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Insula and anterior cingulate GABA levels in post-traumatic stress disorder: Preliminary findings using magnetic resonance spectroscopy

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Abstract

Background—Increased reactivity of the insular cortex and decreased activity of the dorsal anterior cingulate (ACC) are seen in functional imaging studies of post-traumatic stress disorder (PTSD), and may partly explain the persistent fear- and anxiety-proneness that characterize the disorder. A possible neurochemical correlate is altered function of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). We report results from what we believe is the first study applying proton magnetic resonance spectroscopy (¹H-MRS) to measure brain GABA in PTSD.

Methods—Thirteen adults with DSM-IV PTSD and 13 matched healthy control subjects underwent single voxel ¹H-MRS at 4 Tesla. GABA was measured in the right anterior insula and dorsal anterior cingulate, using MEGAPRESS spectral editing. Subjects were interviewed with the Structured Clinical Interview for DSM-IV and the Clinician Administered PTSD Scale, and also completed the State and Trait Anxiety Inventory.

Results—Insula GABA was significantly lower in PTSD subjects than in controls, and dorsal ACC GABA did not differ significantly between the groups. Insula GABA was not significantly associated with severity of PTSD symptoms. However, lower insula GABA was associated with significantly higher state and trait anxiety in the subject sample as a whole.

Conclusions—PTSD is associated with reduced GABA in the right anterior insula. This preliminary evidence of the ¹H-MRS GABA metabolite as a possible biomarker of PTSD encourages replication in larger samples and examination of relations with symptom dimensions. Future studies also should examine whether insula GABA is a marker of anxiety proneness, cutting across clinical diagnostic categories.

Keywords

post-traumatic stress disorder; magnetic resonance spectroscopy; anxiety; insular cortex; GABA

INTRODUCTION

Post-traumatic stress disorder (PTSD) is a debilitating anxiety disorder in which an acute fear response to a traumatic event does not abate. Instead, patients continue to experience a number of emotional and behavioral dysregulation symptoms, including intrusive recollections of the trauma, hyperarousal and hypervigilance, emotional numbing, and avoidance of trauma reminders. Neurocircuitry models propose that PTSD involves hyper-reactivity of brain regions that mediate fear expression and emotional responsivity, along with hypo-reactivity of brain regions that attenuate fear and other emotional responses.^[1–6]

The neurochemical correlates of abnormal regional brain excitability in PTSD are unclear, but one prominent candidate is altered gamma-aminobutyric acid (GABA), the brain's principal inhibitory neurotransmitter and a modulator of neuronal excitability.^[7] Numerous lines of evidence implicate GABA in anxiety disorders, including PTSD.^[8] Preclinical studies that model aspects of PTSD have shown that benzodiazepines, which enhance GABA(A) receptor function, inhibit the startle response induced by predator stress in animal models.^[9] In addition, exposure to chronic stress reduces presynaptic GABAergic interneuron activity in the rodent ACC, possibly reflecting a decrease in the number of GABA terminals or a reduced release of GABA into the synapse.^[10] In receptor imaging studies, PTSD patients show reduced benzodiazepine receptor binding in a number of brain regions.^[7] Moreover, there is some evidence, albeit inconsistent, that lower serum GABA levels predict the development of PTSD after trauma exposure, as well as a more chronic course of the illness.^[11] At the same time, it is unclear how serum GABA relates to brain GABA levels. It is possible that GABA alterations may vary in their presence and nature across brain regions implicated in PTSD, and might differentially predict clinical phenomenology.

With the combination of metabolite-specific spectral-editing techniques and high field imaging, *in vivo* spectroscopy has been able to reliably detect brain GABA in psychiatric populations.^[12–19] However, there are few published ¹H-MRS GABA studies in anxiety disorders and none, of which we are aware, in PTSD. The first study in anxiety disorders found reduced GABA in the occipital cortex of panic disorder patients.^[14] In more recent studies of panic disorder, GABA was significantly reduced in the ACC^[20, 21] and basal ganglia of patients versus healthy subjects,^[20] but not different in dorsomedial or ventromedial areas of the prefrontal cortex.^[16] A ¹H-MRS study in social anxiety disorder found reduced GABA in a matrix of limbic brain voxels in patients compared with matched controls.^[22] Pollack and colleagues (2008) also reported that whole brain GABA levels increased with anticonvulsant treatment in social anxiety patients. Altogether, this burgeoning literature suggests that ¹H-MRS measures of GABA may be neurobiological markers of clinical anxiety and its treatment.

In this study of regional brain ¹H-MRS GABA, we focused on two of the most consistently implicated brain areas in functional imaging studies of PTSD, as identified in a meta-analysis by Etkin and Wager (2007): the insular cortex and dorsal anterior cingulate (ACC).^[1] Specifically, in this meta-analysis of functional imaging studies of symptom provocation and negative emotional processing, PTSD patients were found to have a hyperactive right insula and hypoactive dorsal ACC.^[1] Both regions are considered part of the “salience network,” which is involved in identifying internal and external stimuli that are most homeostatically-relevant to an individual, in order to guide motivated behavior.^[23] Moreover, the right anterior insula may be particularly important for the integration of homeostatic and sensory information into a subjective awareness and prediction of aversive bodily states associated with anxiety.^[4] Functional activity in both the ACC and insula has been associated with many functions relevant to anxiety, including threat perception and

anticipation,^[24] and is correlated with measures of anxiety proneness such as trait anxiety.^[25–29] We hypothesized that GABA would be lower in PTSD compared with control subjects in the right anterior insula and higher in dorsal ACC, and that altered GABA would be correlated with worse PTSD symptoms. We also predicted that there would be negative correlations between insula GABA and dimensional measures of state and trait anxiety in the sample as a whole (i.e., less GABA, more anxiety).

METHODS

Participants

We studied 13 PTSD patients and 13 healthy comparison (HC) subjects between the ages of 18 and 55, recruited via advertisements in the local Boston community (Table 1). All subjects provided written informed consent after a full explanation of study procedures, and received financial compensation for their participation. The study was approved by McLean Hospital's Institutional Review Board. Subjects received the Structured Clinical Interview for DSM-IV-TR Axis I Disorders – Patient Version (SCID-I/P^[30]). Healthy adult subjects and patients were excluded from participation if they had 1) medical conditions that might affect brain structure (e.g., head trauma, loss of consciousness, seizure disorder); 2) current substance use disorder; 3) current nicotine use; 4) use of benzodiazepine or other anxiolytic, anticonvulsant, mood stabilizing or neuroleptic medication within 4 weeks of the study; 5) history of substance abuse within the past five years; 6) lifetime history of substance dependence; 6) structural abnormalities on MRI scan or contraindications for MR scanning (e.g., metal implants, pregnancy); 7) urine toxicology positive for recent psychoactive drug use or HCG status on the day of scan. In addition, healthy subjects had no history of Axis I diagnoses. Subjects were asked to refrain from alcohol consumption for 48 hours prior to their scan and their abstinence was verified by self-report. Because menstrual cycle stage can affect ¹H-MRS GABA levels,^[31] female subjects provided information on their menstrual cycle to determine whether they were in the follicular or luteal phase and the two subject groups were matched on this variable.

Clinical interviews and measures

PTSD diagnoses were made according to DSM-IV-TR criteria as determined by the SCID-I/P. PTSD symptom scores were established using the Clinician-Administered PTSD Scale (CAPS).^[32] A doctoral-level, licensed psychologist (IMR) administered both interviews. PTSD participants included survivors of accidents (4), physical assault (4), childhood physical abuse (4), childhood sexual abuse (2), combat exposure (2), rape (4); seven of the PTSD patients experienced more than one traumatic event that met Criterion A for PTSD. The mean age at onset of PTSD symptoms was 21 years (standard deviation (SD) = 9) and the mean duration of illness was 13 years (SD = 12). One PTSD patient met criteria for current comorbid major depressive disorder (MDD), and another PTSD patient had comorbid panic disorder without agoraphobia. Past Axis I diagnoses in the PTSD group were major depressive disorder (5), cannabis abuse (2), and alcohol abuse (1). All participants completed the Beck Depression Inventory, 2nd version (BDI-II),^[33] as well as the State-Trait Anxiety Inventory (STAI-S/T).^[34] Only PTSD subject 6 (Table 2) was taking psychotropic medication (fluoxetine 60mg, stable dose for greater than 8 weeks); this subject is shown as an asterisk marker in Figure 2. All other subjects were free of psychotropic medications.

MRI and MRS Data Acquisition

Magnetic resonance imaging (MRI) and ¹H-MRS were performed using a 4.0 Tesla (T) Varian Unity/INOVA MR scanner (Varian Inc., Palo Alto, CA, USA) and a volumetric, 16-element single-tuned (170.3 MHz) birdcage-design radio-frequency (RF) head coil (XLR

Imaging, London, Canada). Participants were positioned inside the bore of the magnet with their head firmly padded to minimize head motion. After global shimming, high-contrast three-dimensional fast low-angle shot T1-weighted images were collected to guide voxel placement. The insula voxel ($15 \times 30 \times 20 \text{ mm}^3$) was double-obliqued over the right insular cortex using the anatomical sagittal and axial images, and the voxel's anterior face was aligned with the anterior edge of the insula (Figure 1). The ACC voxel ($30 \times 20 \times 20 \text{ mm}^3$) was centered on the midline in the axial plane, and in the sagittal plane was positioned with its inferior side abutting the surface of the corpus callosum and with its anterior side aligned with the descending portion of the genu of the corpus callosum (Figure 1).

For each voxel, proton spectroscopy implemented a Mescher-Garwood Point-Resolved Echo Spectroscopy Sequence (MEGAPRESS).^[35] The MEGAPRESS sequence collected 68-millisecond (ms) echo-time spectra in an interleaved fashion where the GABA-editing pulse was applied on every second transient. Additional acquisition parameters were: TR = 2s, spectral bandwidth = 2kHz, readout duration = 512ms, number of excitations = 384, total scan duration = 13min. Manual shimming of the magnetic field within each prescribed voxel achieved water linewidths ranging from 9–12 Hz. Following the automated optimization of water suppression power and tip angles, the transmitter frequency was set onto the creatine resonance at 3.00ppm to minimize chemical-shift displacement artifact for each spectral acquisition.

Proton MRS processing—A physicist (JEJ) conducted all spectroscopic data processing and analyses blind to all subject information and group assignment. For each spectral pair, the individual “ON” and “OFF” spectra were first frequency- and phase-corrected by using the NAA resonance of the interleaved “OFF” spectra as a navigator. Individual spectra within the time-series were first averaged in groups of 16 to increase signal-to-noise for this purpose. The phase- and frequency-correction factors from the OFF NAA resonance for each averaged group were then used for both the ON and OFF datasets. This was necessary since the NAA resonance was saturated in the “ON” spectra due to the frequency-selective Gaussian editing pulse applied at 1.89 ppm. This strategy provides effective tracking and correcting of motion artifacts throughout the 13-min acquisition, which otherwise would result in imperfect subtraction and contamination of the difference-edited GABA signal with creatine (Cr) at 3.00ppm. All corrected ON and OFF spectra were then averaged separately to produce single 68ms spectra, which were then subtracted to produce the optimized, difference-edited GABA spectrum. In order to obtain the best estimate of the area under the 3.00ppm difference-edited GABA resonance, the difference-edited spectra were first filtered with 5Hz exponential filtering and then Fourier-Transformed. The magnitude spectrum was then obtained and the area under the GABA resonance numerically integrated from 2.9–3.2ppm. In order to obtain total creatine area, the 68ms OFF sub-spectra were fitted using LCModel^[36] and basis sets acquired with phantoms at 4T.

All fitted metabolite areas were normalized to total Cr (creatine and phosphocreatine combined) from the 68ms spectrum after determining that the patient and control groups did not differ in terms of Cr/total proton signal (Table 3). Thus, Cr from the 68ms spectrum was used as a nonbiased internal standard (denominator) for this study.

Image segmentation—All image segmentation was performed blind to all subject identifying data. For voxel tissue partial-volume estimation, high-resolution T1-weighted axial images were first automatically segmented into cortical grey matter (GM), white matter (WM) and cerebral spinal fluid (CSF) compartments using the segmentation tool in the software package FSL 4.1 (FMRIB Software Library; Oxford, UK). This package allows for optimized and fully automated cortical tissue segmenting. Segmented images were then reformatted for input into an automated voxel co-registration and partial-volume analysis

program written in-house in C-code. Subsequently, the volumetric tissue contribution for each oblique voxel was determined and contributions of GM, WM, and CSF were calculated.

Statistical Analyses

All demographic, behavioral and imaging variables were tested for normality and equality of error variances. Our hypotheses of between-group differences in GABA in the right anterior insula and dorsal ACC were tested using independent t-tests with Bonferroni-adjusted two-tailed alpha of .025. Significant group differences were followed by post hoc stepwise multiple regressions to examine the effects of age, grey/white/CSF partial volume, and sex as potential covariates based on prior studies.^[37–39] These regressions entered group and the aforementioned covariates using a mixed entry procedure with entry and removal probabilities of .05. Bivariate relationships were examined using Pearson's r , only for MRS variables found to differ between groups (in order to limit the number of follow-up analyses). Specifically, we examined correlations with clinical variables, as well as the hypothesized associations of GABA with state and trait anxiety. Secondary analyses compared N-acetyl aspartate (NAA) (normalized to Cr) between groups. The JMP statistical package (SAS Institute, Cary, NC) was used for all statistical analyses.

RESULTS

Demographic and clinical variables

The two groups were matched on major demographic variables (Table 1). In addition, the groups were matched for proportion of female subjects in the follicular (50%) and luteal phase (50%) of their menstrual cycle. PTSD patients reported mild depression levels on the BDI-II, while healthy controls endorsed no depression symptoms. State and trait anxiety scores were log-transformed to obtain normal distributions, and PTSD patients scored significantly higher than controls on both measures. Patients' CAPS scores reflect PTSD symptoms in the moderate-to-severe range (Table 1).

GABA in PTSD and control subjects

In the insula voxel, PTSD patients had 30% less GABA/Cr than healthy comparison subjects, corresponding to a significant group difference and large effect size (Cohen's $d = -1.13$, $p = .003$; Table 3). Follow-up stepwise regressions with diagnostic group as the independent variable showed that none of the potential covariates met criteria for entry into the regression (i.e., not age, sex, GM proportion, WM proportion, or CSF proportion). Moreover the two groups did not differ in terms of relative tissue proportions within the insula voxel (Table 3). The group difference remained statistically significant and the effect size slightly increased if the patient (PTSD subject #6, Table 2) on fluoxetine was removed from the analysis ($t(24) = -3.19$, $p = .004$, $d = -1.28$), and if the two patients with comorbid MDD and panic disorder were also removed ($t(22) = -2.94$, $p = .008$, $d = -1.22$). Finally, secondary analyses revealed that insula NAA/Cr did not differ significantly between groups (Table 2).

For the ACC voxel, there were no significant group differences in GABA/Cr, relative tissue contributions, or NAA/Cr (Table 3).

Correlations with anxiety and clinical measures

Insula GABA/Cr was not significantly associated with age at onset ($r = -0.41$, $df = 13$, $p = .16$) or illness duration ($r = 0.01$, $df = 13$, $p = .96$) of PTSD, and insula GABA/Cr did not differ between patients with one versus multiple traumatic events ($t = -1.77$, $df = 13$, $p = .10$).

11). Insula GABA/Cr was not correlated with PTSD subjects' current CAPS ($r = 0.16$, $df = 13$, $p = .61$), lifetime CAPS ($r = 0.24$, $df = 13$, $p = .42$), or BDI-II scores ($r = -0.06$, $df = 13$, $p = .85$).

Within the whole sample, insula GABA/Cr was significantly negatively correlated with log-transformed measures of state anxiety ($r = -0.63$, $df = 24$, $p = .0005$) and trait anxiety ($r = -0.51$, $df = 24$, $p = .007$) (Figure 2).

DISCUSSION

To our knowledge, this is the first ^1H -MRS study reporting altered regional brain GABA in PTSD. We found that GABA in the right insula was significantly lower in PTSD patients relative to matched healthy subjects, whereas there were no significant group differences in ACC GABA. In addition, we found a significant negative correlation of insula GABA with state and trait anxiety in the whole sample. These findings are consistent with a growing literature indicating that GABA assessed with ^1H -MRS is sensitive to clinical anxiety, and further suggest that it may be sensitive to variation along a continuum of subclinical to pathologic anxiety symptoms. Reduced insula GABA may be a substrate of insula hyper-reactivity in PTSD, and more broadly, insula GABA levels may relate to individual differences in anxiety proneness.

GABA was significantly lower in the right insula of PTSD subjects versus controls, and this was not due to group differences in gray-white matter tissue proportions within the MRS voxel. In addition, we can be confident that the GABA difference was not due to psychotropic medication effects, as all but one of the patients were unmedicated. A GABA reduction in the insula could reflect either an alteration intrinsic to the pathology of PTSD, or an adaptation to that pathology. The insula is a key component of fear circuitry^[40] and is consistently hyperactive in PTSD subjects across a variety of functional imaging paradigms, including symptom provocation and processing of threatening stimuli.^[1] Moreover, the right anterior insula appears especially important for interoception and conscious awareness of anxious states.^[4, 41, 42] Unlike GABA levels, insula NAA and creatine levels did not differ significantly between PTSD patients and control subjects. NAA is used as an indicator of neuronal integrity in most neuropsychiatric pathologies^[43] due to its high correlation with axon density.^[44] Creatine is depleted along with NAA in pathologies involving cell loss.^[45, 46] Thus, the absence of concomitant and substantial decreases in NAA and creatine suggests that the insula GABA reduction is not consequent to neuronal injury or loss. Instead, the GABA deficit may reflect an abnormality of metabolism, such as decreased synthesis of GABA. GABA is formed from the alpha-decarboxylation of glutamate by glutamic acid decarboxylase (GAD).^[47] Thus, lower GABA could result from decreased GAD activity, which is thought to be the principal regulator of steady-state *in vivo* GABA concentration.^[48] Alternatively, deficient precursors could also limit GABA synthesis. Two previous studies have reported associations between polymorphisms in GAD genes and anxiety disorders,^[49, 50] although neither study included PTSD subjects, leaving this as an enticing question for future investigation.

Insula GABA was negatively correlated with trait and state anxiety in the combined subject sample, indicating that it relates to individual differences in the anticipation and experiencing of anxiety. Trait anxiety is a measure of anxiety proneness and predicts an individual's propensity to anticipate and experience state (situational) anxiety. Functional neuroimaging research has consistently implicated the insula in anticipating and responding to anxiety-provoking stimuli,^[51–56] particularly in subjects selected to be anxiety-prone or clinically anxious,^[57–61] including PTSD patients.^[1, 62, 63] Given the insula's roles in mediating homeostatic responses to stress and in predicting aversive body responses,^[4] it

seems concordant to find a relationship between insula neurochemistry and psychological mechanisms that influence the probability, intensity and duration of these responses. In the context of trauma exposure, it would be interesting to investigate whether insula chemistry predicts vulnerability or resilience to protracted fear responses.

Contrary to our predictions, dorsal ACC GABA did not differ significantly between PTSD patients and control subjects, and the effect size for this comparison was small. Functional imaging studies have often found altered dorsal ACC excitability in PTSD, although the directionality has included both hypoactivity in emotional paradigms and hyperactivity in certain cognitive paradigms and at rest.^[1, 6, 64, 65] It may be that altered function of the dorsal ACC reflects alterations in neurochemical mechanisms other than GABA metabolism. Alternatively, it is possible that our bilateral voxel masked a unilateral alteration in dorsal ACC GABA. This would be consistent with prior receptor imaging work in PTSD showing reduced benzodiazepine-receptor binding in the right ACC only.^[17]

Several methodological issues merit consideration in the interpretation of our results. The main limitation of this study is the small sample, which is in part a consequence of our strict exclusion criteria for potentially confounding influences, including drug and nicotine use.^[66, 67] In addition, all but one of the PTSD patients were free of psychotropic drug use, such that our results were not confounded by current medication effects. Unlike ¹H-MRS studies of major depression,^[13, 68–70] we did not find a relationship of GABA with depression scores; however our PTSD patient group was only mildly depressed such that restricted variance in BDI scores may have precluded a significant finding. Menstrual cycle stage also can be a source of variability in GABA among female subjects,^[31] and we were able to match the groups on proportion of women in each menstrual stage. In addition, the significant diagnostic group difference in insula GABA was maintained and the effect size even increased ($d = -1.9$) when analyses were repeated in only male subjects. Use of creatine as an internal reference is appropriate only when it is not altered by the pathologic condition of interest.^[71] In this study, PTSD patients and control subjects did not differ in terms of creatine levels in either of the two voxels examined, and there are no reports of altered creatine in these regions in previous MRS studies of PTSD.^[72, 73] Importantly, current ¹H-MRS methods detect total brain GABA concentration averaged over a relatively large area of interest that includes both intracellular and extracellular compartments of brain GABA.^[74] At the same time, ¹H-MRS GABA is sensitive to drug manipulation^[22, 67] and behavioral challenge,^[75] suggesting that it is at least correlated with neuromodulatory pools of GABA.^[74] Finally, the GABA resonance overlaps with that of macromolecules^[76] and we cannot rule out that a group difference in macromolecules contributed to our GABA findings; however, there is no reason to suspect that PTSD would be associated with differential and substantial alterations of these heterogeneous lipid molecules.

In summary, ¹H-MRS GABA in the right insula was reduced in PTSD patients versus matched healthy subjects, and was negatively correlated with state and trait anxiety in the whole sample. These findings may be consistent with well-replicated evidence of insula hyperactivity in functional imaging studies of PTSD, and with the insula's role in subjective awareness and prediction of aversive emotional states. No group difference in GABA was found in the dorsal ACC, though this may be due to the small sample and bilateral ACC voxel. Our findings motivate study into whether insula GABA alterations might predict responses to trauma exposure and/or be sensitive to therapeutic interventions in PTSD.

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Figure 1. T1-weighted images depicting voxel placement as well as difference-edited and 68ms spectra from the ACC (a, b) and insula (c, d) of a representative subject.

Figure 2.

Associations of GABA/Cr in right anterior insula with state anxiety (A) and trait anxiety (B) in the whole sample. Circles correspond to PTSD patients (asterisk indicates the single medicated patient) and triangles are control subjects.

TABLE 1

Demographic and clinical data

	PTSD n = 13	Controls n = 13	t/Chi-square	p
Age	33 ± 12	32 ± 12	0.36	.72
Female	6 (46%)	6 (46%)	0	1
Caucasian	4 (31%)	5 (39%)	0.20	.65
BDI-II ^a	16 ± 11	0.4 ± 0.9	4.94	<.0001
State Anxiety ^b	43 ± 12	25 ± 4	7.79	<.0001
Trait Anxiety ^b	46 ± 12	32 ± 4	3.92	.001
CAPS ^c current	62 ± 24	–		
CAPS ^c lifetime	76 ± 20	–		

Note: Mean ± SD or N(%); Beck Depression Inventory, 2nd version;^[33]^b State and Trait Anxiety Inventory;^[34]^c Clinician Administered PTSD Scale.^[32]

TABLE 2

Insula and anterior cingulate cortex GABA levels in adults with PTSD and healthy control subjects

Subject	Insula GABA/Cr		ACC GABA/Cr	
	PTSD	Control	PTSD	Control
1	0.064	0.112	0.104	0.072
2	0.083	0.091	0.069	0.116
3	0.112	0.154	0.110	0.092
4	0.031	0.136	0.073	0.104
5	0.129	0.105	0.091	0.115
6	0.082	0.078	0.069	0.063
7	0.052	0.088	0.138	0.140
8	0.100	0.138	0.105	0.124
9	0.083	0.111	0.120	0.110
10	0.087	0.106	0.158	0.074
11	0.105	0.130	0.090	0.079
12	0.085	0.100	0.063	0.082
13	0.035	0.149	0.087	0.071

TABLE 3
Group Differences in MRS and MRI data between PTSD patients and healthy controls, mean $\pm$ SD

Voxel	MRS/MRI variable	PTSD n = 13	Controls n = 13	t	p
Insula	GABA/Cr	0.081 $\pm$ 0.029	0.115 $\pm$ 0.024	-3.32	.003
	NAA/Cr	1.17 $\pm$ 0.13	1.27 $\pm$ 0.19	-1.71	.10
	Grey matter %	53.38 $\pm$ 6.93	50.30 $\pm$ 5.60	1.25	.22
	White matter %	35.85 $\pm$ 9.03	40.06 $\pm$ 8.69	-0.96	.34
	Cerebrospinal fluid %	10.73 $\pm$ 4.45	9.80 $\pm$ 4.24	0.55	.59
Anterior Cingulate	Cr/total proton signal	0.33 $\pm$ 0.04	0.29 $\pm$ 0.04	0.61	.55
	GABA/Cr	0.098 $\pm$ 0.028	0.096 $\pm$ 0.024	0.26	.79
	NAA/Cr	1.11 $\pm$ 0.09	1.12 $\pm$ 0.09	-0.18	.86
	Grey matter %	53.09 $\pm$ 4.21	52.01 $\pm$ 6.42	0.49	.63
	White matter %	38.40 $\pm$ 4.65	35.45 $\pm$ 6.90	1.54	.23
	Cerebrospinal fluid %	8.5 $\pm$ 3.31	11.22 $\pm$ 6.38	- 1.31	.21
	Cr/total proton signal	0.32 $\pm$ 0.09	0.29 $\pm$ 0.05	-0.44	.66