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Reduced Medial Prefrontal N-Acetyl-Aspartate Levels in Pediatric Major Depressive Disorder: A Multi-Voxel In Vivo ¹H Spectroscopy Study

Rene Luis Olvera^{a,b,*}, Sheila C. Caetano^c, Jeffrey A. Stanley^d, Hua-Hsuan Chen^e, Mark Nicoletti^f, John P. Hatch^{b,g}, Manoela Fonseca^{b,h}, Steven R. Pliszka^{a,b}, and Jair C. Soares^f

^a Division of Child and Adolescent Psychiatry, Department of Psychiatry, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

^b Department of Psychiatry, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

^c Department of Psychiatry, University of São Paulo Medical School, São Paulo, Brazil

^d Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI, USA

^e Department of Radiology, The University of Cincinnati, Cincinnati, OH, USA

^f Department of Psychiatry, The University of Texas Health Science Center at Houston, Houston, TX, USA

^g Department of Orthodontics, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

^h Psychiatry Research Unit, Federal University of Rio Grande do Sul, School of Medicine, Porto Alegre, Brazil

1. Introduction

Although uncommon in early childhood, MDD reaches a point prevalence of 6–9% and lifetime prevalence of 33% in adolescents, (Lewinsohn et al., 1993; Schachar and Tannock, 1995). This disorder has a chronic course and is associated with severe psychosocial impairment (Lewinsohn et al., 1995; Birmaher et al., 1996). Numerous magnetic resonance imaging (MRI) studies show volumetric reductions in the frontal cortex and its subregions in adults with MDD (Drevets et al., 1997; Rajkowska et al., 1999; Botteron et al., 2002; Bremner et al., 2002; Sheline, 2003). Post-mortem studies of adults with MDD show reductions in glial cell density of the DLPFC (Cotter et al., 2002; Uranova et al., 2004) and orbitofrontal regions (Drevets et al., 1997; Ongur et al., 1998; Rajkowska et al., 1999). Reviews of functional studies suggest hypo-functioning of the PFC (Seminowicz et al., 2004) in particular the DLPFC (Fitzgerald et al., 2006).

*Corresponding author: Rene L. Olvera, M.D., M.P.H., Division of Child and Adolescent Psychiatry, Department of Psychiatry, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl, Dr., San Antonio, TX, 78229, USA, olverar@UTHSCSA.edu.

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The few MRI studies done in children and adolescents with MDD, also suggest evidence of frontal lobe involvement. Decreased frontal volume and increased ventricular size was seen in depressed children compared to psychiatric controls (Steingard et al., 1996). In a later study, smaller whole brain volumes and smaller frontal white matter volumes were noted in depressed adolescents compared to controls (Steingard et al., 2002). After finding no differences in PFC volumes between untreated adolescents with MDD and healthy controls, Nolan et al. (2002) further subdivided their sample and found smaller left sided PFC gray matter volumes in patients with familial MDD compared to patients without a family history of MDD. Furthermore, subjects without a family history of MDD had larger left-sided total PFC volumes and PFC white matter volumes compared to patients with familial MDD and controls (Nolan et al., 2002). There was no significant difference in either total orbitofrontal cortex (OFC) volume or total gray matter OFC volume between twenty-seven medication naïve children and adolescents with MDD patients compared to healthy controls (Chen et al., 2008).

In vivo proton (^1H) spectroscopy is a non-invasive and non-radioactive neuroimaging tool, which can measure the levels of major neurochemicals in vivo such as NAA, GPC + PC, myo-inositol (mI), PCr + Cr and glutamate (Glu) (Stanley et al., 2000; Stanley, 2002). These in vivo measurements provide insights into the neurochemical properties of selected brain regions. Of these neurochemicals, NAA is considered a marker of neuronal integrity, mitochondrial energy metabolism, and a developmental marker of dendritic and synaptic proliferation (Tsai and Coyle, 1995; Pouwels and Frahm, 1998; Barker, 2001; Baslow, 2003). GPC + PC are breakdown products and precursors of membrane phospholipids, respectively (Stanley et al., 2000; Stanley, 2002) and PCr + Cr are high-energy phosphate metabolites related to the cellular energy metabolism via the creatine kinase high-energy phosphate reaction (Hemmer and Wallimann, 1993; Kemp, 2000; Aubert and Costalat, 2002).

^1H spectroscopy studies of adults with MDD provide mixed results. Within the frontal lobes, decreased NAA/PCr + Cr ratios were found bilaterally in depressed adults (Gruber et al., 2003) and in the left frontal lobe of elderly MDD patients with more deep white matter hyperintensities compared to MDD subjects with fewer white matter hyperintensities (Murata et al., 2001). Higher GPC + PC/PCr + Cr and myo-inositol/PCr + Cr ratios were reported in the dorsolateral white matter of elderly MDD patients (mean age 69.9 years) compared to healthy control subjects (Kumar et al., 2002). Decreased glutamate plus glutamine (Glu + Gln) and Glu alone was noted in the AC of depressed patients compared to controls (Auer et al., 2000). In two separate studies of depressed patients, reduced Glu levels in the DLPFC (Michael et al., 2003) and left cingulum (Pfleiderer et al., 2003) were seen pre-electro convulsive therapy (ECT) compared to healthy controls. These levels no longer differed from control subjects in patients who responded to ECT. Subjects whose depression was in remission did not differ from healthy subjects on any metabolite as measured using in vivo ^1H spectroscopy in voxels placed in the ventromedial prefrontal cortex and dorsomedial/dorsal anterolateral prefrontal cortex (Hasler et al., 2005).

Most studies using in vivo ^1H spectroscopy in children and adolescents with MDD have focused on the AC, noting reduced Glu (Mirza et al., 2004; Rosenberg et al., 2004; Rosenberg et al., 2005) and lower PCr + Cr (Mirza et al., 2004; Rosenberg et al., 2004) levels. Additional studies encompassing a variety of frontal cortex regions have found higher levels of GPC + PC in the OFC (Steingard et al., 2000), and elevated right prefrontal GPC + PC/PCr + Cr ratios in a gray matter voxel close to the anterior cingulate (Mac Master and Kusumakar, 2006). There are two studies of the DLPFC, with one noting increased GPC + PC in the left DLPFC of youth with MDD compared with healthy controls (Farchione et al., 2002) while Caetano et al. (2005) found significantly lower levels of GPC + PC and

higher levels of myo-inositol in the left DLPFC of adolescents with MDD compared to healthy controls. In vivo ^1H spectroscopy studies of the thalamus have found decreased Glu and NAA in the right medial thalamic area of MDD adolescent vs. controls (Mirza et al., 2006) and increased medial thalamic GPC + PC levels bilaterally in patients with Obsessive Compulsive Disorder compared with both healthy control subjects and patients with MDD (Smith et al., 2003). In the amygdala, MDD adolescents had decreased GPC + PC/PCr + Cr ratios in the left amygdala region compared with controls (Kusumakar et al., 2001).

We used a 2D, multi-voxel or chemical shift imaging (CSI) method to assess levels of NAA, GPC + PC, and PCr + Cr in frontal areas of children and adolescents with and without MDD. A multi-voxel technique has the advantage of obtaining multiple spectra simultaneously therefore evaluating multiple regions of interest during one measurement. Smaller voxels result in less contamination from neighboring tissues. Pitfalls with CSI however include potential smearing across voxels caused by smoothing, decreased signal-to-noise, and residual field inhomogeneities, all of which make processing more complex (Maudsley et al., 1983; Sauter et al., 1991; McRobbie et al., 2003). In contrast, single voxel techniques allow for precise localization using larger voxels which allow for more homogeneity, and easier water suppression. The downside to using a large single voxel, is the voxel may encompass multiple brain areas and tissue types and only one region can be assessed at a time therefore even basic comparison between hemispheres require multiple scans (Maudsley et al., 1983; Sauter et al., 1991; Duijn et al., 1992).

We sought to replicate our prior work (Caetano et al., 2005) finding lower levels of GPC + PC levels in the DLPFC of MDD subjects compared to healthy controls. Based on prior child studies we also expected decreased PCr + Cr in the anterior cingulate (Mirza et al., 2004; Rosenberg et al., 2004). The MPFC has not been examined in pediatric populations therefore our goal is exploratory at this point. For regions that had significant differences in neurochemicals, we explored the effects of clinical status including duration of illness, CDRS scores, medication status and positive family history of mood disorders on these levels. Given the unique nature of a multi-voxel technique, we used the occipital region as a control area where no differences between MDD and control subjects were expected.

2. Methods

2.1 Subjects

We recruited sixteen children and adolescents with MDD and thirty-eight healthy controls, between 8 and 17 years old, without serious medical problems, who were magnetic resonance compatible, from news advertisements and received referrals from local psychiatrists. MDD subjects were included if they met DSM-IV criteria for the disorder assessed with the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL) (Kaufman et al., 1997) administered separately to the children and their parent(s) or guardian(s). Final diagnoses were made via consensus of our diagnostic team after integrating the child and parent interview and all other available records. Exclusion criteria were a lifetime diagnosis for psychotic disorders, bipolar disorder, developmental disorders, substance abuse/dependence, eating disorders, tic disorders, or mental retardation. Exclusion criteria for healthy controls were a history of current psychiatric disorder, and a history of any Axis I psychiatric disorder in first-degree relatives. The severity of depression was rated using the Children's Depression Rating Scale, revised (CDRS) (Poznanski and Mokros, 1996) and the Hamilton Depression Rating Scale (HAM-D) (Clark and Donovan, 1994). Puberty status was assessed through the Pubertal Development Scale – Petersen Scale (Petersen et al., 1988). Global Assessment of Functioning Scale (GAF) was recorded as part of the diagnostic interview. Family socioeconomic status (SES) was measured by the Hollingshead Four Factor Index of Social

Status (Hollingshead, 1975). Laterality was assessed with the Oldfield scale (Oldfield, 1971). This study was approved by the Institutional Review Board of The University of Texas Health Science Center at San Antonio. Written informed consent was obtained from all subjects' parents or legal guardians and written assent was obtained from each child and adolescent.

2.2 Multi-voxel ^1H Spectroscopy

We acquired five T_1 -weighted scout images in order to achieve appropriate placement of the multi-voxel axial slice. In the sagittal plane, we selected the inferior slice adjacent to the superior border of the corpus callosum as this allowed us to examine portions of the MPFC, the dorsal AC and DLPFC. To exclude the sinus, the slice angle was carefully adjusted to be parallel with the anterior commissure-posterior commissure line (Figure 1). Acquisition parameters were as follows: repetition time/echo time = 1.5 s/272 ms; field of view = 24 cm; spectral bandwidth = 2,000 Hz; complex data points = 1024; CSI matrix size 16×16 ; nominal voxel size 1.5×1.5 cm; dimension of the region of interest localized by the point-resolved spectroscopy sequence (PRESS) sequence within the CSI = $119.14 \pm 12.89 \times 146.61 \pm 16.12 \times 2$ cm³, number of measurements = 2; and acquisition time = 16 min. To reduce lipid contamination from the anterior, posterior and lateral scalp, we positioned four outer volume saturation bands. In order to obtain absolute quantification, we also collected water unsuppressed data using 8×8 phase-encoding steps, which was zero-filled to 16×16 (Stanley et al., 1995).

2.3 Post-acquisition data processing

We zero-filled the unsuppressed data to match the 16×16 matrix; and then performed a Fourier transformation before transferring the data to the workstation. Following the data acquisition, we used the Spec-Tool program, version 3.2 (Philips Medical Systems) that runs under the Philips Research Imaging Development Environment (PRIDE) in order to reposition the voxels in predefined (left and right) brain areas (Figure 1): the MPFC, the anterior cingulate and the DLPFC. This process was applied to the data related to water suppressed spectra, as well as to those related to water unsuppressed spectra. We used the PRIDE to extract the complex time-domain signal of the shifted voxels, which was then quantified as a separate spectrum. Prior to the spectral fitting, we removed any residual water or lipid signals using Hankel-Lanczos singular value decomposition (de Beer et al., 1992). We modeled the NAA, PCr + Cr and GPC + PC peaks in the time domain using Gaussian modulated sinusoidal functions and the nonlinear Levenberg-Marquardt algorithm (Marquardt, 1963).

The proportion of gray and white matter tissue, and CSF/extra-cortical space were estimated for each extracted ^1H spectroscopy voxel. In a fully automated procedure, the T_1 -weighted images were co-registered to the axial scout images, corrected for any B_1 field bias, the brain was extracted and the images segmented into partial volume maps of gray and white matter tissue, and CSF/extra-cortical space using FreeSurfer and FSL tools (Dale et al., 1999; Smith et al., 2004). The tissue fractions were then estimated by extracting from the segmented images the region of interest matching the coordinates and size of the ^1H spectroscopy voxel using FSL tools.

We obtained the absolute metabolite levels (mmol/kg wet weight) using the gray and white matter tissue, and CSF voxel content values, and the quantified water peak from the data related to water unsuppressed spectra (Stanley et al., 1995). Because of the time limitation in scanning children, additional measurements were not conducted to estimate the T_1 and T_2 relaxation values of the metabolites in all of the participants. Therefore, the T_1 and T_2 relaxation values necessary for absolute quantification (Stanley et al., 1995) were assumed

to be constant for all subjects and reflected values of adults (Kreis et al., 1993). Spectra were systematically rejected if the chemical shift was not within the ± 2 ppm window of the NAA, GPC + PC and PCr + Cr peaks (2.01 ppm, 3.02 ppm and 3.20 ppm, respectively); as was any spectral peak with a line width ≥ 50 Hz (18% of voxels were rejected).

2.4 Statistical Data Analysis

Statistical analyses were conducted using SPSS software version 14 (SPSS, Inc., Chicago, IL). Analysis of covariance with age and gender as covariates was performed. We adopted a two-tailed significance level of $p < 0.05$. We selected to examine the DLPFC, MPFC and AC without adjusting for multiple comparisons. Pearson's correlation coefficients were used to examine linear association between chemical measures and age, and Spearman's correlation coefficients were used for clinical variables that did not follow a normal distribution (duration of illness, CDRS).

3. Results

Our two groups did not differ in age, gender, pubertal status, years of education, handedness or socioeconomic status (Table 1). In the DLPFC, we did not see significant differences in GPC + PC metabolite levels with trends for lower NAA in the left DLPFC gray matter and right DLPFC white matter. In the right AC we saw significantly decreased NAA ($F=4.76$, $df=1, 46$, $P=0.03$) and GPC + PC ($F=7.98$, $df=1, 46$, $P=0.007$), with a trend for decreased PCr + Cr, in MDD subjects compared to healthy controls (see Table 2). Examination of the MPFC revealed significantly lower levels of NAA in the right MPFC ($F=5.06$, $df=1, 46$, $P=0.03$) and a trend for decreased GPC + PC in MDD subjects compared to healthy controls (see Table 2). As expected we found no metabolite differences in the occipital region. We examined the effects of medication status (medicated vs. unmedicated subjects) on metabolite levels in regions that were statistically significant and found no significant differences. Similarly correlations between clinical variables and metabolite levels in these areas did not reach statistical significance (see Table 3).

4. Discussion

This study found decreased levels of NAA in the right MPFC and decreased NAA and GPC + PC in the right dorsal AC of depressed adolescents compared to healthy controls. Although prior child MRS studies failed to find decreased NAA in PFC areas (Steingard et al., 2000; Mac Master and Kusumakar, 2006) ours is the first pediatric study to specifically examine the MPFC. Studies of the AC have mainly focused on Glu + Gln but did not report other metabolite concentrations (Rosenberg et al., 2004; Rosenberg et al., 2005). The exception is the AC study by Mirza et al (2004) who reported decreased Glu + Gln and PCR + Cr but no difference in NAA or GPC + PC in depressed children and adolescents. Of note decreased NAA/PCr + Cr ratios have been reported in the MPFC of adult (Gruber et al., 2003) and elderly subjects with depression (Murata et al., 2001).

We did not replicate our findings of lower GPC + PC levels in the left DLPFC of depressed patients (Caetano et al., 2005). Our failure to replicate our prior study is of interest as many of the same subjects were scanned using both the SVS and CSI method. The main difference between these studies is the the CSI voxel is smaller which would effect both the placement and composition of the brain examined. These potential differences are evident in this sample as the MDD subjects' GPC + PC levels in the left DLPFC gray matter are slightly higher compared to controls whereas in the DLPFC white matter the GPC + PC levels were slightly lower in MDD subjects (see Table 2). The same methodological differences could explain our novel AC findings as prior spectroscopy studies of the AC in depressed children and adolescents used a large central single voxel and averaged metabolite

levels across hemispheres (Mirza et al., 2004; Rosenberg et al., 2004; Rosenberg et al., 2005).

4.1 The Frontal Cortex in Mood Regulation

Our main findings encompass differences in two PFC regions, namely the right MPFC and right AC. The prefrontal cortex is a complex region with multiple cortico-striato-thalamo-cortical connections (or loops) to the orbito-frontal cortex (that in turn connect to sensory visual, auditory, somatosensory, olfactory and gustatory inputs) and outputs to the visceromotor system (thalamic, hypothalamic and brainstem) (Hamer et al., 1993; Phillips et al., 2008). Given this extensive network, it has been postulated that the MPFC and AC function in the monitoring and regulation of emotional states; with growing evidence to this effect based on converging evidence from neuroimaging and neuropathological studies (Drevets, 2007; Levesque et al., 2004; Yurgelun-Todd, 2007). Our voxel is placed in the dorsal AC which is a transitional area encompassing Brodmann areas 32 and 24c, 24b associated with both emotional and cognitive control (Bush et al., 2000). Our significant findings of decreased metabolites on the right MPFC and AC are of interest as fMRI studies have shown right sided prefrontal activation is associated with decreased cerebral blood flow in the amygdala (Hariri et al., 2000) and when trying to suppress the sad feelings in healthy subjects (Levesque et al., 2004; Beauregard et al., 2006). Decreased NAA in the MPFC and AC is suggestive of disrupted neuronal integrity in these regions. NAA is present in axons, dendrites and synaptic terminals, and changes in NAA levels in early development concomitant with dendritic arborization and the formation of synaptic connections suggest NAA is a marker of functioning neuroaxonal tissue (Barker, 2001; Baslow, 2003; Horska et al., 2002; Pouwels and Frahm, 1998; Tsai and Coyle, 1995). GPC + PC are breakdown products and precursors of membrane phospholipids, respectively (Stanley et al., 2000; Stanley, 2002) therefore our finding of lower GPC + PC levels could be interpreted as evidence of diminished cell growth or myelination in MDD compared to healthy controls.

4.2 Limitations

Limitations of this study are different exposure to psychotropic medication and the presence of comorbid diagnoses. These circumstances however, are common to clinical populations of pediatric MDD. Our subjects also had moderate depressive symptoms, therefore, it is unclear if our findings were a result of their mood state or if these are underlying differences that predispose subjects to a mood disorder. One must also acknowledge that the use of a long TE time for data acquisition. This raises the possibility of a difference in T₂ relaxation value between subjects groups driving the significant difference in metabolite levels between groups. Further limitations include a small sample size and failure to control for multiple comparisons, therefore our findings must be interpreted as preliminary. A meta-analysis of existing ¹H spectroscopy studies (Yildiz-Yesiloglu and Ankerst, 2006) highlights the inconsistent findings in MDD, however most of the studies used single voxel techniques. With the advances in field strength and signal-to-noise ratio, further studies with the CSI technique are warranted before final conclusions can be drawn.

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Figure 1.
CSI Placement and Spectra
Locations: 1= Medial Prefrontal cortex, 2 = Dorsolateral Prefrontal Cortex, 3 = Anterior cingulate,

Table 1

Demographic and Clinical Characteristics of subjects

		Pediatric MDD patients (N = 16)	Healthy controls (N = 38)
Age (years) (sd)		13.19 (2.45)	13.88 (2.66)
Male Gender (%)		11 (69%)	19 (50%)
Education (years) (sd)		7.07 (2.79)	7.89 (2.72)
Petersen Puberty score (sd)		6.75 (2.98)	7.74 (2.91)
Right Handed (%)		15 (92%)	35 (92%)
Years of schooling		7.07 (2.79)	7.89 (2.72)
Hollingshead SES		46.58 (12.56)	45.21 (14.60)
Age of onset (years) (sd)		10.31 (2.50)	-
Duration of illness (months) (sd)		28.50 (11.50)	-
Number of episodes(sd)		1.64 (1.64)	-
Medicated (%)		7 (44%)	-
CDRS score(sd)		42.56 (16.77)	-
HAM-D		11.50 (6.42)	-
CGI Depression		2.75 (1.48)	-
CGI Mania		1.25 (0.78)	-
Family History of Mood disorder (%)		15 (94%)	0
First Degree relative with a Mood Disorder (%)		15 (94%)	0
Ethnicity	(%)		
	Hispanic	6 (38%)	26 (68%)
	Non-Hispanic White	8 (50%)	6 (16%)
	African American	1 (6%)	4 (10%)
	Other	1 (6%)	2 (5%)

Table 2

Metabolite levels of Anterior Brain Regions

Region	Chemical	MDD Subjects (N=16)		Healthy Controls (N=38)	
		mean±SD (mmol/kg)	p	mean±SD (mmol/kg)	F
Left DLPFC Gray Matter	NAA	22.78±4.20	0.092	25.85±6.79	2.961
	GPC + PC	3.94±2.54	0.016	3.88±1.30	0.016
	PCr + Cr	19.15±5.87	0.330	20.28±8.92	0.330
Right DLPFC Gray Matter	NAA	27.08±7.71	0.426	28.91±7.76	0.645
	GPC + PC	4.90±2.64	0.213	3.98±1.53	1.592
	PCr + Cr	18.89±6.71	0.106	22.82±7.89	2.709
Left DLPFC White Matter	NAA	16.82±5.71	0.347	18.28±6.24	0.900
	GPC + PC	3.02±0.97	0.135	3.38±1.08	2.308
	PCr + Cr	14.22±5.35	0.385	15.48±5.70	0.768
Right DLPFC White Matter	NAA	16.81±4.26	0.091	19.38±6.08	2.967
	GPC + PC	3.43±1.42	0.378	3.71±1.40	0.793
	PCr + Cr	13.53±3.01	0.278	15.01±5.76	1.205
Left Medial PFC	NAA	21.22±5.50	0.216	24.20±7.75	1.576
	GPC + PC	3.21±0.99	0.092	4.01±1.90	2.966
	PCr + Cr	20.00±6.56	0.290	18.04±7.85	1.145
Right Medial PFC	NAA	17.46±4.08	0.029	22.00±7.59	5.056
	GPC + PC	3.01±0.91	0.051	3.72±1.28	4.020
	PCr + Cr	17.20±7.04	0.596	18.48±7.22	0.284
Left Anterior Cingulate	NAA	21.25±6.56	0.935	21.72±6.86	0.007
	GPC + PC	4.04±1.00	0.593	4.19±1.24	0.290
	PCr + Cr	15.88±4.71	0.631	16.88±5.34	0.233
Right Anterior Cingulate	NAA	17.71±4.40	0.034	21.28±6.37	4.768
	GPC + PC	3.41±0.66	0.007	4.22±1.34	7.986

		MDD Subjects (N=16)	Healthy Controls (N=38)		
Region	Chemica l	mean±SD (mmol/kg)	mean±SD (mmol/kg)	F	p
	PCr + Cr	13.40± 4.14	16.39± 7.05	3.065	0.086
Left Occipital Cortex	NAA	24.92± 7.56	23.38± 9.43	0.188	0.667
	GPC + PC	3.90± 2.78	3.44± 2.97	0.056	0.815
	PCr + Cr	18.90± 10.10	16.95± 8.53	0.429	0.516
Right Occipital Cortex	NAA	20.02± 4.68	22.44± 6.97	1.426	0.239
	GPC + PC	2.84± 1.71	3.20± 2.02	0.599	0.444
	PCr + Cr	20.72± 12.28	16.83± 8.62	1.130	0.294

Table 3

Correlation Between Clinical Variables and Metabolite Levels in selected regions for MDD Subjects

	NAA	GPC + PC	PCr + Cr
Right Anterior Cingulate			
Age	−0.06	0.03	−0.33
CDRS	0.07	−0.23	0.015
CGI Mania	−0.26	−0.36	−0.14
CGI Depression	−0.06	−0.15	0.01
HAM-D	0.22	−0.15	0.13
Number of episodes	0.05	0.04	0.02
YMRS	−0.07	−0.11	−0.03
Right Medial Prefrontal Cortex			
Age	0.36	−0.05	0.48
CDRS	−0.00	−0.19	−0.15
CGI Mania	−0.08	−0.00	−0.29
CGI Depression	−0.01	−0.22	−0.15
HAM-D	−0.08	−0.26	−0.19
Number of episodes	0.39	0.22	0.03
YMRS	−0.40	−0.52	−0.14