

RESEARCH ARTICLE

Cortico-Striatal GABAergic and Glutamatergic Dysregulations in Subjects at Ultra-High Risk for Psychosis Investigated with Proton Magnetic Resonance Spectroscopy

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Abstract

Background: Dysregulations of the major inhibitory and excitatory amino neurotransmitter systems of γ -aminobutyric acid and glutamate, respectively, have been described in patients with schizophrenia. However, it is unclear whether these abnormalities are present in subjects at ultra-high risk for psychosis.

Methods: Twenty-three antipsychotic naïve subjects at ultra-high risk and 24 healthy control subjects, matched for age, sex, handedness, cigarette smoking, and parental education, underwent proton magnetic resonance spectroscopy scans in the dorsal caudate bilaterally and the medial prefrontal cortex at 3T. Levels of γ -aminobutyric acid and of the combined resonance of glutamate and glutamine (Glx) were obtained using the standard J-editing technique and expressed as peak area ratios relative to the synchronously acquired unsuppressed voxel water signal.

Results: Higher levels of γ -aminobutyric acid ($P < .001$) and Glx ($P = .007$) were found in the dorsal caudate of the subjects at ultra-high risk than in the healthy controls. In the medial prefrontal cortex, likewise, both γ -aminobutyric acid ($P = .03$) and

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Glx ($P = .006$) levels were higher in the ultra-high risk group than in the healthy controls. No group differences were found for any of the other metabolites (*N*-acetylaspartate, total choline, or total creatine) in the 2 regions of interest.

Conclusions: This study presents the first evidence of abnormal elevations, in subjects at ultra-high risk, of γ -aminobutyric acid and Glx in 2 brain regions that have been implicated in the pathophysiology of psychosis, warranting longitudinal studies to assess whether these neurotransmitter abnormalities can serve as noninvasive biomarkers of conversion risk to psychosis as well as of illness progression and treatment response.

Keywords: GABA, glutamate, ^1H MRS, ultra-high risk, psychosis, schizophrenia

Introduction

Schizophrenia is a highly debilitating neuropsychiatric disorder of young adulthood onset and a leading cause of disability worldwide (Whiteford et al., 2013). Therefore, there is great interest in investigating subjects at ultra-high risk (UHR) for psychosis to evaluate the dynamic phenotypic and genotypic changes that occur with illness progression to gain a better understanding of the mechanisms of schizophrenia pathogenesis necessary for assessing the risk of conversion to full psychosis or for identifying new treatment targets (Yung et al., 1998; Singh et al., 2012).

Recent research in schizophrenia has focused on dysregulations of the major inhibitory and excitatory amino neurotransmitter systems of γ -aminobutyric acid (GABA) and glutamate, respectively (Benes, 1995; Lewis et al., 2005). Using proton magnetic resonance spectroscopy (^1H MRS), a number of studies have sought to document these glutamatergic and GABAergic dysregulations in vivo in patients with schizophrenia. Most, but not all, of these studies have found elevations of glutamatergic compounds (ie, glutamate, glutamine, glutamate+glutamine (Glx) or glutamine/glutamate ratios) (Bartha et al., 1997; Theberge et al., 2002; Bustillo et al., 2010; Goto et al., 2012; Kegeles et al., 2012; Ota et al., 2012; Kraguljac et al., 2013) and of GABA (Kegeles et al., 2012) in unmedicated subjects in different phases of psychosis, while decreased or unchanged levels of the 2 neurotransmitters have been reported in chronic disease or in subjects treated with antipsychotic medication (Theberge et al., 2003; Goto et al., 2009; Reid et al., 2010; Yoon et al., 2010; Bustillo et al., 2011; Kegeles et al., 2012; Kraguljac et al., 2012; de la Fuente-Sandoval et al., 2013b; Rowland et al., 2013).

Recently, we and others reported the results of ^1H MRS studies that found elevated glutamate levels in the dorsal caudate of subjects at UHR (de la Fuente-Sandoval et al., 2011) and of those at familial high risk for schizophrenia (Tandon et al., 2013), suggesting the involvement of this excitatory amino acid neurotransmitter in the early phases of schizophrenia. However, confirmation studies are needed, since not all prior ^1H MRS studies had found such elevations in UHR subjects (Stone et al., 2009, 2010; Wood et al., 2010; Natsubori et al., 2014). Moreover, no prior ^1H MRS study has, to our knowledge, measured in vivo brain GABA levels in subjects at UHR. The primary objectives of the present study were, therefore, to use ^1H MRS to test, for the first time, the hypothesis that, as in schizophrenia (Kegeles et al., 2012), prefrontal GABA levels are abnormally elevated in the early stages of schizophrenia and to replicate our previous observation of abnormal elevations of glutamate levels in antipsychotic-naïve UHR subjects (de la Fuente-Sandoval et al., 2011).

METHODS

Participants

The present study, conducted between May 2012 and March 2013, was approved by the Ethics and Scientific Committees of

the National Institute of Neurology and Neurosurgery (INNN) of Mexico. Written informed consent of all subjects was obtained, as were written consent of both parents and assent of subjects younger than 18 years of age, prior to participation.

Together, 47 subjects participated in the study and of these 23 met the criteria for UHR, as assessed with the Structured Interview for Prodromal Syndromes (SIPS) (Miller et al., 2003). The UHR participants were recruited through the Emergency Service and the Adolescent Program of Neuropsychiatric and Imaging Study of the INNN. All UHR participants included in this study were treatment seeking and were excluded if they (1) had a concomitant medical or neurological illness, current substance abuse, or history of substance dependence (excluding nicotine), or comorbidity of any other axis I disorders; (2) were considered to be at high risk for suicide; or (3) presented with psychomotor agitation. All UHR participants, furthermore, were required to be antipsychotic medication-naïve, and use of benzodiazepines, mood stabilizers, or antidepressants within 12 weeks of the scans was exclusionary. Information about previous use of psychoactive medication, including antipsychotics, was collected as part of the diagnostic interviews of the participants and their family members.

Twenty-four age- and sex-matched healthy subjects, as established using the Structured Clinical Interview for DSM-IV (First et al., 1997), were recruited to serve as the normal comparison group. Participants in the control group with a history of psychiatric illness or family history of schizophrenia were excluded.

All enrolled subjects were screened for drugs of abuse (cannabis, cocaine, heroin, opioids, and benzodiazepines) at screen and at 1 hour prior to the ^1H MRS scans. Working memory performance was assessed in all subjects with the Letter-Number Span test (Wechsler, 1997).

Magnetic Resonance Neuroimaging Procedures

All the neuroimaging studies were conducted at the INNN on a 3T GE Signa EXCITE MRI system (GE Healthcare, Milwaukee, WI) equipped with an 8-channel head coil (Invivo, Orlando, FL). To ensure reproducible scan prescription and minimize head motion, each participant's head was positioned along the cantho-meatal line and then immobilized with a forehead strap.

Structural MRI

A 3-plane localizer imaging series was obtained, followed by a volumetric T_1 weighed spoiled gradient-recalled (SPGR) echo acquisition ($TE = 5\text{ms}$, $TR = 12\text{ms}$, $TI = 450\text{ms}$, flip angle = 20° , $FOV = 25.6\text{cm}$, 256×256 matrix, 186 slices, and a slice thickness = 1mm) oriented above and parallel to the anterior-posterior commissures line. The resulting 3D SPGR images were reformatted to sagittal and coronal views and used for optimal ^1H MRS voxel placement and brain tissue segmentation.

¹H MRS

In vivo brain GABA and Glx spectra were obtained from a 3.0×2.5×2.5-cm³ medial prefrontal cortex (MPFC) voxel (including portions of Brodmann areas 24, 32 and 10, and the pregenual anterior cingulate cortex) (Figure 1A) and from a 4.5×2.5×2.0-cm³ voxel that was prescribed to include primarily the dorsal caudate bilaterally (the lower end of voxel was located 3mm dorsal to the anterior commissure, including the maximum amount of gray matter and with a dorsal extension (thickness) of 2 cm (Figure 1C). Levels of GABA and Glx in each voxel were recorded with the standard J-edited spin echo difference method (Rothman et al., 1993; Sailasuta et al., 2001), as fully described recently (Geramita et al., 2011) and illustrated in Figure 1B. Briefly, a pair of a frequency-selective inversion pulses was inserted into the standard point-resolved spectroscopy method and then applied on the GABA C-3 resonance at 1.9 ppm on alternate scans using TE/TR 68/1500 ms. This resulted in 2 subspectra (Figure 1B, traces a-b) in which the GABA C-4 resonance at 3.03 ppm and Glx C-2 at 3.71 ppm were alternatively inverted or not inverted.

Subtracting these 2 subspectra yielded a spectrum consisting only of the edited GABA C-4 and Glx C-2 resonances, with all overlapping resonances eliminated (Figure 1B). For each voxel, the data were acquired in 13.4 minutes using 256 interleaved excitations (512 total), with the editing pulse on or off. While, in principle, the J-editing method cannot separate the individual components of the Glx peak, our recent data suggest that this resonance is predominantly glutamate due to the relatively low concentration of glutamine and the poor editing efficiency of the method for both compounds (Shungu, 2013; Shungu et al., 2013). The resulting raw 8-channel phased-array coil data were combined into a single regular 1D signal using the relative coil sensitivities derived from the unsuppressed voxel tissue water signal acquired with each receiver coil. The magnetic field homogeneity for the acquisitions was typically ≤12Hz, as assessed from the full-width at half maximum of the unsuppressed water resonance.

¹H MRS Data Processing and Quantification

Details of the MRS data quality assessment criteria and procedures used in this study to retain or reject spectra for inclusion in group analyses are provided in the [supplementary Material](#). The areas under the individual spectral peaks, which are proportional to the concentrations of the associated metabolites, were obtained as illustrated in Figure 1B (traces a-f) by frequency-domain fitting of each resonance to a Gauss-Lorentz (ie, pseudo-Voigt) function using a highly optimized public-domain Levenberg-Marquardt nonlinear least-squares minimization routine (Markwardt, 2009). The levels of GABA and Glx in the edited spectra were then expressed as ratios of peak areas relative to the synchronously acquired and similarly fitted unsuppressed voxel water signal (W), a method shown to have a high test-retest reliability for both GABA and Glx (Kegeles et al., 2006). Levels of N-acetylaspartate, total choline, and total creatine were likewise derived by fitting the subspectra recorded with the editing pulses turned off (Figure 1B, trace a).

Voxel Tissue Heterogeneity

To estimate the proportions of gray matter, white matter, and cerebrospinal fluid contained in each voxel of interest, the volumetric SPGR MRI data were segmented using the SPM8 software (Friston et al., 1995). In-house software developed in MATLAB (MathWorks, Natick, MA) was then implemented to generate a segmentation mask for each voxel, from which the proportions of gray matter, white matter, and cerebrospinal fluid were determined. These were then compared between the groups and, in case of significant differences, included in the statistical model as covariates.

Statistical Analysis

To identify potential confounding factors, unequal variance t tests compared the groups for age, gender, and voxel tissue heterogeneity. Factors that differed ($P < .05$) or trended toward

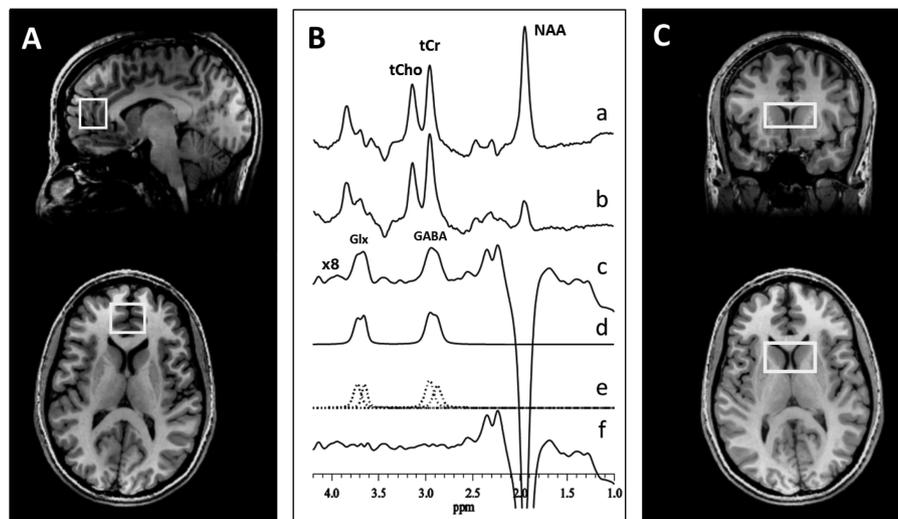


Figure 1. (A) Sagittal (top) and axial localizer images showing the size and location of the medial prefrontal cortex voxel of interest. (B) Demonstration of in vivo human brain γ -aminobutyric acid (GABA) and glutamate+glutamine (Glx) detection by proton magnetic resonance spectroscopy (¹H MRS) in the dorsal caudate: (a-b), single-voxel subspectra acquired in 13.4 minutes with the editing pulse on and off and 256 (512 total) interleaved averages; spectrum (c), difference between spectra (a) and (b) showing the edited brain GABA and Glx resonances; spectrum (d), model-fitting of the experimental spectrum (c) to obtain the GABA and Glx peak areas; spectrum (e), individual components of the fits; spectrum (f), residual of the difference between spectra (c) and (d). (C) Coronal (top) and axial localizer images showing dorsal caudate voxel size and location. NAA, N-acetyl-aspartate; tCho, total choline; tCr, total creatine. Comparable spectral quality and signal-to-noise ratios were also obtained for the MPFC voxel.

a significant difference ($.05 < P \leq 0.1$) between the groups were entered as covariates in between-group comparisons of the outcome measures (GABA/W, Glx/W) using ANCOVA. Additional ANCOVA tests were performed when demographic or clinical variables differed between the groups. Posthoc analyses were performed using Tukey's honest significant difference, with correction for multiple comparisons.

Exploratory associations between MRS and clinical variables were assessed using Pearson's product-moment correlation (r) or, for nonnormally distributed data, Spearman ρ . All the statistical comparisons were deemed significant at $P < .05$, and $P = .05/4$ was set as the significance threshold level for comparisons of the clinical scales (SIPS positive, negative, disorganization, general) for the UHR group.

RESULTS

Demographic and Clinical Characteristics

Demographic data for the study participants are provided in Table 1. The level of education was higher in the control group than in the UHR group ($t(45) = 3.61, P = .001$). The 2 groups did not differ on age, gender, handedness, parental education, or cannabis or tobacco use. These factors were therefore not included as covariates when comparing the 2 groups. The UHR group had a mean duration of prodromal symptoms of 46 ± 35 weeks (range 2–112 weeks).

Voxel Tissue Composition, Spectral Quality, and Regional Neurometabolite Levels

The MPFC ^1H MRS data for one UHR subject and those in the caudate voxel for another UHR subject were excluded due to poor quality as determined by our quality assessment criteria (see [supplementary Material](#)). Neither the levels of the unsuppressed reference tissue water signal (W) nor the proportions of gray matter, white matter, or cerebrospinal fluid in the MPFC or caudate voxel (Table 2) differed between the groups, indicating that our partial inclusion of the lateral ventricle in the caudate voxel (Figure 1C) did not result in differences in cerebrospinal fluid content between the groups in this voxel, thereby eliminating

Table 1. Demographic and Clinical Characteristics of the Sample.

	Control Subjects	UHR Group
Age (\pm SD) years	21.4 (3.3)	20.7 (4.1)
Gender (male/female)	19/5	15/8
Education (\pm SD) years	14.5 (2.9)	11.5 (2.7) *
Parental education (\pm SD) years	12.5 (4.2)	12.6 (4.7)
Handedness (right/left)	24/0	23/0
Tobacco (ever used)	4/24	5/23
Cannabis (ever used)	1/24	2/23
Length of illness (\pm SD) weeks		45.6 (34.9)
SIPS		12.0 (4.0)
Positive symptoms		
SIPS		11.7 (7.0)
Negative symptoms		
SIPS		8.2 (4.3)
Disorganization symptoms		
SIPS		7.3 (3.8)
General symptoms		

* $P < .05$.

Table 2. Means (\pm SD) for Each Metabolite, Tissue Composition, and Spectral Quality in the Two Regions of Interest in Ultra-High Risk for Psychosis and Healthy Control Groups.

Region	Group	Metabolites							Tissue Composition			Spectral Quality		
		GABA/W	Glx/W	NAA/W	tCho/W	tCr/W	Internal water	CSF fraction	GM fraction	WM fraction	FWHM	SNR		
MPFC	UHR	2.12 (0.33) $\times 10^{-3}$ *	1.77 (0.25) $\times 10^{-3}$ *	1.80 (0.14) $\times 10^{-2}$	1.16 (0.13) $\times 10^{-2}$	1.29 (0.11) $\times 10^{-2}$	1.72 (0.19) $\times 10^{-12}$	0.202 (0.026)	0.530 (0.044)	0.263 (0.043)	12.02 (2.17)	18.93 (1.50)		
	Control	1.91 (0.29) $\times 10^{-3}$	1.58 (0.20) $\times 10^{-3}$	1.84 (0.23) $\times 10^{-2}$	1.15 (0.13) $\times 10^{-2}$	1.26 (0.16) $\times 10^{-2}$	1.69 (0.19) $\times 10^{-12}$	0.211 (0.028)	0.523 (0.032)	0.261 (0.036)	12.20 (2.30)	19.82 (2.15)		
Dorsal Caudate	UHR	2.21 (0.36) $\times 10^{-3}$ *	1.49 (0.24) $\times 10^{-3}$ *	1.58 (0.23) $\times 10^{-2}$	1.10 (0.20) $\times 10^{-2}$	1.15 (0.18) $\times 10^{-2}$	1.77 (0.13) $\times 10^{-12}$	0.158 (0.058)	0.356 (0.049)	0.483 (0.079)	8.53 (2.66)	22.41 (5.02)		
	Control	1.80 (0.27) $\times 10^{-3}$	1.28 (0.25) $\times 10^{-3}$	1.58 (0.26) $\times 10^{-2}$	1.04 (0.22) $\times 10^{-2}$	1.11 (0.20) $\times 10^{-2}$	1.82 (0.13) $\times 10^{-12}$	0.187 (0.067)	0.354 (0.036)	0.456 (0.049)	7.84 (1.83)	22.87 (2.92)		

GM, grey matter; NAA, N-acetyl-aspartate; tCho, total choline; tCr, total creatine; WM, white matter; CSF, cerebrospinal fluid; FWHM, full-width at half maximum; SNR, signal-to-noise ratio of the NAA peak.
* $P < .05$.

group differences in voxel tissue composition and water content as potential confounds and allowing GABA/W and Glx/W to be referred to as simply GABA or Glx. The full-width at half maximum and signal-to-noise ratio values for both groups in the MPFC and caudate voxels are given in Table 2. No group differences in full-width at half maximum or signal-to-noise ratio values were found in either voxel.

In the MPFC, both GABA ($t(44)=2.22$, $P=.03$) and Glx ($t(44)=2.88$, $P=.006$) were higher in the UHR group than in the control group (Figure 2A). In the caudate voxel, likewise, both GABA ($t(44)=4.43$, $P<.001$) and Glx ($t(44)=2.83$, $P=.007$) levels were higher in the UHR group than in the control group (Figure 2B). On the other hand, no group differences in the levels of any of the major metabolites (N-acetylaspartate, total choline, total creatine) were found in either voxel.

Regional Correlations between GABA and Glx

Across all study participants, there was a positive correlation between GABA and Glx levels in both the MPFC ($\rho(44)=0.51$; $P<.001$) (Figure 1A in supplementary Material) and the caudate ($\rho(44)=0.45$, $P=.002$) (Figure 1B in supplementary Material). Within the UHR group, there was a strong positive correlation between GABA and Glx levels in the MPFC ($\rho(20)=0.57$; $P=.006$) (Figure 3A), but not in the caudate ($\rho(20)=0.06$; $P=.79$) (Figure 2B in supplementary material), whereas within the control group the significance of this correlation was regionally reversed: GABA and Glx levels were strongly correlated in the caudate ($\rho(22)=0.65$; $P=.001$) (Figure 3B), but not in the MPFC ($\rho(22)=0.29$; $P=.17$) (Figure 2A in supplementary Material). Lastly, exploratory correlational analyses between regions and metabolites by group (4 correlations) revealed a single strong negative correlation between caudate GABA and MPFC Glx ($\rho(20)=-0.58$, $P=.006$; correlation is significant at $P<.012$ [$P<.05/4$]) in the UHR group (Figure 4A), but not in the control group ($\rho(22)=-0.13$, $P=.54$) (Figure 3A in supplementary Material).

Associations between Neurotransmitter Levels and Clinical Measures

Levels of Glx were significantly correlated with the overall SIPS score ($P<.013$ [$0.05/4$]) in the caudate, with posthoc analyses revealing a negative correlation between caudate Glx and the SIPS positive subscore ($\rho(20)=-0.60$, $P=.004$) (Figure 4B). The UHR group performed significantly lower than the control group on the Letter-Number Span test ($t(45)=2.12$, $P=.04$), and working

memory performance in the UHR group was negatively correlated with Glx levels in the caudate ($\rho(22)=-0.36$, $P=.02$). However, the latter correlation vanished after correction for multiple comparisons. No significant correlations were found between GABA and Glx levels and clinical measures in the MPFC (eg, Figure 3B in supplementary Material), and none was found between GABA and clinical variables in the caudate.

Discussion

The present ^1H MRS study is, to our knowledge, the first to investigate simultaneously potential regional glutamatergic and GABAergic abnormalities in antipsychotic-naïve subjects at UHR. In an independent cohort, and using a different MRS technique, this study has replicated our previously reported elevations of glutamatergic compounds in the caudate of UHR subjects (de la Fuente-Sandoval et al., 2011). In addition, we have here extended that prior observation by documenting elevations of Glx also in the MPFC, and finding, for the first time, elevations of GABA in both the caudate and the MPFC in UHR subjects.

GABA and Glx Elevations

The present ^1H MRS findings of abnormal elevations of caudate and MPFC Glx levels in UHR subjects are in general agreement with prior studies that measured glutamatergic compounds in medication-naïve, unmedicated, or minimally medicated patients with schizophrenia (Bartha et al., 1997; Theberge et al., 2002; Bustillo et al., 2010; Goto et al., 2012; Kegeles et al., 2012; Ota et al., 2012; Kraguljac et al., 2013). On the other hand, among the few published ^1H MRS studies that have measured glutamatergic compounds only in UHR subjects, the findings have been less consistent, with studies reporting regionally decreased (Stone et al., 2009, 2010), increased (de la Fuente-Sandoval et al., 2011), or unchanged levels (Wood et al., 2010; Natsubori et al., 2014), and in our recent longitudinal study, subjects at UHR who progressed to develop full psychosis had higher glutamate levels in the caudate than both nonconverter UHR and control subjects (de la Fuente-Sandoval et al., 2013a). While the reasons for these discrepant findings are unclear and methodological differences cannot be ruled out, regional differences in neurotransmitter abnormalities and/or failure to take into account the medication status of the subjects may be significant contributors (vide infra).

Pathophysiologically, and as recently suggested (Kegeles et al., 2012), the reported elevations of glutamatergic compounds

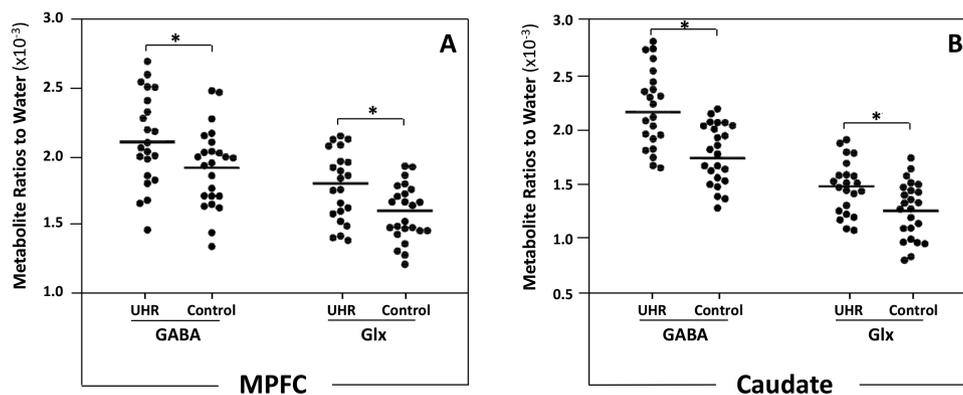


Figure 2. Levels of γ -aminobutyric acid (GABA) and glutamate+glutamine (Glx) (A) in the medial prefrontal cortex (MPFC) and (B) in the dorsal caudate of ultra-high risk (UHR) and control subjects. The short horizontal bars represent the mean value for each group; * denotes significant differences at $P<.05$.

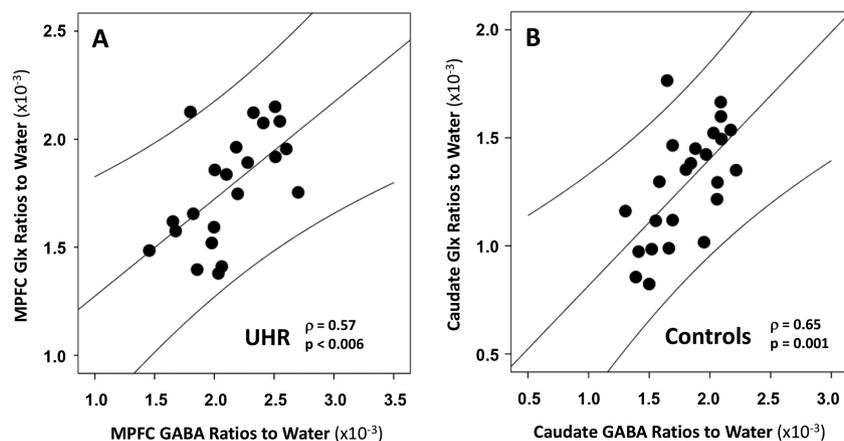


Figure 3. Scatterplots with linear regression fit for the associations between γ -aminobutyric acid (GABA) and glutamate+glutamine (Glx): (A) in the medial prefrontal cortex (MPFC) within the ultra-high risk (UHR) group, and (B) in the dorsal caudate within the control group. The curves bracketing the data points represent the 95% confidence interval.

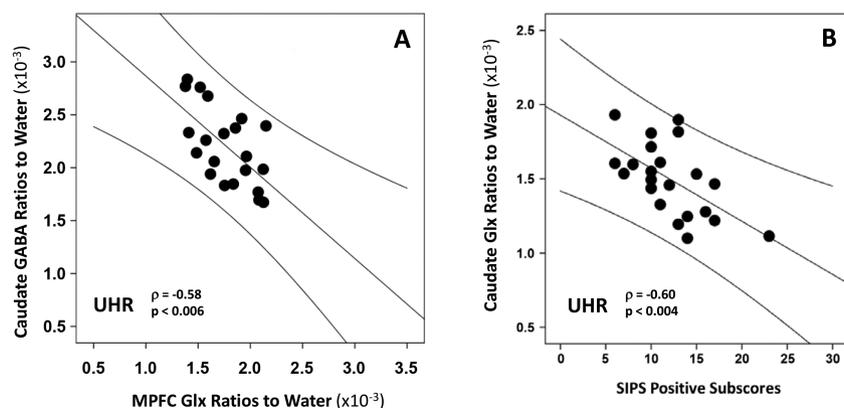


Figure 4. (A) Scatterplot with linear regression fit for the association between γ -aminobutyric acid (GABA) in the dorsal caudate and glutamate+glutamine (Glx) in the medial prefrontal cortex (MPFC) within the ultra-high risk (UHR) group. (B) Scatterplot with linear regression fit for the association between Glx in the caudate and the Structured Interview for Prodromal Syndromes (SIPS) positive subscores within the UHR group. The curves bracketing the data points represent the 95% confidence interval.

in psychosis are potentially consistent with the N-methyl-D-aspartate receptor hypofunction model of the schizophrenia (Olney and Farber, 1995), for which one of the most consistently replicated supporting evidence is a rapid surge in extracellular glutamate in response to antagonism of the receptor by sub-anesthetic doses of ketamine (Moghaddam et al., 1997; Kegeles et al., 2013; Schobel et al., 2013; Milak et al., 2015).

In contrast to the results on glutamatergic compounds, ^1H MRS studies of GABA in schizophrenia, which have been few and primarily in medicated patients to date, have yielded mixed results. There have been reports of regional deficits (Goto et al., 2009; Yoon et al., 2010; Rowland et al., 2013), elevations (Ongur et al., 2010; Kegeles et al., 2012), or unchanged levels of GABA (Tayoshi et al., 2010; Kegeles et al., 2012). However, on close examination, these discrepancies appear to reflect regional differences and/or whether the patient cohorts had prior exposure to antipsychotics (Goto et al., 2009; Tayoshi et al., 2010; Yoon et al., 2010; Rowland et al., 2013) or anticonvulsants (Ongur et al., 2010). Supporting this view are the results of a recent study (Kegeles et al., 2012) that measured GABA and Glx levels in both the MPFC and dorsolateral prefrontal cortex of medicated and unmedicated schizophrenia patients and found significant elevations of both neurotransmitters only in the MPFC in the unmedicated patients compared with both

the medicated and control subjects. These findings, which are consistent with the MPFC elevations reported in the present study in medication-naïve UHR subjects, strongly suggest the existence of regional differences in GABA and Glx levels, as well as the possibility of reductions or normalization of the levels of the 2 neurotransmitters with antipsychotic medication treatment.

While the GABA elevations in UHR subjects reported in this study are both novel and in agreement with a recent MRS study in unmedicated schizophrenia patients (Kegeles et al., 2012), they seem inconsistent with postmortem data, which have generally suggested decreased GABA levels in the disorder (Lewis et al., 2005). Potential sources of this discrepancy between in vivo MRS and postmortem data could be that (1) the MRS studies, in which total tissue levels are measured, and postmortem studies may not measure the same pools of GABA, (2) the detection of markers of GABA elevations in postmortem brain may be confounded by long periods of exposure to antipsychotic medication treatment, and (3) postmortem deficits in the GAD67 enzyme, responsible for GABA synthesis (Hashimoto et al., 2008), which is one of the strongest pieces of postmortem evidence supporting a GABA deficit, may be compensated for by other classes of interneurons with an unimpaired GAD67 enzyme (Kegeles et al., 2012).

Within-Region Associations among Neurotransmitters

In this study, we found a positive correlation between GABA and Glx across all study participants in both the MPFC and the caudate, replicating, at least for the MPFC, the results of a recent study that found such a correlation within this brain region across a full sample consisting of medicated and unmedicated patients with schizophrenia and matched control subjects (Kegeles et al., 2012). However, we have here extended this observation by finding intriguing within-group regional differences in correlations between Glx and GABA. Within the UHR group, Glx and GABA levels were positively correlated in the MPFC but not in the caudate, while within the control group the 2 regions were reversed, with Glx and GABA showing a positive correlation in the caudate but not in the MPFC. These within-group regional correlation differences seem to add a layer of regional complexity to the reported abnormal elevations of cortical GABA and Glx that may account for the discrepancies among the reported ^1H MRS measures of the 2 neurotransmitters.

The cortico-striatal Glx and GABA elevations found in this study in subjects at UHR and in unmedicated schizophrenia patients (Kegeles et al., 2012), and the positive regional correlations between the 2 neurotransmitters, seem to associate psychosis with a hyperglutamatergic and hyperGABAergic state. Although such simultaneous elevations of GABA and Glx would be inconsistent with previous suggestions that disinhibition of pyramidal cells by GABAergic interneurons is responsible for the N-methyl-D-aspartate receptor blockade that leads to glutamate elevations in psychosis or following ketamine administration (Moghaddam et al., 1997; Lorrain et al., 2003), a recent study using a knockout mouse model for the serine racemase gene, which exhibits NMDAR hypofunction and cognitive impairments (Basu et al., 2009), found frontal elevations of both GABA and glutamate measured by 9.4 T ^1H MRS, likely due to compensatory upregulation in non-fast-firing GABA neurons as an endogenous attempt to overcome the downregulation of fast-firing GABA interneurons (Puhl et al., 2014). Moreover and in agreement with the above preclinical study, our own recent studies monitoring the response of GABA and Glx in healthy human subjects (Kegeles et al., 2013) and in depressed patients (Milak et al., 2015) following ketamine administration have found concerted and robust elevations of both GABA and Glx. As discussed below, such inconsistencies in neurotransmitter levels between postmortem, preclinical, and emerging in vivo ^1H MRS data may be due, in part, to differential cerebral responses to inhibitory and excitatory inputs across brain regions (Carlsson and Carlsson, 1989, 1990).

Associations across Brain Regions and with Clinical Variables

Another significant association found in this study is a negative across-regions correlation between MPFC Glx and caudate GABA levels within the UHR group, which was not present within the control group. This finding suggests regional interactions, whereby decreased cerebral response to inhibitory neurotransmission in the caudate would lead to increased cerebral response to excitatory neurotransmission in the MPFC in UHR subjects. Conversely, increased cortical Glx levels may exert inhibitory influence on striatal response (Jinno and Kosaka, 2004). Supporting the existence of such across-region interactions in psychosis is the finding in rodents of increased glutamate release in the prefrontal cortex after subcortical blockade

of the N-methyl-D-aspartate receptors in GABAergic interneurons, which was accompanied by a GABA decrease and disinhibition of glutamatergic neurons projecting into the cortex (Moghaddam et al., 1997; Lorrain et al., 2003). Moreover, abnormal functional connectivity between the MPFC and the dorsal caudate has been described in both individuals at UHR (Dandash et al., 2014) and in patients with schizophrenia (Tu et al., 2012). Such regional interactions could possibly explain the apparent discrepancy between recent human brain ^1H MRS studies in which, on one hand, rapid and robust surges of both MPFC Glx and GABA levels were reported in patients with depression (Milak et al., 2015) and in healthy subjects (Kegeles et al., 2013) following ketamine administration, while on the other hand, only MPFC glutamate elevations with no changes in subcortical GABA levels were found in healthy subjects administered ketamine (Stone et al., 2012).

The preceding seemingly discrepant observations could be explained, at least partially, by one of the most intriguing results of present study, which is that GABA and Glx are positively correlated within the same region (MPFC) but inversely correlated across regions (MPFC vs caudate) in subjects at UHR. Specifically, we found a positive correlation between GABA and Glx in the MPFC but not in the caudate within the UHR group (Figure 3A), while at the same time finding a negative across-region correlation between MPFC Glx and caudate GABA levels (Figure 4A). Furthermore and interestingly, we found these same correlations to be regionally reversed within the control group, with GABA and Glx showing a positive correlation in the caudate but not in the MPFC (Figure 3B). These differences in regional associations between GABA and Glx in UHR and healthy subjects warrant further investigation as potentially specific indices of psychotic illness progression, treatment response, or as biomarkers of conversion risk in subjects at UHR.

Lastly, the abnormal and regionally reversed correlations between GABA and Glx found in this study could potentially account for our finding of a strong negative correlation between caudate Glx levels and the SIPS positive subscores (Figure 4B), which was unexpected, since prior studies in schizophrenia (Kegeles et al., 2012) and healthy subjects administered ketamine (Stone et al., 2012) had reported positive correlations between Glx levels and overall positive symptoms of PANSS. Further studies are clearly needed to explain fully the preceding observations and potential mechanisms.

Limitations

The present study has a number of limitations. First, direct measurement of GABA and glutamate neurotransmissions is not feasible with ^1H MRS, since the technique can measure only total tissue levels, including metabolic, synaptic, and vesicular. Second, the relatively low brain concentration of GABA requires sampling of relatively large voxels for reliable quantification at 3T; consequently, associated partial volume effects, especially for a heterogeneous brain structure like the dorsal caudate with large white matter and cerebrospinal fluid proportions, could confound interpretation. Third, the contribution of macromolecules that are known to coedit with GABA was not taken into account and is thus also a potential confound. Finally, while our exclusion of UHR subjects with comorbid Axis I disorders minimized sample heterogeneity, this quite likely affected the generalizability of our results due to the high prevalence of mood and anxiety disorders among individuals at UHR (Fusar-Poli et al., 2014).

Conclusion

This study has replicated our prior finding of increased glutamatergic compounds in the caudate of UHR subjects and extended it by reporting similar elevations of Glx in the MPFC and providing the first evidence of elevations of both GABA and Glx in the 2 brain regions. Since a recent meta-analysis found that approximately 36% of subjects at UHR will develop a primary psychotic illness within 3 years of clinical follow-up (Fusar-Poli et al., 2012), the present results of elevated MPFC and caudate GABA and Glx levels in UHR subjects provide a compelling rationale for longitudinal studies to evaluate in vivo changes in the 2 neurotransmitters as potential noninvasive biomarkers of risk of conversion to full psychosis, as well as of disease progression and of therapeutic response to novel glutamate- or GABA-modulating treatments that may present lower risk than current antipsychotic medication.

Supplementary Material

For supplementary material accompanying this paper, visit <http://www.ijnp.oxfordjournals.org/>

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Interest Statement

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