

A magnetic resonance spectroscopy investigation of obsessive–compulsive disorder and anxiety

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Abstract

The aim of the current study was to use proton magnetic resonance spectroscopy (MRS) to investigate potential irregularities in neurochemical compounds in obsessive–compulsive disorder (OCD) and the extent to which these irregularities are related to state anxiety. Single voxel MRS was used to image the head of the caudate nucleus (HOC) and orbitofrontal white matter (OFWM) bilaterally in adult patients with OCD and a control group. The results indicated that patients with OCD had increased levels of a combined measure of glutamate and glutamine (Glx/Cr) and *N*-acetyl-*l*-aspartic acid (NAA/Cr) relative to creatine in the right OFWM and reduced levels of myo-inositol relative to creatine (ml/Cr) in the HOC bilaterally. Correlational analyses indicated that Glx/Cr in the OFWM was related to OCD symptoms, while ml/Cr in the HOC was related to trait and/or state anxiety. Reanalysis of the significant group differences controlling for state anxiety symptoms erased three of the four group differences. These results are discussed in context of the methodological difficulties facing this area of research.

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1. Introduction

Neuroimaging studies in obsessive–compulsive disorder (OCD) have the potential to increase our understanding of the connection between the observable symptoms and associated neurobiology, and perhaps lead to improvements in treatment and in matching treatment to patient needs (Rauch, 2000). With respect to neurobiology, brain regions involved in processing threat cues and routine complex motor programming have been implicated in OCD. For instance, the orbitofrontal

cortex (OFC) and the caudate nucleus are thought to be involved in the mediation of emotional responses to stimuli associated with biologically based threats, a process that appears to be disrupted in OCD (Saxena et al., 2001). Much of the evidence supporting the presence of OCD-specific dysfunction in the OFC and caudate comes from studies employing positron emission tomography (PET) and single photon emission computed tomography (SPECT) to compare patients with OCD with healthy volunteers. The most consistent differences between patients with OCD and healthy controls identified by this research have been in the orbital gyrus and the head of the caudate nucleus (Saxena et al., 2001; Whiteside et al., 2004).

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More recently, researchers (Ebert et al., 1997; Bartha et al., 1998; Ohara et al., 1999; Fitzgerald et al., 2000; Rosenberg et al., 2000; Russell et al., 2003) have begun using magnetic resonance spectroscopy (MRS) to evaluate potential differences in regional brain metabolites between patients with OCD and healthy controls. MRS has been used primarily to measure concentrations of metabolites in brain tissue such as *N*-acetyl-l-aspartate (NAA; a marker of neuronal viability), combined glutamate and glutamine (Glx; a marker for excitatory neurotransmitters), choline (Cho; a marker of cell membrane turnover), myo-inositol (mI: involved in phospholipid metabolism), and creatine (Cr; a marker of cellular energetics, commonly used as a reference level).

Findings from the PET and SPECT literature have begun to be replicated through the use of MRS. Although no studies have found identical results, two have identified unilaterally decreased NAA in the striatum of adults with OCD (relative to Cr in the right striatum: Ebert et al., 1997; relative to water in the left striatum: Bartha et al., 1998), and two have identified increased absolute concentrations of choline bilaterally in the medial thalami (Rosenberg et al., 2001; Smith et al., 2003) of children with OCD. In addition, Rosenberg et al. (2000) found that elevated levels of Glx concentrations relative to water in the left caudate decreased after successful pharmacotherapy of pediatric OCD. Finally, compared with findings in healthy controls, absolute levels of NAA have been shown to be increased in the right, but not left, dorsolateral prefrontal cortex (Russell et al., 2003) and the absolute Glx level has been found to be decreased in the anterior cingulate of children with OCD (Rosenberg et al., 2004).

In the present study, we used MRS to examine levels of neurochemicals in the head of the caudate nucleus and orbitofrontal white matter (OFWM) in adults with OCD versus a healthy control group. First, we sought to determine whether the previously observed lesser amounts of NAA levels found in the striatum (Ebert et al., 1997; Bartha et al., 1998) could be localized to the head of the caudate and whether increased levels of Glx in children (Rosenberg et al., 2000) could be extended to adults. Differences in these areas would be consistent with the functional neuroanatomy theory of OCD proposed by Saxena et al. (2001) that implicates the ventromedial caudate nucleus which receives projections from the OFC, as well as with the results of our meta-analysis, which found consistent differences in the head of the caudate nucleus (Whiteside et al., 2004). To our knowledge, MRS has not yet been used to study the head of the

caudate in adult OCD. Second, we sampled the OFWM since, despite frequent findings of abnormalities in the orbital gyrus using other imaging techniques (Saxena et al., 2001), only one study has used MRS to examine the prefrontal cortex in pediatric OCD (Russell et al., 2003). To our knowledge, no studies have used MRS to evaluate the prefrontal cortex in adult OCD. Since it is not possible to reliably image the orbital gyrus or OFC due to methodological constraints, we sampled the broader OFWM with a voxel that incorporated a large portion of the orbital gyrus and expected to find increased levels of Glx. Third, we investigated whether increased levels of Cho in OCD extended to the head of caudate and OFWM.

One limitation of the existing neuroimaging literature is that no studies have ruled out the possibility that observed differences in brain chemistry between OCD patients and controls might be related to between-group differences in levels of state (or trait) anxiety, as opposed to the presence of OCD per se. If differences in observed brain chemistry are associated with general anxiousness, this would suggest that such altered chemistry is a feature of excessive anxiety in general rather than OCD specifically. Only two studies to date, both utilizing SPECT (Lucey et al., 1995, 1997), have measured anxiety during the scanning process. One of these studies found that the anxiety ratings correlated with whole brain blood flow (Lucey et al., 1997).

Since no OCD studies utilizing MRS have controlled for the level of anxiety experienced during the imaging procedure, we included a measure of state anxiety (the Subjective Units of Distress Scale; SUDS) as a possible mediator of the relationship between OCD and neuroimaging findings. We hypothesized that differences in levels of state anxiety during the scan would account for decreased levels of NAA in the striatum and increased levels of Glx in the head of caudate and OFWM in OCD patients compared with control values.

2. Methods

2.1. Participants

Thirty Caucasian adults, aged 31–62, participated in the study, which was approved by the Mayo Clinic institutional review board. The clinical group consisted of 15 patients with OCD who were screened after presenting for treatment at an outpatient anxiety disorders clinic at Mayo Clinic. Fifteen age- and sex-matched healthy controls were recruited through print advertising. A total of 18 patients with OCD and 17

potential controls were screened. All participants were provided a small stipend as compensation for their time.

All participants had a diagnostic interview by a trained doctoral level psychologist using the Structured Clinical Interview for DSM-IV Axis I and II Disorders (SCID-IV; First et al., 1996). The OCD group was also administered the Yale-Brown Obsessive Compulsive Scale (Y-BOCS). Exclusion criteria were (a) current substance abuse, (b) major underlying neurological or medical problems, (c) active psychosis, or (d) acute risk for suicide or danger to others. Participants in the OCD group met SCID-IV criteria for OCD and had a score of 18 or higher on the Y-BOCS, but were excluded if they had a current diagnosis of major depression, bipolar disorder, schizophrenia, mental retardation, or substance abuse, or a history of a failed trial of cognitive behavior therapy for OCD. Finally, as the Expert Consensus Guidelines for the treatment of OCD (Frances et al., 1997) consider 8 to 13 weeks to be an adequate medication trial, the eight OCD patients who were receiving treatment with psychotropic medication were required to have been on a stable dose of their medication for at least 3 months.¹ Of the seven patients who were not receiving psychotropic medications, three had received them in the past, and four were treatment naïve. Individuals in the healthy control group had no current or past history of psychiatric diagnoses or psychiatric or psychological treatment. None were taking psychotropic medication. Table 1 presents demographic and clinical information for both groups.

2.2. Design and general procedure

The study employed a two-group case–control design. Research technicians were blind to diagnostic status of participants. Following a review of the study procedures and provision of consent, potential participants received the diagnostic interview. Participants who met the inclusion criteria provided a urine sample to screen for recreational drug use and received the MRS scan. The MRS technician asked the participants to rate their anxiety using the Subjective Unit of Distress Scale (SUDS; described below) at the following times: (a) before entering the scanning room, (b) after being placed

¹ Medications included Trazodone, Remeron, Celexa, clomipramine, clonazepam, Prozac, Paxil, Ritalin, Anafranil, Wellbutrin, and Luvox.

Table 1
Participant demographic and clinical information

	Experimental group		t-value
	OCD (n=15)	Control (n=15)	
Age	41.2 (7.1)	41.4 (7.9)	0.07
Males	9 (60%)	9 (60%)	–
Education (years)	16.7 (3.2)	16.14 (4.0)	0.11
Y-BOCS			
Total	24.13 (3.2)	–	–
Obsessions	11.73 (1.5)	–	–
Compulsions	12.40 (2.5)	–	–
Sheehan Disability Scale	17.57 (6.1)	–	–
Hamilton Depression Scale	8.8 (4.4)	–	–
Age of onset	18.27 (8.0)	–	–
Duration of symptoms in years (n=11)	23.09 (9.8)	–	–
Anxiety in scanner	31.36 (14.3)	7.27 (6.9)	5.87 *
STAI-T	49.43 (6.9)	30.43 (9.2)	6.18 *
BDI	13.07 (7.7)	1.08 (1.66)	5.48 *
OCI-R	27.5 (10.3)	1.69 (2.32)	8.85 *

Y-BOCS = Yale-Brown Obsessive Compulsive Inventory; STAI-T = State-Trait Anxiety Inventory-Trait Form; BDI = Beck Depression Inventory; OCI-R = Obsessive–Compulsive Inventory-Revised.

* $P < 0.001$.

in the scanner before the first imaging sequence, and (c) following each imaging sequence (11 times) during the scan.

2.3. Assessment

The OCD patients had an interview that included the structured interview described below. All participants completed the self-report measures.

2.3.1. Clinical interview

Structured Clinical Interview for DSM-IV (SCID-IV; (First et al., 1996): The SCID-IV was used to make diagnoses according to DSM-IV criteria. Both part I (for Axis I disorders) and part II (for personality disorders) were employed.

Yale-Brown Obsessive Compulsive Scale (Y-BOCS; Goodman et al., 1989a,b): The Y-BOCS is a valid and comprehensive 10-item clinician interview of OCD symptoms considered to be the “gold standard” severity measure. It yields a total score of 0 to 40, has an internal consistency of 0.91, is sensitive to treatment effects, and has good convergent validity with other OCD measures (Taylor, 1995).

Hamilton Depression Scale (HAM-D; Hamilton, 1960): The HAM-D is a 17-item interview measure of vegetative symptoms of depression. Interrater reliability

is high (0.84), and the scale has good convergent validity with other depression measures.

2.3.2. Self-report measures

Beck Depression Inventory (BDI; Beck et al., 1961): This is a 21-item measure that is widely used to assess depressive symptoms. Scores on the BDI range from 0 (no symptoms) to 63 (very severe symptoms). The sound psychometric properties of the scale are supported by an extensive literature (Beck et al., 1988).

State-Trait Anxiety Inventory-Trait Version Form Y (STAI-T; Spielberger et al., 1983): The STAI-T is a 20-item inventory that assesses trait anxiety, defined as the enduring tendency to experience anxiety. The STAI-T has demonstrated high test–retest reliability, internal consistency, and concurrent validity with other anxiety questionnaires (Spielberger et al., 1983).

Obsessive–Compulsive Inventory-Revised (OCI-R; Foa et al., 2002): The OCI-R is an 18-item self-report questionnaire. Respondents rate the degree to which they have been bothered or distressed by 18 common

symptoms of OCD in the past month. OCI-R total scores have demonstrated excellent psychometric properties and validity (Foa et al., 2002).

2.3.3. Assessment during imaging

Subjective Units of Distress Scale (SUDS): Participants were asked to rate their current level of anxiety during the scan on a scale of 0 to 100, where 0 indicates that they feel no anxiety whatsoever and 100 indicates that they are extremely anxious, experiencing maximum distress. Anchors were created by having the participants identify situations that corresponded to 0, 50, and 100. The 12 ratings obtained while the patient was in the scanner were averaged to produce a single score representing overall anxiety experienced during the scan.

2.4. Neuroimaging procedures

To eliminate inter-scanner variability, all imaging was performed on a single clinical 1.5-Tesla MRI scanner

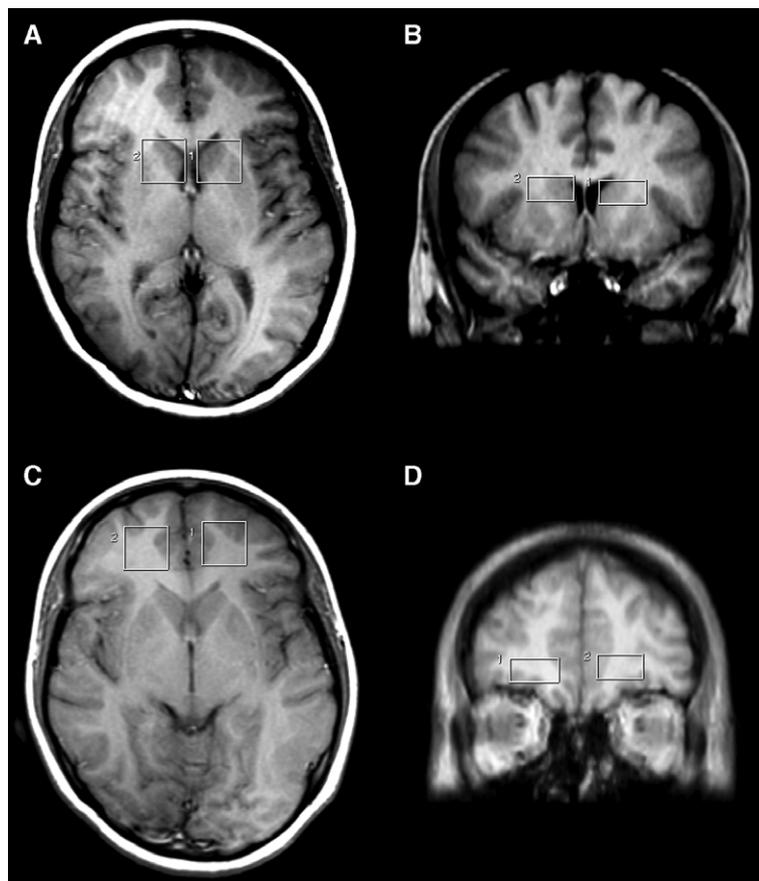


Fig. 1. Axial (A, C) and coronal reformatted (B, D) images showing the representative locations of the MRS voxels. A, B: locations of the left and right caudate head voxels. C, D: locations of the orbitofrontal cortex voxels.

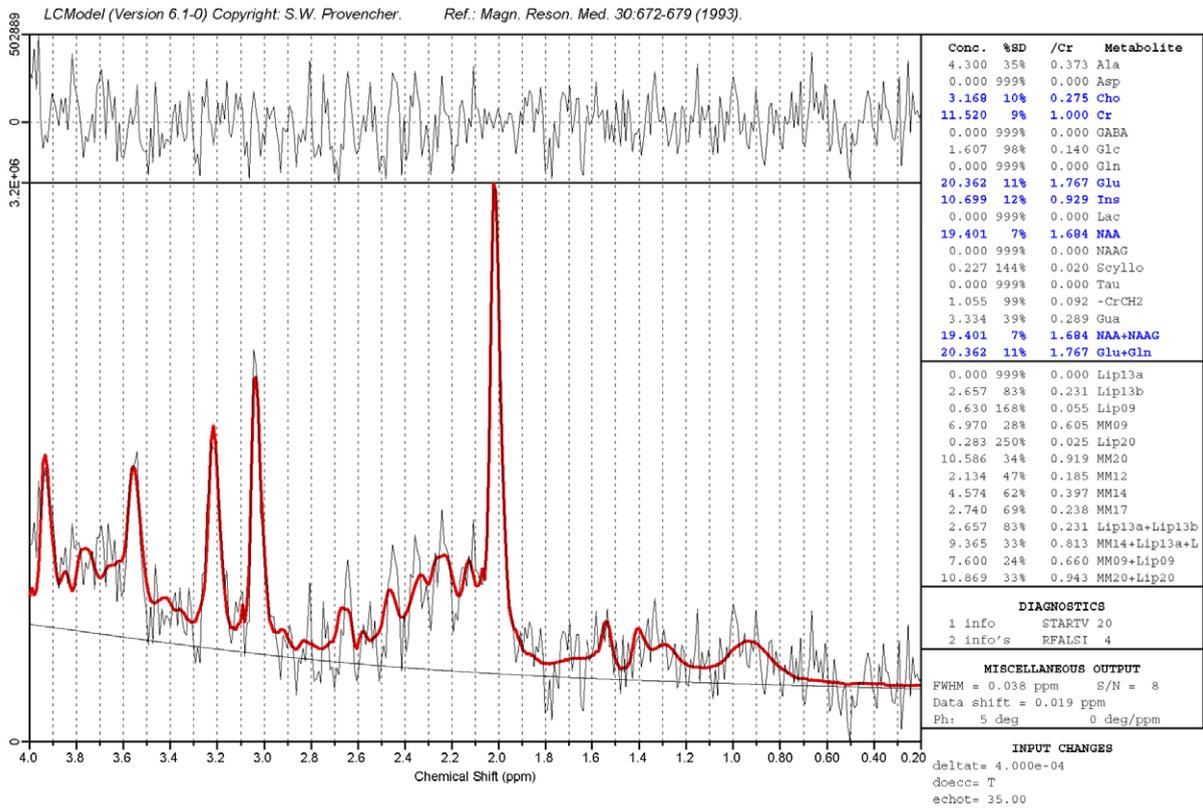


Fig. 2. Typical spectrum as analyzed by LCModel. Note: This spectrum is from a voxel located in the left orbitofrontal white matter of Subject 10. The concentrations of the metabolites and their associated Cramer-Rao bounds are listed in the box on the right.

(GE Medical Systems, Milwaukee, Wisconsin) at the Mayo Clinic. A clinical MR scan was obtained, followed by four single-voxel short-echo-time MRS scans (PROBE-P, TR=2000, TE=35, 128 scans, 2 cm A-P × 2 cm R-L × 1 cm S-I for a nominal voxel volume of 4 cc). Voxels were relatively “flat” to 1) reduce intravoxel dephasing by excluding brain regions affected by susceptibility artifacts from the adjacent ethmoid sinuses and orbits, and 2) better fit the selected brain structures. The total imaging time was approximately 1 h.

Caudate head voxels were positioned to encompass the entire caudate head. Unfortunately, given the relatively large size of the voxel as compared with the size of the caudate head, a small portion of the adjacent anterior horn of the lateral ventricle, the surrounding periventricular and internal capsule white matter, and the anterior aspect of the putamen were also included in the “caudate head” voxel. However, these voxels consist primarily of caudate head gray matter. OFWM voxels were positioned to include the lateral aspect of the orbital gyrus in the medial portion of the voxel, and the adjacent orbitofrontal cortex in the lateral portion of the voxel. Unfortunately, given the severe susceptibility

artifacts from the ethmoid sinuses and orbits, on average the OFWM voxels contained a large quantity of subcortical white matter adjacent to orbitofrontal cortex. Occasionally these voxels would need to be repositioned to obtain usable spectra, and in several cases no usable spectra could be obtained. Fig. 1 demonstrates representative voxel placement.

Following the scans, participant information was removed from the images and a subject number assigned; the images and spectra were then sent to a

Table 2
Percentage of acceptable voxel data by location and metabolite

Metabolite	Region of interest			
	Head of caudate		Orbitofrontal white matter	
	Left	Right	Left	Right
Cho/Cr	95	100	90	83
mI/Cr	80	90	73	78
NAA/Cr	98	100	90	85
Glx/Cr	76	88	56	56

board-certified neuroradiologist (JDP) for clinical interpretation and further analysis. Single voxel MRS data were processed using LCModel (Provencher, 1993) to quantify the metabolites; a typical LCModel spectrum and fitting are shown in Fig. 2. The quantitative metabolite data placed into a spread sheet, provided that 1) the spectrum for a given voxel was well shaped and free of artifact, and 2) the LCModel Cramer-Rao bounds for a given metabolite were less than 20%. Metabolite values that did not meet these criteria were entered as “missing.” Table 2 presents the percentages of acceptable voxel data by location and metabolite. Overall, data integrity for the caudate voxels was excellent, while data integrity for the OFWM voxels was not optimal due to susceptibility artifacts from the ethmoid sinuses which invalidated some of the spectra. The results of the clinical assessment and group designation were placed into a spread sheet using the assigned subject number. To preserve the blinded nature of the study, clinical information was not revealed until all spectral analysis was complete.

The primary outcome variables were the ratios of NAA (NAA+NAAG data from LCModel), Cho, Glx (Glu+Gln data from LCModel) and mI relative to Cr in each brain region of interest. NAAG was included in addition to NAA to reduce the variability in the data. NAAG is the aminated form of NAA, and both are neuronal markers. Ratios to Cr were used to “correct” for the amount of CSF in the voxel; at the time this analysis was carried out, formal tissue volume correction software was not available to explicitly compensate for the CSF volumes in the voxels.

2.5. Overview of the analyses

To test the hypotheses that the OCD patients have lower relative NAA, as well as higher relative Glx and choline levels, in the head of the caudate and OFWM compared with controls, we conducted independent sample *t*-tests comparing these two groups. In addition, we conducted *t*-tests to look for differences in relative levels of myo-inositol (mI). To test the hypothesis that these compounds are related to OCD symptomatology, we conducted bivariate correlational analyses between neurochemical levels and scores on the Y-BOCS. To test the hypothesis that neuroimaging findings in OCD are related to state anxiety, we a) conducted bivariate correlations between anxiety and relative levels of NAA, mI, choline, and Glx; and b) conducted analyses of covariance on any chemical levels for which *t*-tests indicated significant between-group differences. Before

conducting the above-mentioned analysis, we inspected the data for outliers to increase the normality of the data distribution and to reduce the chance of single subjects disproportionately affecting the results. Outliers were defined as any value greater than two standard deviations from the group (OCD or control) mean. No more than one data point was removed per group, and removal of outliers improved the normality of the variable distributions.

3. Results

3.1. Preliminary analyses

Preliminary analyses were conducted to determine if the OCD and control participants differed on demographic and clinical variables. As expected given our matching procedure, the groups did not differ on age, years of education, or gender composition. As expected, we observed significant differences in the level of depressive symptoms, trait anxiety, state anxiety in the scanner, and level of OCD symptoms (Table 1). OCD patients scored higher on these clinical variables relative to control participants.

Table 3
Relative values of neurochemicals for the experimental groups

	Experimental group				<i>t</i> -value
	OCD		Control		
	<i>N</i>	Mean	<i>N</i>	Mean	
Head of caudate					
Left					
Cho/Cr	14	0.24 (0.030)	15	0.26 (0.043)	1.61
mI/Cr	13	0.69 (0.241)	11	1.01 (0.369)	2.56*
NAA/Cr	14	1.27 (0.113)	15	1.30 (0.314)	0.32
Glx/Cr	12	2.03 (0.335)	9	2.24 (0.610)	1.01
Right					
Cho/Cr	14	0.25 (0.028)	14	0.27 (0.032)	1.72 [†]
mI/Cr	14	0.66 (0.179)	12	0.85 (0.219)	2.43*
NAA/Cr	13	1.17 (0.140)	15	1.24 (0.216)	0.93
Glx/Cr	14	1.98 (0.285)	12	2.06 (0.294)	0.69
Orbitofrontal white matter					
Left					
Cho/Cr	14	0.30 (0.070)	13	0.31 (0.076)	0.42
mI/Cr	10	1.26 (0.425)	9	1.31 (0.515)	0.22
NAA/Cr	14	1.65 (0.365)	13	1.65 (0.345)	0.03
Glx/Cr	7	2.95 (0.843)	9	2.67 (1.087)	0.57
Right					
Cho/Cr	12	0.34 (0.066)	10	0.30 (0.033)	1.81 [†]
mI/Cr	11	1.08 (0.328)	12	1.09 (0.278)	0.09
NAA/Cr	11	1.65 (0.200)	12	1.43 (0.237)	2.38*
Glx/Cr	8	2.76 (0.687)	7	2.09 (0.470)	2.17*

**P*<0.05, results in bold type. [†]*P*<0.10, results are italicized.

3.2. Between-group differences in neurochemical variables

Independent sample *t*-tests were conducted to test whether relative levels of NAA, Glx, Cho, and ml differed between groups. These results are presented in Table 3. As predicted, the level of Glx/Cr in the right OFWM was significantly greater in OCD patients than in healthy controls, $t(13)=2.17$, $P<0.05$. In addition, there was a nonsignificant trend for levels of Cho/Cr to be lower in the right caudate and greater in the right OFWM in OCD patients compared with healthy controls, $t(26)=1.72$, $P=0.10$, and $t(20)=1.81$, $P=0.09$, respectively. Contrary to our hypothesis, the level of NAA/Cr in the right OFWM was greater in patients with OCD than in healthy controls, $t(21)=2.38$, $P<0.05$. Additional analyses indicated that in patients with OCD, the levels of ml/Cr in the left and right caudate were significantly less than levels in healthy controls, $t(22)=2.56$, $P<0.05$, and $t(24)=2.43$, $P<0.05$, respectively. No other between-group differences were detected.

Further analyses were conducted to determine if subgroups of individuals differed on neuroimaging variables and thus would need to be accounted for in the overall analyses. Independent sample *t*-tests indicated that males and females did not differ reliably on any of the clinical

or neuroimaging measures. However, among OCD patients, the level of NAA/Cr in the left OFWM was greater in patients taking medication ($M=1.83$, $S.D.=0.29$) than in those not taking medication ($M=1.42$, $S.D.=0.33$), $t(12)=2.50$, $P<0.05$, and there was a marginally significant trend for patients taking medication to have lower levels of ml/Cr in the right OFWM than patients not taking medication, $t(9)=2.22$, $P=0.05$. As neither sex nor medication status had a substantial effect on the dependent variables, we did not conduct multivariate ANOVAs including these variables and neurochemical values.

3.3. Relationships between neurochemical variables and symptom severity

Correlational analyses were conducted to determine if neurochemical variables were related to clinical symptoms (Table 4). For measures that were administered to both experimental groups, the relative level of Glx in the right OFWM was related to OCD symptoms as measured by the OCI-R. Relative levels of ml in the head of the caudate were negatively related to trait anxiety as measured by the STAI-T bilaterally, to state anxiety as measured by the STAI-T bilaterally and to state anxiety as measured by the SUDS on the left side. Within the OCD sample, depressive symptoms as measured by the HAM-D were related to ml/

Table 4
Correlations (sample size) between neurochemical levels and symptomatology

	Y-BOCS obs.	Y-BOCS comps.	Y-BOCS total	OCI-R	STAI-T	SUDS	BDI	HAM-D
Head of caudate								
Left								
Cho/Cr	-0.20 (14)	-0.01 (14)	-0.09 (14)	-0.23 (26)	0.09 (27)	-0.18 (29)	0.01 (27)	-0.37 (13)
ml/Cr	0.46 (13)	0.28 (13)	0.40 (13)	-0.37 (21)	-0.44* (22)	-0.42* (24)	-0.38 (22)	0.29 (12)
NAA/Cr	-0.18 (14)	-0.21 (14)	-0.25 (14)	0.1 (26)	0.04 (27)	-0.06 (27)	-0.15 (22)	-0.02 (13)
Glx/Cr	-0.26 (12)	-0.21 (12)	-0.27 (12)	-0.20 (18)	-0.29 (19)	-0.15 (21)	-0.27 (19)	0.47 (11)
Right								
Cho/Cr	-0.14 (14)	0.19 (14)	0.09 (14)	-0.18 (25)	-0.16 (26)	-0.17 (28)	-0.33 (26)	0.31 (13)
ml/Cr	0.00 (14)	0.50 (14)	0.41 (14)	-0.31 (23)	-0.44* (24)	-0.22 (26)	-0.30 (24)	0.64* (13)
NAA/Cr	-0.04 (13)	-0.31 (13)	-0.26 (13)	-0.26 (25)	-0.21 (26)	-0.10 (28)	-0.17 (26)	0.35 (12)
Glx/Cr	0.26 (14)	-0.39 (14)	-0.20 (14)	-0.14 (23)	-0.11 (24)	-0.02 (26)	-0.01 (24)	0.32 (13)
Orbitofrontal white matter								
Left								
Cho/Cr	0.38 (14)	-0.25 (14)	-0.03 (14)	0.01 (24)	-0.07 (25)	-0.20 (27)	-0.08 (25)	0.03 (13)
ml/Cr	0.14 (10)	0.30 (10)	0.30 (10)	-0.11 (18)	-0.17 (19)	0.11 (19)	-0.19 (18)	-0.10 (10)
NAA/Cr	-0.25 (14)	0.05 (14)	-0.08 (14)	-0.01 (24)	0.04 (25)	0.04 (27)	0.02 (25)	0.37 (13)
Glx/Cr	-0.14 (7)	0.42 (7)	0.24 (7)	0.48 (14)	0.20 (15)	0.09 (16)	0.37 (14)	0.27 (7)
Right								
Cho/Cr	0.34 (12)	-0.48 (12)	-0.25 (12)	0.37 (20)	0.23 (20)	-0.02 (22)	0.10 (21)	-0.27 (11)
ml/Cr	-0.12 (11)	-0.05 (11)	-0.09 (11)	0.09 (21)	-0.26 (21)	-0.07 (23)	0.02 (22)	0.35 (10)
NAA/Cr	-0.86* (11)	-0.02 (11)	-0.30 (11)	0.33 (21)	0.43 (21)	0.41 (23)	0.40 (22)	0.27 (10)
Glx/Cr	0.37 (8)	0.46 (8)	0.53 (8)	0.58* (14)	0.47 (15)	0.37 (15)	0.51 (14)	-0.17 (7)

Y-BOCS = Yale-Brown Obsessive Compulsive Inventory; OCI-R = Obsessive-Compulsive Inventory-Revised; STAI-T = State-Trait Anxiety Inventory-Trait Form; SUDS = Subjective Units of Distress Scale, BDI = Beck Depression Inventory; HamD = Hamilton Depression Scale.

* $P<0.05$. [†] $P<0.01$. Significant results in bold type.

Cr in the right head of the caudate. OCD symptom severity as measured by the Y-BOCS was negatively related to the level of NAA/Cr in the right OFWM.

3.4. Between-group differences controlling for anxiety level

Given that anxiety was related to relative metabolite levels (principally mI), we conducted analyses of covariance (ANCOVA) to determine whether the aforementioned differences between OCD and control groups remained after controlling for the amount of anxiety experienced during the scanning procedure, as measured by SUDS scores. These analyses indicated that the between-group differences in levels of Glx/Cr and NAA/Cr in the right OFWM, and in levels of mI/Cr in the left head of caudate, were no longer significantly different after controlling for SUDS level, $P_s > 0.10$. However, the difference in level of mI/Cr in the right caudate head remained significant after controlling for SUDS scores, $F(1, 23) = 5.23$, $P < 0.05$.

4. Discussion

Previous research has identified the caudate and OFC as potential areas of the brain involved in OCD (e.g., Saxena et al., 2001; Whiteside et al., 2004). However, the present study is the first to use MRS to image the head of the caudate nucleus and the OFC in adult OCD patients. This study is also the first to thoroughly explore the relation of neuroimaging findings to state anxiety and depressive symptoms in OCD.

We found that within the right OFWM, relative levels of Glx and NAA were increased in patients with OCD compared with healthy controls. Moreover, greater levels of Glx/Cr were associated with more severe OCD symptoms. Although *greater* levels of NAA/Cr were found in OCD patients than in controls, NAA/Cr was *negatively* related to the severity of obsessions within the sample of OCD patients. We found that in the head of the caudate nucleus, relative values of mI were decreased in OCD patients bilaterally, and that lower levels of mI/Cr were associated with greater anxiety, but not with OCD symptom severity. Of the four significant group differences, only the differences in mI/Cr in the right head of the caudate remained significant after statistically controlling for state anxiety during the scanning process. We also found that the relative level of mI in the right head of the caudate was positively associated with depressive symptoms within the OCD sample.

The current findings of elevated Glx/Cr in the OFWM of OCD patients could be consistent with the PET and SPECT literature in finding patient–control differences in the orbital gyrus. PET and SPECT are thought to be markers of brain activity; and although it is unknown whether Glx concentrations are also a marker of brain activity, Glx has been found to have a direct relation to glucose metabolism as measured by PET (Pfund et al., 2000). If Glx is indeed a marker of brain activity, the current results coupled with previous findings of elevated Glx relative to water in the caudate (Rosenberg et al., 2000) lend support to theorized overactivation of the cortico-striato-thalamo-cortical circuit in OCD (Saxena et al., 2001). This theory would be consistent with positive correlations found between activation in the head of the caudate and the orbital cortex in OCD patients before, but not after, successful treatment (Baxter et al., 1992). A similar theory was posited by Rosenberg et al. (2004) to explain decreased absolute Glx in the anterior cingulate in the context of previous studies finding increased Glx relative to water in the caudate.

Although relative levels of Glx in the OFWM were positively associated with OCD symptoms, state anxiety appears to be an important variable in this relationship since the differences between OCD patients and controls did not remain significant after controlling for anxiety. Moreover, relative levels of Glx in the right OFWM were moderately correlated with the severity of anxiety and depressive symptoms, although none of these reached significance, perhaps due to low statistical power. Similarly, the trend toward differences in caudate Cho/Cr levels between OCD patients and healthy controls in the head of the caudate and the OFWM is consistent with previous studies implicating absolute choline disturbances in the thalamus in OCD (Rosenberg et al., 2001), but did not reach significance in this study.

Differences in relative NAA levels were also found in the right OFWM, but they are more difficult to interpret. Although relative levels of NAA were *increased* in OCD patients compared with controls, within the OCD sample greater levels of NAA/Cr were associated with *decreased* obsessive symptoms. However, this correlation is based on only 11 patients and thus should be interpreted with caution. In fact, increased levels of NAA/Cr in the right OFWM are similar to results found by Russell et al. (2003) that found increased absolute NAA in the dorsolateral prefrontal cortex in pediatric OCD. Increased relative levels of NAA found in the present study may be consistent with biologically based theories of OCD. Specifically, NAA is thought to be a marker of neuronal

viability, and thus our finding may reflect an overabundance of neurons. As such, increased NAA/Cr in the OFWM could be consistent with neurobiological models of OCD postulating increased activity in the cortex (Saxena et al., 2001) and/or neurodevelopmental theories of OCD involving inefficient neuronal pruning (Rosenberg and Keshavan, 1998).

This study represents the first to find differences in relative mI levels between OCD patients and healthy controls. However, mI disturbances have been implicated in other mental illnesses including bipolar disorder (Davanzo et al., 2001) and attention deficit/hyperactivity disorder (Courvoisier et al., 2004). In the current study, relative levels of mI were related to general anxiety rather than to OCD symptoms per se. Specifically, the level of left caudate mI/Cr did not differ between groups after controlling for state anxiety. In addition, although the differences in mI/Cr in the right caudate remained significant after controlling for state anxiety, the relative level of this neurochemical was significantly correlated with trait anxiety, but not with OCD symptoms. The *positive* correlation between mI/Cr and depression within the OCD group is inconsistent with the finding that the OCD group had relatively greater levels of depression, but *lower* mI/Cr levels compared with the control group. The possibility that depressive symptoms are related to group differences should be entertained as previous studies with MRS have found differences in the OFC between controls and depressed patients (Steingard et al., 2000). Again, the present finding should be interpreted with caution as they may be idiosyncratic to our relatively small sample.

Our results suggest that anxiety is an important (and we believe understudied) variable in understanding the relationship between neuroimaging findings and OCD symptoms. Specifically, out of the four group differences we found in neurochemical variables, only one remained significant after controlling for state anxiety during the scanning procedure, and levels of that neurochemical were correlated with anxiety. These findings indicate that the observed group differences in brain-imaging profiles may be better accounted for by levels of general anxiety than by OCD per se. This notion challenges neurobiological theories that propose that particular brain abnormalities specifically produce OCD symptoms. Instead of reflecting aspects of the brain that are specific to OCD, pathological, and stable, our data raise the possibility that what is being measured is actually a normal, transient, and nonspecific process. Additional research is, of course, needed to clarify these relationships.

Related to this issue is the fact that the neuroimaging literature is often interpreted as demonstrating significant progress toward identifying a neurological *etiology* of OCD symptoms (e.g. Saxena et al., 2001). However, the designs of existing neuroimaging studies are cross-sectional (correlational), and such research designs cannot answer questions regarding cause and effect. That is, although OCD patients and controls consistently show *differences* associated with neural activation in the head of the caudate and the orbital gyrus, there are three equally plausible explanations for these findings. First, the alterations in functioning of certain brain regions might cause OCD. Second, alterations in functioning in certain brain regions might be *caused by* OCD symptoms. Third, it is possible that OCD symptoms and observed alterations in functioning might both be influenced by a separate variable(s). The results of the present study lend some support to the third explanation and raise the possibility that observed differences in neural functioning between OCD patients and healthy individuals found in the imaging literature reflect the presence of greater state (or trait) anxiety among OCD patients. If so, it is premature to conclude from results of neuroimaging studies that observed findings point to a specific neurobiological signature of OCD.

On the basis of our findings, we propose that general anxiety mediates the relation between OCD and imaging findings, and that altered functioning or metabolite levels in the caudate and OFC may not be specific to OCD, but more general to anxiety. Moreover, we suggest that researchers further examine this important hypothesis by directly comparing OCD patients to other psychiatric groups (e.g., those with other anxiety disorders; Smith et al., 2003) as well as to healthy controls. In addition, examining the relation of imaging results to continuous variables, such as the STAI-T, BDI, or Y-BOCS, rather than dichotomous variables, such as diagnostic groups, will capitalize on naturally occurring variance and increase our ability to partial out the contributions of multiple emotional factors. Finally, comparing OCD patients with healthy controls who are experiencing distress (i.e., using symptom provocation paradigms) could help to determine if neuroimaging findings are reflecting “abnormalities” or heightened activation of normally working circuits. Interestingly, existing symptom-provocation studies (e.g., Cottraux et al., 1996) have reported results that are consistent with our assertion.

Our study has a number of limitations that should be considered. First, the small sample size may have restricted our power to detect differences. However, this

is a common shortcoming in the field of neuroimaging. For instance, previous OCD MRS studies have typically included only 12 or 13 patients. Given the expense, equipment, and time required to complete these studies, a potential solution is multi-site collaboration.

A second limitation is the large number of statistical comparisons we computed. By examining multiple outcome variables (four compounds in four regions of interest), this study and similar ones increase the likelihood of finding significant differences based on chance. Moreover, these metabolite levels can be quantified in numerous manners, including absolute concentrations, relative levels to creatine, and relative levels to water. The potential for these different approaches to yield disparate results is illustrated in two articles by Rosenberg et al. first identifying decreased levels of NAA relative to Cho or to Cr among OCD patients (Fitzgerald et al., 2000) and then decreased absolute levels of choline in a subsequent reanalysis of this data (Rosenberg et al., 2001). In addition, one of the aims of this study was to localize previous differences in NAA found in the striatum to the head of the caudate. However this extension is complicated by the fact that these studies quantified NAA relative to different values (water: Bartha et al., 1998; Cr: present study and Ebert et al., 1997). Without an accepted standard practice for processing and analyzing data, investigators must utilize multiple approaches, which increases the chance of finding a significant difference even if there are no true differences between the two populations.

Moreover, focusing on the apparent similarity in findings among studies and ignoring the risk of Type I error (i.e., spurious statistically significant results) can potentially lead to complex theories based on nothing more than a smattering of chance findings. For example, although our current results appear to be consistent with the theories of OCD and previous research regarding disturbances in the caudate, there are more inconsistencies than consistencies among the studies available to date. Specifically, the current study did not find differences in NAA levels in the head of the caudate, although differences in NAA have been found previously in the striatum (Ebert et al., 1997; Bartha et al., 1998). Moreover, Rosenberg et al. (2000) also did not find differences in NAA in the caudate, but they did find differences in Glx, a finding that did not emerge in the current study or the two previous studies of the striatum. Comparing these studies is difficult due to differences in the size of the region of interest, quantification of metabolite levels, and the age of subjects (adults vs. children), but the fact remains that there have been few replicated findings in this area. Without replication, the possibility that results are due to random chance,

idiosyncrasies of small samples, or characteristics of the experimental environment rather than true group differences cannot be ruled out. Thus, we believe that it is important to conceptualize these studies as exploratory, and place an emphasis on replication.

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