms (apathy, social withdrawal, decreased attention, decreased executive function, abnormal psychomotor speed of processing and impairment of verbal memory) (APA, 2000; Rajji *et al*, 2009). Despite treatment advances, schizophrenia remains as a seriously disabling, lifelong illness, that is among the world's top ten causes of long-term disability (Saraceno, 2002). The onset of schizophrenia is usually preceded by a 'prodromal phase', characterized by subthreshold psychotic symptoms, a high likelihood of a family history of schizophrenia, and a decline in everyday functioning (Yung and McGorry, 1996). Longitudinal studies, in developed countries, have found that between 19% and 35% of individuals with prodromal symptoms experience conversion to a primary psychotic illness across 1- to 2.5-year follow-up intervals (Yung *et al*, 2003; Cannon *et al*, 2008; Ruhrmann *et al*, 2010), which provides an ideal window of opportunity to assess biomarkers associated with progression of full-blown psychotic illness.

At the present time, pharmacological management of schizophrenia is based on antagonists or partial agonists of the dopamine D_2 receptors (Kapur *et al*, 2000; Seeman and Kapur, 2000; Mamo *et al*, 2007). Although pharmacotherapy improves psychotic symptoms in most patients, improvement in negative and cognitive symptoms is at best minimal (Lieberman *et al*, 2005), and specific therapeutic strategies have yet to be tested in adequately powered samples to fully assess their impact on the management and conversion rate of the prodromal phase (Cadenhead *et al*, 2010).

The clinical effect of dopamine antagonists has been the basis for 'The dopamine hypothesis of schizophrenia'

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(Howes and Kapur, 2009a), which posits an aberrant function of the dopaminergic system in patients with schizophrenia (Sato *et al*, 1992; Laruelle *et al*, 1996; Abi-Dargham *et al*, 1998; Hietala *et al*, 1999; Kapur, 2003). The hypothesis has been bolstered by the recent observation of an elevated striatal (18)F-dopa uptake in subjects with prodromal symptoms (Howes *et al*, 2009b).

Although the dopamine hypothesis has been a useful model in our understanding and study of the psychotic state, it does not explain the deteriorating course in terms of cognition and function seen in the first few years of schizophrenia. Glutamate antagonists are well known to induce positive and negative psychotic symptoms more akin to schizophrenia than the positive symptoms induced by dopamine agonists alone (Javitt, 2007). It has been proposed that this deterioration course may be partially explained by cortical neuronal toxicity secondary to enhanced glutamate exposure (Sharp *et al*, 2001), which in turn is thought to reflect a compensatory increase in cortical glutamatergic activity due to hypofunction of the *N*-methyl-D-aspartate (NMDA) receptor (Olney and Farber, 1995).

The interaction between glutamate and dopamine is widely documented (Cepeda and Levine, 1998; Levine and Cepeda, 1998; Kulagina *et al*, 2001; West *et al*, 2003; David *et al*, 2005). In schizophrenia, dopaminergic dysregulation is thought to be the final common pathway resulting from an altered glutamatergic neurotransmission (Carlsson and Carlsson, 1990; Javitt and Zukin, 1991; Olney and Farber, 1995). Disruption of the cortical glutamatergic afferents induce decreased tonic dopamine release with a subsequent disinhibition of phasic dopamine release, causing abnormal responses to insignificant stimuli (Grace, 1991, 1993, 2000).

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) studies in humans have provided evidence that non-competitive glutamate NMDA receptor antagonists, such as ketamine, increase amphetamine-induced dopamine release (Kegeles *et al*, 2000), and decrease $D_{2/3}$ binding in the posterior cingulate cortex (Aalto *et al*, 2005) and striatum (Breier *et al*, 1998; Smith *et al*, 1998; Vollenweider *et al*, 2000). Other studies though have not supported this finding in the striatum (Aalto *et al*, 2002; Kegeles *et al*, 2002).

Recent developments in proton magnetic resonance spectroscopy (^{1}H-MRS) permit the *in vivo* study of the glutamatergic system (Di Costanzo *et al*, 2003; Abbott and Bustillo, 2006; Di Costanzo *et al*, 2007).

We therefore used ^{1}H-MRS to compare the glutamate levels in antipsychotic-naïve subjects with prodromal symptoms, antipsychotic-naïve patients with a first episode of psychosis and sex-and-age similar healthy controls. We selected two regions of interest for our analysis: the precommissural dorsal caudate, which is characterized by a high density of dopamine receptors and dopamine afferents, and the cerebellum, a brain region with negligible quantity of dopamine receptors and absence of dopamine afferents. It was recently shown that both patients with schizophrenia and prodromal subjects have higher dopamine levels in the associative striatum (Howes *et al*, 2009b; Kegeles *et al*, 2010), of which the precommissural dorsal caudate (henceforth, dorsal caudate) is the main component (Mawlawi *et al*, 2001). Our hypothesis was that subjects with prodromal symptoms and patients with a first episode of psychosis would show higher glutamate than controls in the dorsal caudate as measured by ^{1}H-MRS, and no differences of glutamate between groups in the cerebellum.

MATERIALS AND METHODS

Clinical Sample

The study was approved by the Ethics and Scientific Committees of the National Institute of Neurology and Neurosurgery of Mexico (INNN). All the subjects were included following successful completion of an informed consent procedure, and with written consent of both parents for subjects under 18 years old.

Eighteen subjects with ultra high-risk for schizophrenia–or prodromal symptoms (UHR), and 18 patients during their first non-affective psychotic episode (FEP) were recruited from the inpatient psychiatric service, first psychotic episode clinic, and the Adolescent Program of Neuropsychiatric and Imaging Study (PIENSA) of the INNN. All subjects were interviewed using the structured clinical interview for DSM-IV (First *et al*, 1997) and the UHR group met Structured Interview for Prodromal Syndromes (SIPS) criteria (Miller *et al*, 2003) for study entry. Both groups were antipsychotic naïve, and were capable to grant informed consent. Patients were excluded if they had the following: any concomitant medical or neurological illness, current substance abuse or history of substance dependence (excluding nicotine), comorbidity of any other axis I disorders, were considered to be at high risk for suicide, or show psychomotor agitation. Use of psychotropic medications was not permitted for the duration of the study. Forty right-handed age- and gender-matched healthy controls were also recruited. The control subjects were assessed in the same manner as the patients and any subject with a history of psychiatric illness or positive familiar history for schizophrenia was excluded. All participants were screened for drugs of abuse (eg, cannabis, cocaine, heroin, opioids and benzodiazepines) before the ^{1}H-MRS studies.

Magnetic Resonance Studies

The ^{1}H-MRS studies were performed in a 3T GE (GE Healthcare, Milwaukee, WI) whole-body scanner with a high-resolution 8-channel head coil (Invivo, Orlando, FL). The participant's head was positioned along the cantho-meatal line and immobilized by means of a forehead strap. ^{1}H-MRS spectra were obtained using point-resolved spectroscopy (PRESS; TE = 35 ms, TR = 2000 ms, spectral width = 5000 Hz, 4096 data points used, 128 water-suppressed, and 16 water-unsuppressed averages) in volume elements (voxels) of 8 ml ($2 \times 2 \times 2$ cm) centered in the right dorsal caudate nucleus and right cerebellar cortex in all studied subjects. This acquisition allowed for the quantification of glutamate (Glu), *N*-acetyl-aspartate (NAA), creatine (Cr), phosphocreatine (PCr), glycerophosphocholine (GPC), phosphocholine (PCh), and myo-inositol (mI).

The spectra were shimmed to achieve full width at half maximum (FWHM) $\leqslant$12 Hz (measured on the unsuppressed water signal from the voxel), and spectra with larger

FWHM were excluded. The voxels were defined in a T_1 weighed volumetric image in axial projection (SPGR, TE = 5.7 ms, TR = 13.4 ms, TI = 450 ms, flip angle = 20°, FOV = 25.6 cm, 256 × 256 matrix, slice thickness = 1.2 mm) and oriented above and parallel to the anterior (AC) to the posterior commissure (PC).

The lower end of the dorsal caudate (or associative-striatum) voxel was located 3 mm dorsal to the AC, including the maximum amount of gray matter and with a dorsal extension (thickness) of 2 cm. The cerebellar voxel was located in the cerebellar cortex below the inferior cerebellar peduncle avoiding the midline (Figure 1). All spectra were analyzed with the Linear Combination Model included in the LCModel program, version 6.2-1T (Provencher, 1993). The metabolites included in the basis set were as follows: L-alanine (Ala), aspartate (Asp), Cr, γ-aminobutyric acid (GABA), glucose (Glc), glutamine (Gln), Glu, GPC, PCh, L-lactate (Lac), mI, NAA, *N*- acetylaspartylglutamate acid (NAAG), *Scyllo*-inositol (Scyllo), taurine (Tau), Cr methylene group (–CrCH2), guanidinoacetate (Gua), GPC + PCh, NAA + NAAG, Glu + Gln, lipids (Lip), and macromolecules (MM): Lip13a, Lip13b, Lip09, MM09, Lip20, MM20, MM12, MM14, MM17, Lip13a + Lip13b, MM14 + Lip13a + Lip13b + MM12, MM09 + Lip09, and MM20 + Lip20. All metabolites with Cramer-Rao lower-bound >20% reported by LCModel were excluded.

SPGR scans used for localization of the voxels were subsequently segmented into gray matter, white matter, and cerebrospinal fluid (CSF) using Statistical Parametric Mapping 8 (Friston *et al*, 1995) (SPM8, Wellcome Department of Cognitive Neurology, London; http://www.fil.ion.ucl.ac.uk/spm). Then, the size and location of each area from the spectra file headers were extracted to calculate the percentage of gray, white, and CSF content within the voxel, allowing for correction of the CSF fraction of the spectroscopic values. CSF correction assumed zero metabolite concentration in the CSF using the equation $SV_{corr} = SV/(1-CSF)$, where SV are the spectroscopic values, CSF is the CSF fraction within the voxel and SV_{corr} are the corrected spectroscopic values.

Statistical Analysis

The results are presented in means and standard deviations (± SD). Statistical analyses were performed using SPSS v16.0 software (SPSS, Chicago, IL). Demographic and clinical characteristics of the sample were compared between controls, FEP, and UHR groups with analysis of variance (ANOVA), with the exception of frequency data. Frequency data were analyzed using χ^2 or Fisher's exact tests. Metabolite measures between groups were compared using a general linear model (GLM). The percentages of gray matter in the dorsal caudate and cerebellum, as well as age were included as covariates in the GLM. On the basis of significant main effects per metabolite and region, *post hoc* comparisons with Bonferroni correction were performed. The statistical comparisons were carried out at a significance level set at $p < 0.05$.

Partial Spearman correlations controlling for the effect of gray matter were conducted to examine the relationship between clinical scales and Glu concentration for each region. Spearman correlations (non-parametric) rather than Pearson coefficients were used due to the relatively small sample size. The statistical threshold was established with $p \leqslant 0.05$ to control for multiple comparisons, $p = 0.05/4$ clinical scales for UHR group (SIPS positive, negative, disorganization, general), and $p = 0.05/3$ clinical scales for FEP (PANSS positive, negative, and general).

RESULTS

Demographic and Clinical Characteristics

The UHR group was younger than the FEP ($F_{[2,73]} = 3.60$, $p = 0.03$), but both groups did not differ from controls. Education was higher in controls compared with FEP and UHR groups ($F_{[2,73]} = 9.59$, $p < 0.001$). One subject in the UHR and two in the FEP groups previously used cannabis (Fisher's exact test = 6.12, $p = 0.03$). Four subjects in the UHR group were taking a selective serotonin reuptake inhibitor (two fluoxetine, one paroxetine, one sertraline) at the time of the study (Fisher's exact test = 9.26, $p = 0.005$). UHR and FEP groups did not differ in gender, handedness, or tobacco use compared with controls (see Table 1).

Cerebral Metabolites

There was a significant difference in Glu levels in the dorsal caudate between groups ($F_{[2,73]} = 5.99$, $p = 0.004$). *Post hoc* tests revealed higher Glu in both UHR and FEP groups than controls ($p = 0.04$ and $p = 0.01$, respectively), with no statistical difference between FEP and UHR groups ($p = 0.64$). On the contrary, cerebellar Glu concentrations did not differ significantly between the three groups

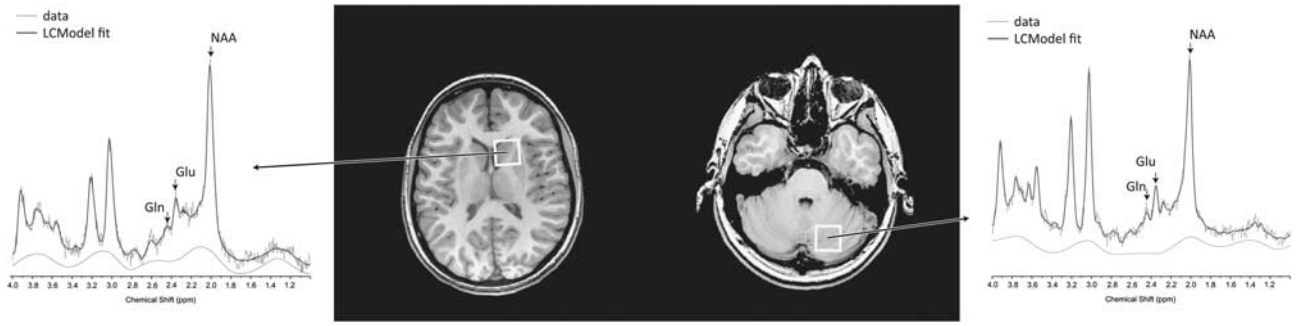

Figure 1 Spectroscopic voxel placement in right dorsal caudate and right cerebellum and representative spectra for each region. Glu, glutamate; Gln, glutamine; NAA, *N*-acetylaspartate.

Table 1 Demographic and Clinical Characteristics of the Sample

	Control subjects	UHR	FEP	Statistic
Age (±SD) years	21.83 ± 4.47	19.56 ± 3.46	23.44 ± 4.93[a]	$F_{[2,73]} = 3.60$, $p = 0.03$
Gender (male/female)	28/12	14/4	10/8	Fisher's = 2.09 NS
Education (±SD) years	14.47 ± 3.32	10.67 ± 2.61[b]	12.11 ± 3.51[b]	$F_{[2,73]} = 9.60$, $p < 0.001$
Handedness (right/left)	40/0	18/0	18/0	NS
Length of illness (±SD) weeks	NA	16.56 ± 12.28	18.72 ± 18.14	NS
Tobacco (ever used)	9/40	1/18	6/18	Fisher's = 4.32 NS
Cannabis (ever used)	0/40	1/18	2/18	Fisher's = 6.12, $p = 0.03$
Use of antipsychotic treatment	0/40	0/18	0/18	NS
Use of SSRIs	0/40	4/18	0/18	Fisher's = 9.26, $p = 0.005$
PANSS positive symptoms			22.17 ± 3.94	
PANSS negative symptoms			26.72 ± 6.87	
PANSS general symptoms			51.44 ± 15.23	
SIPS positive symptoms		12.72 ± 3.95		
SIPS negative symptoms		17.94 ± 5.73		
SIPS disorganization symptoms		9.28 ± 3.14		
SIPS general symptoms		8.94 ± 3.55		

Abbreviations: NA, not applicable; NS, not significant; SSRIs, selective serotonin reuptake inhibitors.
Significant *post hoc* pair-wise comparisons with Bonferroni correction ($p < 0.05$) are indicated: [a]*vs* UHR; [b]*vs* controls

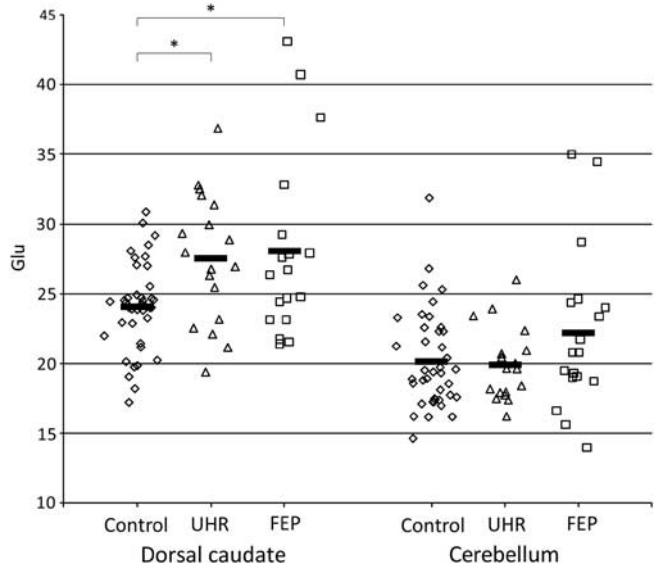

Figure 2 Glutamate (Glu) levels of each participant in the dorsal caudate and cerebellum of controls, ultra high-risk for schizophrenia (UHR), and first episode-psychosis patients (FEP). Bars represent the mean for that group. *vs control, $p < 0.05$.

($F_{[2,73]} = 1.41$, $p = 0.25$) (Figure 2). Glutamine (Gln) was not analyzed due to poor spectra fitting in the caudate (number of spectra rejected in controls = 37, UHR = 18, and FEP = 17) and the cerebellum (number of spectra rejected in controls = 40, UHR = 18, and FEP = 18).

NAA levels in the dorsal caudate were higher among patient groups ($F_{[2,73]} = 6.72$, $p = 0.002$), with higher NAA in the UHR group compared with controls ($p = 0.002$). Although NAA was also higher in the FEP group, this finding did not survive correction for multiple comparisons (Table 2). We also found a difference in NAA concentration between groups in the cerebellum ($F_{[2,73]} = 7.65$, $p = 0.001$), with higher NAA in the FEP group *vs* both controls ($p = 0.003$) and the UHR group ($p = 0.001$).

GPC + PCh concentrations, for which we had no *a priori* hypotheses, in the dorsal caudate and cerebellum were different between groups ($F_{[2,73]} = 7.57$, $p = 0.001$ and $F_{[2,73]} = 4.03$, $p = 0.02$, respectively), with higher GPC + PCh in the FEP group compared with controls in the dorsal caudate ($p = 0.002$), as well as the cerebellum ($p = 0.03$).

No differences in Glu + Gln were found between groups in dorsal caudate ($F_{[2,73]} = 2.11$, $p = 0.13$). Although an effect was found in Glu + Gln in the cerebellum ($F_{[2,73]} = 3.39$, $p = 0.04$), *post hoc* analysis did not show any differences between groups.

The results of metabolite comparisons did not change when age was included as a covariate (data not shown). No significant correlations were found in the dorsal caudate or cerebellum between any clinical measure in UHR or FEP groups and all the metabolites concentrations.

The percentages of gray matter in the dorsal caudate and cerebellum were different between the three groups ($F_{[2,73]} = 3.44$, $p = 0.04$, $F_{[2,73]} = 9.38$, $p = 0.001$, respectively). The *post hoc* analysis showed that the gray matter in the dorsal caudate was lower in the UHR group than in FEP ($p = 0.03$). The gray matter in the cerebellum was lower in the FEP in comparison with controls ($p = 0.002$) and with UHR ($p = 0.001$). Besides the differences of the percentage of gray matter found in dorsal caudate and cerebellum between the groups, the results of metabolites comparisons did not change whether or not the percentages of gray matter were included as a covariate (data not shown).

Glu and NAA levels were correlated in the caudate and cerebellum of all participants ($r = 0.77$, $p \leqslant 0.001$ and $r = 0.71$, $p \leqslant 0.001$, respectively). Moreover, Glu and NAA

Table 2 Means (±SD) for Each Metabolite in Dorsal Caudate and Cerebellum

	Region	Dorsal caudate			Statistic	Cerebellum			Statistic
	Group	Control	UHR	FEP		Control	UHR	FEP	
Metabolite									
Glu		24.08 ± 3.16	27.54 ± 4.67[a]	27.79 ± 4.29[a]	$F_{[2,73]} = 5.99$	20.25 ± 3.01	19.78 ± 2.13	22.94 ± 4.57	$F_{[2,73]} = 1.41$
		$n = 40$	$n = 18$	$n = 18$	$p = 0.004$	$n = 40$	$n = 18$	$n = 18$	$p = 0.25$
Glu+Gln		32.47 ± 3.48	34.26 ± 5.13	35.38 ± 5.13	$F_{[2,73]} = 2.11$	25.54 ± 5.06	25.16 ± 5.18	29.34 ± 5.51	$F_{[2,73]} = 3.39$
		$n = 40$	$n = 18$	$n = 18$	$p = 0.13$	$n = 40$	$n = 18$	$n = 18$	$p = 0.04$
NAA		21.45 ± 2.30	24.69 ± 2.52[a]	23.09 ± 3.02	$F_{[2,73]} = 6.72$	15.45 ± 2.75	14.56 ± 2.07	19.03 ± 3.94[a,b]	$F_{[2,73]} = 7.65$
		$n = 40$	$n = 18$	$n = 18$	$p = 0.002$	$n = 40$	$n = 18$	$n = 18$	$p = 0.001$
GPC+PCh		4.24 ± 3.22	4.65 ± 0.28	4.89 ± 0.70[a]	$F_{[2,73]} = 7.57$	4.63 ± 0.83	4.57 ± 0.42	5.43 ± 1.13[a]	$F_{[2,73]} = 4.03$
		$n = 40$	$n = 18$	$n = 18$	$p = 0.001$	$n = 40$	$n = 18$	$n = 18$	$p = 0.02$
mI		9.01 ± 1.87	8.87 ± 1.72	10.14 ± 1.21	$F_{[2,73]} = 3.11$	14.68 ± 2.43	12.81 ± 1.71	14.46 ± 2.66	$F_{[2,73]} = 3.01$
		$n = 39$	$n = 18$	$n = 18$	$p = 0.05$	$n = 40$	$n = 18$	$n = 18$	$p = 0.06$
Cr+PCr		17.35 ± 1.83	18.22 ± 2.14	18.69 ± 3.02	$F_{[2,73]} = 2.11$	19.76 ± 3.03	18.30 ± 2.19	20.06 ± 3.19	$F_{[2,73]} = 1.43$
		$n = 40$	$n = 18$	$n = 18$	$p = 0.13$	$n = 40$	$n = 18$	$n = 18$	$p = 0.25$

Abbreviations: Cr, creatine; FEP, first episode of psychosis group; Gln, glutamine; Glu, glutamate; GPC, glycerophosphocholine; mI, myo-inositol; n, number of spectra analyzed; NAA, *N*-acetyl-aspartate; PCh, phosphocholine; PCr, phosphocreatine; UHR, ultra high-risk for schizophrenia group.
Significant *post hoc* pair-wise comparisons with Bonferroni correction ($p < 0.05$) are indicated: [a]*vs* controls; [b]*vs* UHR.

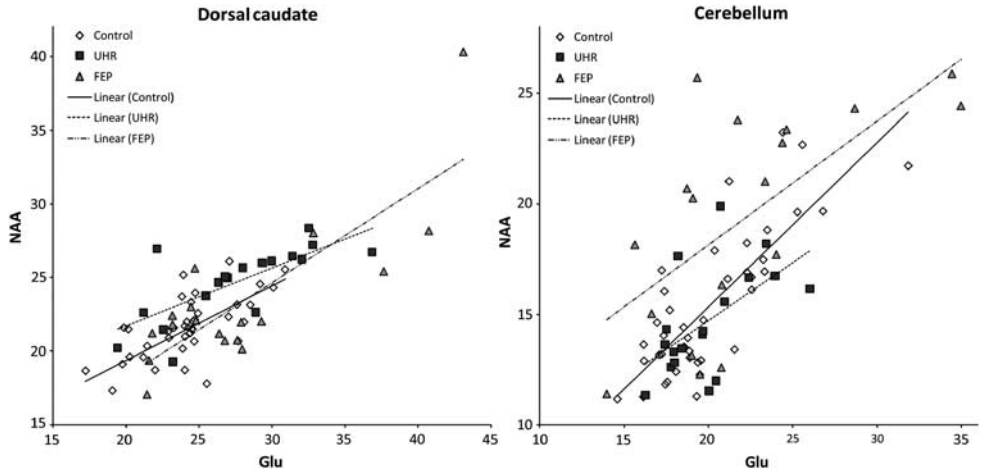

Figure 3 Correlation between glutamate (Glu) and *N*-acetylaspartate (NAA) in the dorsal caudate and cerebellum of the participants. Dorsal caudate: Pearson's $r = 0.70$, $p \leqslant 0.001$ in controls, Pearson's $r = 0.74$, $p \leqslant 0.001$ in ultra high-risk for schizophrenia (UHR), Pearson's $r = 0.78$, $p \leqslant 0.001$ in first episode-psychosis patients (FEP). Cerebellum: Pearson's $r = 0.81$, $p \leqslant 0.001$ in controls, Pearson's $r = 0.56$, $p = 0.01$ in UHR, Pearson's $r = 0.64$, $p = 0.002$ in FEP.

levels were correlated in the caudate of controls ($r = 0.70$, $p \leqslant 0.001$), UHR ($r = 0.74$, $p \leqslant 0.001$), and FEP groups ($r = 0.78$, $p \leqslant 0.001$), and the cerebellum of controls ($r = 0.81$, $p \leqslant 0.001$), UHR ($r = 0.56$, $p = 0.01$), and FEP groups ($r = 0.64$, $p = 0.002$) (Figure 3).

DISCUSSION

Summary of Main Results

Our results confirmed our hypothesis that subjects with prodromal symptoms of schizophrenia and unmedicated subjects experiencing a first episode of psychosis, have increased Glu in the dorsal caudate but not in the cerebellum. The absence of differences in the cerebellum suggests that the alterations in Glu in subjects with psychotic and pre-psychotic symptoms are not ubiquitous within the brain, and that the differences may be restricted to brain dopamine-rich regions such as the associative striatum, a region thought to be involved in the pathophysiology of schizophrenia (Howes *et al*, 2009b; Kegeles *et al*, 2010).

The associative striatum (or cognitive striatum) (Mawlawi *et al*, 2001) includes the rostral and dorsal part of the caudate nuclei or precommissural dorsal section, also known as neostriatum (Brodal, 2004). This structure is rich in dopamine afferents and D_2 receptors, and is frequently included in the quantification of *in vivo* occupancy of

antipsychotics (Farde *et al*, 1988; Graff-Guerrero *et al*, 2008). In addition, the associative striatum establishes major connections with the frontal lobe (Lehericy *et al*, 2004). The frontal cortex has been implicated in the neurocognitive deficits seen in schizophrenia (Villalta-Gil *et al*, 2006), deficits which are also present in subjects with prodromal symptoms and FEP patients (Cadenhead, 2002; Jahshan *et al*, 2010).

The associative striatum, and especially the precommissural dorsal caudate, has shown the highest D_2 receptor availability after acute pharmacologically induced dopamine depletion in antipsychotic-free patients with schizophrenia (Kegeles *et al*, 2010). In this same study, no differences in receptor availability were seen in the other functional subdivisions of the striatum (limbic and sensorimotor striatum), suggesting that schizophrenia is associated with elevated dopamine function in associative regions of the striatum. Moreover, UHR subjects had elevated DOPA decarboxylase activity in this same functional region, suggesting that the subcortical dopamine synthesis is enhanced before the expression of psychosis (Howes *et al*, 2009b).

Although our main interest was to explore the differences in Glu associated with a dopamine rich region, we included the cerebellar cortex for comparison. The cerebellar cortex has a negligible amount of dopamine receptors and has no dopamine afferents (De Keyser *et al*, 1988; Camps *et al*, 1989). On the other hand, both the dorsal caudate and the cerebellar cortex are abundant in glutamatergic cells (Brodal, 2004) and cortical afferents from the frontal cortex (Schmahmann and Pandya, 1995; Middleton and Strick, 2001; Dum and Strick, 2003; Kelly and Strick, 2003). In this sense, one of the differences between the dorsal caudate and the cerebellum are the dopaminergic afferents, which are restricted to the dorsal caudate. Although it is tempting to speculate that some differences observed in the Glu in the dorsal caudate may be related to the dopaminergic tone, our study was not designed to address this question. Although, in agreement with our results, preclinical studies have shown that elevation in striatal endogenous Glu induces an increase in striatal dopamine release (Segovia *et al*, 1997). Preliminary results from our group (de la Fuente-Sandoval *et al*, 2009) have shown that patients with schizophrenia during an acute psychotic episode and after 6 weeks of antipsychotic treatment presented with higher Glu levels in the dorsal caudate compared with controls, and with no differences in the cerebellum. These data suggest that higher Glu levels are a stable finding in the dorsal caudate regardless of treatment. However, we acknowledge that to confirm the association between higher Glu with dopamine, a direct measure of both Glu and dopamine should be performed in the same subject.

Glutamate-Dopamine Interaction and Schizophrenia

Abnormal interaction between Glu and dopamine neurotransmission systems has been shown in preclinical model of schizophrenia (Grace, 2000). In addition, using SPECT in normal controls, Kegeles *et al*. (Kegeles *et al*, 2000) demonstrated that ketamine administration enhanced an increase in amphetamine-induced dopamine release. In a PET study, ketamine administration resulted in a decrease in striatal [^{11}C]-raclopride binding, reflecting an increase on striatal synaptic dopamine. Moreover, this decrease in [^{11}C]-raclopride binding was similar to the decrease shown after amphetamine administration (Breier *et al*, 1998). Others (Smith *et al*, 1998; Vollenweider *et al*, 2000; Aalto *et al*, 2005) have also documented this interaction, concluding that glutamatergic antagonism is associated to an increase of dopamine release in the cerebral cortex and the striatum, and inducing positive and negative symptoms similar to those observed in schizophrenia. In addition, NMDA-receptor blockade in healthy humans has been shown to increase Gln levels in the anterior cingulate as measured by ^{1}H-MRS (Rowland *et al*, 2005).

Glu system dysfunction has been suggested to have a role in schizophrenia (Carlsson and Carlsson, 1990; Javitt and Zukin, 1991; Olney and Farber, 1995). Uncompetitive NMDA receptor antagonists, such as phencyclidine (PCP) and ketamine, induce the full range of positive and negative psychotic symptoms observed in schizophrenia patients (Javitt, 2007). In rats, ketamine administration produced an increase in glutamate release in prefrontal cortex (Moghaddam *et al*, 1997).

The results of this study, which show higher Glu levels in subjects with pre-psychotic and psychotic symptoms in the dorsal caudate, a brain region previously associated with enhanced dopaminergic transmission in schizophrenia, are consistent with our hypothesis. However, it is important to note that direct measurement of Glu neurotransmission is not possible with ^{1}H-MRS. This technique measures both metabolic and vesicular Glu. A summary of findings from previous studies that measured Glu are presented in Table 3. The reasons for the discrepant findings between studies are unclear; nevertheless, our study illustrates, using a within subject design, that Glu levels are elevated in the dorsal caudate in comparison with the cerebellum.

Glutamate and Neuronal Degeneration

Excessive glutamatergic activity is also associated with neuronal degeneration (Olney *et al*, 1989). Our study revealed a lower percentage of gray matter in caudate voxels of UHR participants, which may be secondary to excessive glutamate levels. Other studies have also reported a decrease in gray matter in UHR subjects in a variety of other brain regions, including the right medial temporal, lateral temporal, orbitofrontal and cingulate cortex, insula, and inferior frontal and superior temporal gyrus (Pantelis *et al*, 2003; Borgwardt *et al*, 2007; Stone *et al*, 2009). Other factors, however, may have also contributed to our finding of reduced gray matter as a lower percentage of gray matter was not observed in FEP patients. Moreover, our study was not intended to compare gray matter differences between regions, which were principally included in this study as a covariate. As noted above, no differences were seen in the metabolites results whether or not we included this measure as a covariate. Interestingly, we found a decrease in cerebellar gray matter of the FEP group compared with UHR subjects and controls. This finding agrees with previous studies of FEP, drug-naïve patients (Jayakumar *et al*, 2005; Chua *et al*, 2007).

Table 3 Summary of Glutamate ^{1}H-MRS Studies

Author (year)	Tesla	Studied group	Medication status	Brain region	Glutamate results
Bartha *et al*, 1997	1.5	Schizophrenia	Antipsychotic-naïve	Medial prefrontal cortex	No differences
Théberge *et al*, 2002	4	Schizophrenia	Antipsychotic-naïve	Anterior cingulate thalamus	No differences
Théberge *et al*, 2003	4	Schizophrenia	Antipsychotic treated	Anterior cingulate	Decreased
Tibbo *et al*, 2004	3	High genetic risk for schizophrenia	Unmedicated	Medial prefrontal cortex	Glu/Gln increased
Ohrmann *et al*, 2005	1.5	Schizophrenia	Antipsychotic treated	Dorsolateral prefrontal cortex	Glu/Gln increased
van Elst *et al*, 2005	2	Schizophrenia	Antipsychotic treated	Dorsolateral prefrontal cortex hippocampus	Increased
Théberge *et al*, 2007	4	First-episode schizophrenia	Antipsychotic-naïve	Anterior cingulate thalamus	No differences
Olbrich *et al*, 2008	2	Schizophrenia	60% antipsychotic treated	Dorsolateral prefrontal cortex	Increased
Purdon *et al*, 2008	3	High genetic risk for schizophrenia	Unmedicated	Medial prefrontal cortex	Increased
Keshavan *et al*, 2009	1.5	High genetic risk for schizophrenia	Unmedicated	Inferior parietal/occipital cortex	Glu+Gln increased
Stone *et al*, 2009	3	Prodromal	18% Antipsychotic treated	Thalamus	Decreased
Lutkenhoff *et al*, 2010	3	Discordant twins for schizophrenia	Antipsychotic treated	Medial prefrontal cortex	Decreased
Bustillo *et al*, 2010	4	Schizophrenia	Minimally treated	Anterior cingulate	Gln/Glu increased
Stone *et al*, 2010	3	Prodromal	Unreported	Hippocampus	Decreased
Wood *et al*, 2010	3	Prodromal	Antipsychotic-naïve	Hippocampus	No differences

Abbreviations: Gln, glutamine; Glu, glutamate.

Other Metabolites

Our results indicate that Glu levels are significantly correlated with NAA in the caudate and cerebellum in all comparison groups. NAA, one of the prominent peaks consistently shown in ^{1}H-MRS, is present almost exclusively in neurons and is thought to be a marker of neuronal functional integrity (Barker, 2001) and axonal mitochondrial metabolism (Bates *et al*, 1996). Higher NAA levels may be driven by increased axonal mitochondrial metabolism to maintain axonal conduction (Ariyannur *et al*, 2008). It is not surprising then that an increase in Glu, an excitatory neurotransmitter, would be associated with an increase in local neuronal metabolism.

We report higher NAA in the cerebellum of FEP patients compared with both controls and UHR subjects. This is inconsistent with the findings of a recent meta-analysis, (Steen *et al*, 2005) which found no difference in NAA levels between patients with schizophrenia and controls. Another study of early schizophrenia patients with minimal antipsychotic exposure also found no differences in the cerebellum compared with controls (Bustillo *et al*, 2008). The reasons for these discrepant findings are unclear, but may be related to the clinical status of patients, previous exposure to antipsychotic medication, spectroscopic acquisition (single-voxel *vs* chemical shift imaging and varying echo time), and the use of ratios (NAA to Cre or Cho) *vs* metabolite concentrations. Future replication studies are necessary to shed light on these discrepant findings.

Elevated choline containing compounds levels (represented collectively as GPC + PCh) have been interpreted as supportive of the 'membrane hypothesis' of schizophrenia (Horrobin *et al*, 1994), suggesting that phospholipid disturbances and an increased myelin degradation supports a generalized membrane disorder in patients with schizophrenia (Auer *et al*, 2001). Our results in elevated choline containing compounds (GPC + PCh) found in the dorsal caudate of FEP patients agrees with previous reports in patients with schizophrenia (Bustillo *et al*, 2002; Lutkenhoff *et al*, 2010), and in childhood-onset schizophrenia (O'Neill *et al*, 2004). Three previous reports have failed to find differences in choline in the cerebellum of patients with schizophrenia (Eluri *et al*, 1998; Tibbo *et al*, 2000; Bustillo *et al*, 2008).

Glycerophosphocholine is one the main products of membrane phospholipid breakdown. The observed increase in the FEP group but not in the UHR suggests increased membrane turnover that occurs after a switch to FEP, and may result from changes in membrane mass or proliferation of dendrites and synaptic connections in these regions (Stanley *et al*, 2006).

Limitations

Limitations of this study need to be considered. First, we did not include cognitive evaluations: we therefore could not address the possibility of an effect of cognition associated with Glu levels. Second, the groups could not be matched for education; education was greater in controls than in FEP and UHR. Parental education and socioeconomic status were not collected but would be better variables for subject matching given that the subjects

groups were young and became ill before reaching full educational potential. Third, the spectroscopic voxels involved functionally dissimilar areas and included different proportions of CSF, gray and white matter, making interpretations regarding tissue specificity problematic. We tried to minimize this limitation by correcting for CSF proportion and using the gray matter as a covariate. Although we found differences in dorsal caudate and cerebellum gray matter percentage between the groups, this did not change the results. Fourth, altered NAAG has been described in schizophrenia. Although NAAG could contribute to the quantification of NAA, it could not be differentiated in a reliable way with the methodology employed in our study (Lutkenhoff *et al*, 2010). Specific techniques to measure NAAG (Edden *et al*, 2007) could be used in future studies. Fifth, Glu-Gln contamination of the NAA peak has been observed at short echo times, including the one used in this study (TE = 35 ms) (Clementi *et al*, 2005). Thus, this could be reflected as an artificial increase of NAA when Glu is increased. However, this is unlikely as at least the dorsal caudate of FEP showed higher Glu with no difference in NAA levels. Further studies using specific techniques to measure Glu, such as multiple echo times (Zhang *et al*, 2007), TE-averaged PRESS (Hurd *et al*, 2004), or constant time PRESS (Mayer and Spielman, 2005) are needed.

Summary

Our results indicate an increase in Glu in the dorsal caudate of antipsychotic naïve subjects with prodromal symptoms of schizophrenia and at a FEP, but not in the cerebellum. Due to the heterogeneity of outcomes in the UHR population, further longitudinal studies are needed to determine the true difference of Glu of those subjects that will convert to psychosis. Replication of our results and the results of longitudinal studies could help to develop a better prediction algorithm for those subjects that will develop a primary psychotic disorder.

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