LOWER POSTERIOR CINGULATE CORTEX GLUTATHIONE LEVELS IN OBSESSIVE- COMPULSIVE DISORDER

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ABSTRACT

Background: Several lines of evidence support the hypothesis that lower cerebral levels of glutathione (GSH), associated with increased oxidative stress, may contribute to obsessive-compulsive disorder (OCD). However, no studies to date have investigated brain GSH levels in individuals with OCD.

Methods: Twenty-nine individuals with OCD and 25 age-, sex-, and race-matched comparison individuals without OCD underwent single voxel 2D J-resolved proton magnetic resonance spectroscopy (MRS) to examine GSH levels in the posterior cingulate cortex (PCC). MRS data were analyzed using LCModel and a simulated basis set. Group metabolite differences referenced to total creatine (Cr), as well as relationships between metabolite ratios and symptom severity as measured by the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS), were analyzed using linear regression with adjustment for age, sex, and race.

Results: One OCD participant failed to produce usable PCC MRS data. We found significantly lower PCC GSH/Cr in OCD participants compared with non-OCD participants ($\beta = -0.027$ [95% CI: -0.049 to -5.9×10^{-3}]; $P = 0.014$). PCC GSH/Cr was not significantly associated with total Y-BOCS score in the OCD group ($\beta = 5.7 \times 10^{-4}$ [95% CI: -4.8×10^{-3} to 5.9×10^{-3}]; $P = 0.83$).

Conclusions: Lower PCC GSH/Cr may be indicative of increased oxidative stress secondary to hypermetabolism in this brain region in OCD. Future MRS studies are warranted to investigate GSH levels in other brain regions that comprise the cortico-striato-thalamo-cortical circuit thought to be abnormal in OCD.

INTRODUCTION

Obsessive-compulsive disorder (OCD), trichotillomania, excoriation disorder, and other pathological grooming disorders such as nail biting, lip biting, and cheek chewing are grouped by the DSM-5 (1) within a family of Obsessive-Compulsive and Related Disorders (OCRD). Despite substantial prior research into these conditions, their pathogenesis remains unclear. Existing treatments are often only partially effective and these conditions may become chronic, resulting in reduced quality of life and often substantial morbidity (2). A better understanding of the underlying pathophysiology of these disorders could yield more efficacious treatments.

Several lines of evidence suggest that lower cerebral levels of glutathione (GSH), a key antioxidant in brain and other tissues (3), and/or increased oxidative stress, contribute to OCRD. First, polymorphisms of genes related to GSH regulation and oxidative stress have been associated with OCRD. These include SLC1A1, the neuronal glutamate transporter (4), which is the primary means of neuronal cysteine uptake and GSH production (5), and the mitochondrial genes superoxide dismutase and uncouple-2, which buffer mitochondrial oxidative stress (6). Second, N-acetylcysteine (NAC), a potent antioxidant and precursor of GSH, has demonstrated efficacy in several OCRD including OCD, trichotillomania, and excoriation disorder (7). Third, levels of peripheral biomarkers for oxidative stress, including superoxide dismutase and malondialdehyde, a reactive oxygen species (ROS) and end-product of lipid peroxidation, as well as GSH, are abnormal in OCD patients (6, 8-13), and have been associated with symptom severity (9). Fourth, imaging studies report increased striatal levels of synaptic dopamine in OCD (14-18), which can be oxidized rapidly to ROS (19), thereby increasing oxidative stress within a key node of the cortico-striato-thalamo-cortical circuit thought to be abnormal in these disorders (20). Moreover, neuroimaging studies of people with OCD report higher

striatal dopamine transporter densities (21, 22), which could increase intracellular dopamine and levels of ROS, thus augmenting oxidative stress (23). Despite this evidence, no studies have used magnetic resonance spectroscopy (MRS) to investigate cerebral GSH levels in individuals with OCD, primarily because prior MRS studies of OCD employed MRS sequences that were not optimized for GSH quantification (24).

We recently completed a study assessing 2D J-resolved proton MRS data from a pregenual anterior cingulate cortex (pgACC) voxel in individuals with OCD compared to age- and sex-matched non-OCD individuals (25). The study found no group differences in any metabolites, including GSH, in the pgACC. Notably, this study generated additional MRS data, not included in our primary analyses, from a second voxel positioned over the posterior cingulate cortex (PCC) – a brain region evidencing structural (26), functional (27-29), and connectivity (30) abnormalities in prior studies of OCD individuals. Given evidence that individuals with OCD display PCC hyperactivation or hypermetabolism, both at rest (31) and during cognitive challenge paradigms (28, 29), which is attenuated by selective serotonin reuptake inhibitors (SSRIs) (31), and which predicts clinical response to SSRIs (32) and anterior cingulotomy (33), we hypothesized that individuals with OCD would have lower GSH levels in PCC than non-OCD comparison individuals. This hypothesis was further stimulated by a recent finding in our center that striatal GSH levels are decreased in the *Sapap3* knockout mouse model of OCD (34). To test this hypothesis, we compared PCC GSH/total creatine (Cr) ratios in individuals with OCD versus those without OCD.

METHODS AND MATERIALS

Participant Selection

Participants age 18 years or greater, right-handed, with a DSM-IV primary diagnosis of OCD, and scoring ≥ 18 on the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) were recruited from the Obsessive-Compulsive Disorders Institute at McLean Hospital, an intensive residential treatment program for severe OCD. Participants received MRI scans within 2-4 days of admission and prior to medication changes or initiation of behavioral therapy. Exclusion criteria included: history of schizophrenia, bipolar disorder, Tourette's syndrome, or an autism-spectrum disorder; substance abuse or dependence (with the exception of nicotine) within three months of enrollment; primary hoarding as the primary OCD symptom; significant neurologic or medical illness; current pregnancy or lactation; or MRI contraindications. Co-morbid mental disorders such as other OCD, major depression, and anxiety disorders were permitted, provided that they were not the primary presenting disorder. Since our original study was designed primarily to examine glutamate-related metabolites in the pgACC, participants taking medications affecting the glutamate system (e.g., memantine, riluzole, lamotrigine, topiramate, and N-acetylcysteine [NAC]) were excluded. Other psychiatric medications were permitted if participants had received a stable dose ≥ 4 weeks prior to scanning.

Community individuals without OCD, age 18 years or greater, right-handed, with no DSM-IV psychiatric diagnosis, taking no psychoactive medications, and with no psychiatric illness among first-degree relatives, were recruited by advertising. Exclusion criteria included: positive urine screen for drugs of abuse prior to MRI scan; significant neurologic or medical illness; current pregnancy or lactation; or MRI contraindications.

Clinical Evaluation

After participants provided informed consent, approved by the McLean Hospital Institutional Review Board, we obtained: demographic information, medical/psychiatric history, diagnoses via the Structured Clinical Interview for DSM-IV (SCID), OCD symptom information via the Yale-Brown Obsessive-Compulsive Checklist, OCD symptom severity via the Y-BOCS, mood symptom severity via the Montgomery-Åsberg Depression Rating Scale (MADRS), and anxiety symptom severity via the Hamilton Anxiety Rating Scale.

Image Acquisition

Imaging was conducted on a Siemens Trio 3-Tesla scanner (Siemens Medical Solutions USA Inc., Malvern, PA) at the McLean Imaging Center with a 32 channel Trans-Imaging Matrix platform upgrade. All participants underwent a routine anatomic MRI scan to screen for structural abnormalities and to guide ^1H -MRS voxel placement.

MRS acquisition and data analysis used a modified ^1H -MRS protocol similar to that described in previous studies from our group (25, 35-38). Briefly, following acquisition of scout images, a 2x2x2 cm voxel was placed midsagittally, posterior to the splenium of the corpus callosum, encompassing both dorsal and ventral subregions of the PCC (39) as well as portions of the precuneus and occipital lobe (Figure 1). Shimming of the magnetic field within the prescribed voxel was done using a machine-automated shimming routine. Following the additional automated optimization of water suppression power, carrier-frequency, tip angles and coil tuning, a modified J-resolved protocol (2D-JPRESS) was used based on prior experience demonstrating reliable fitting of GSH using this method (36). The 2D-JPRESS sequence collected 22 TE-stepped spectra with the echo-time ranging from 35 msec to 350 msec in 15 msec increments (TR = 2 sec, f1 acquisition bandwidth=67 Hz, spectral bandwidth = 2 kHz, readout duration = 512 msec, NEX = 16/TE-step, approximate scan duration=12

minutes) providing enough *J*-resolved bandwidth (67 Hz) to resolve GSH, as well as other metabolites of interest including glutamate and glutamine (Figures 1 and S1).

MRS Data Processing and Quantification

Spectroscopic data processing and analysis was undertaken on a LINUX workstation. To quantify GSH with the JPRESS data, the 22 TE-stepped free-induction decay series was zero-filled out to 64 points, Gaussian-filtered, and Fourier-Transformed using a GAMMA-simulated *J*-resolved basis set modeled for 3T. Every *J*-resolved spectral extraction within a bandwidth of 67 Hz was fit with the spectral-fitting package LCModel (40) and its theoretically-correct template – a method that we have used successfully in prior studies (25, 35, 36, 38). The integrated area under the entire 2D surface for each metabolite was calculated by summing the raw peak areas across all 64 *J*-resolved extractions for each metabolite as described in our previous work (25, 36, 38). GSH levels are expressed as a ratio to total creatine (Cr).

Tissue segmentation of the PCC voxel was performed to rule out systematic differences in the percentage contribution of gray matter (GM), white matter (WM), or cerebrospinal fluid (CSF). Specifically, 3D mpRAGE axial image data sets were converted into NIFTI binary image file format using FSL (FMRIB). FMRIB's Automated Segmentation Tool (FAST; Oxford, UK), was used for tissue-segmentation of the T1-weighted image sets into GM, WM, and CSF. Partial volume contribution for each tissue type was then derived and expressed as a percentage of total tissue contribution for the PCC voxel using in-house software.

Statistical Analyses

For our primary analysis, we assessed the difference in GSH/Cr ratio between groups, as well as the association between GSH/Cr and Y-BOCS scores within the OCD group, using linear regression, with adjustment for age, gender, and race. We also conducted exploratory analyses of other Cr-normalized metabolites, including glutamate, glutamine, glutamine/glutamate, gamma-aminobutyric acid (GABA), NAA, choline, and myo-inositol. Since all metabolites (except for glutamine/glutamate) were expressed as ratios to Cr, we performed an additional analysis of the Cr/total signal ratio to confirm that there were no between-group differences in Cr. Alpha was 0.05, 2-tailed. We performed analyses using Stata 12.0 software.

RESULTS

Participants

Sixty-two participants (31 with OCD and 31 without OCD) completed study procedures. Two participants with OCD and three comparison participants were excluded from analysis due to: 1) technical problems (MRS data for one OCD participant and one comparison participant were lost due to an error in the transfer of data from the MRI scanner and one OCD participant had unusable MRS data); 2) inability to complete scan (one comparison participant requested to stop scanning before complete data could be collected); or 3) conflicting demographic information (one comparison participant was found to have provided discrepant age and date-of-birth information on prior scans performed at our institution). Additionally, given evidence of racial differences in systemic levels of GSH (41), we limited our analysis to Caucasian and African-American participants, as these were the only races represented in our OCD sample. As a result, the sample included in our final analyses comprised 29 participants with OCD and 25 without OCD (see Tables 1 and 2).

¹H-MRS

Cramer-Rao Lower Bounds (CRLBs) for GSH for the entire sample ranged from 4% to 13%, and the overall mean (SD) did not significantly differ between groups (OCD: 8.3 (1.9), non-OCD: 7.6 (1.3); $t(-1.4)$, $P = 0.16$). Therefore, based on the generally accepted upper limit on CRLBs of 20% for reliable fitting using LCModel (42, 43), we did not exclude any MRS data points on the basis of high CRLBs. There were no significant group differences in the mean (SD) percentage of GM (OCD: 65% (6%), non-OCD: 67% (3%); $t(1.6)$, $P = 0.11$), WM (OCD: 26% (5%), non-OCD: 26% (3%); $t(0.20)$, $P = 0.84$), or CSF (OCD: 9% (8%), non-OCD: 7% (3%); $t(-1.3)$, $P = 0.20$).

Spectral quality often varies considerably with in vivo MRS studies, despite best quality control efforts. In an effort to further increase confidence in our reported 2D J-PRESS GSH measures, we conducted a phantom GSH-loading experiment. This experiment demonstrated a linear increase in GSH levels with increasing GSH concentrations (see Figures S2 and S3). Moreover, other metabolites remained stable as their concentrations were not altered between phantoms. Additionally, we examined two main markers of spectral quality in the PCC data featured in this study. Although unsuppressed voxel water linewidths were not recorded, full-width/half-maximum (FWHM) and signal-to-noise (SNR) were outputted for the J=0.0Hz spectral extraction for the N-acetylaspartate (NAA) resonance at 2.02ppm. For all PCC voxels included in the analysis, NAA FWHM was $7.8 \pm 2.1\text{Hz}$ and NAA SNR was 39.9 ± 11.7 . Using the 3x standard deviation criteria, the maximum cutoff FWHM was 14.2Hz with only one scan over this cutoff (15.6Hz). For SNR, the minimum cutoff was 4.7 with no voxels under this threshold (minimum SNR was 15). The single voxel over the FWHM limit was ultimately included as upon visual inspection, the spectrum was deemed of sufficient quality.

With regard to our primary hypothesis, we found significantly lower PCC GSH/Cr in OCD participants compared with non-OCD participants ($\beta = -0.027$ [95% CI: -0.049 to -0.0059]; $P = 0.014$; Cohen's $d = 0.73$) (Figure 2; Table 3).

On further inspection, we noted one OCD individual with a lower GSH/Cr value than the rest of the group (Figure 2). The CRLB for this GSH measurement was 13%, which falls within the generally accepted range for CRLB (42, 43), and thus there was no reason to suspect that this was an invalid measurement. Nevertheless, we performed several sensitivity analyses to examine the influence of this data point. First, we repeated the regression analysis using ranked data (with rank of GSH/Cr as the outcome variable, adjusted for age, sex, and race) and found a result similar to the unranked analysis above ($\beta = -10.2$ [95% CI: -19.0 to -1.44]; $P = 0.023$; $d = 0.67$). Second, we performed further analyses where we deleted various sets of data points to assess their influence. Initially, we removed the lowest GSH/Cr data point in the OCD group, both untransformed and with rank transformation. In these two analyses, the estimated effect sizes for the between-group difference decreased by 28.2% and 20.6%, respectively, with p-values of 0.075 and 0.072, respectively. Next, we removed the lowest data point in the non-OCD group and performed the same analyses. In these analyses, the estimated effect sizes for the between-group difference increased by 31.8% and 38.7%, respectively. Finally, we removed the lowest GSH/Cr from both groups. The estimated effect sizes for the between-group difference increased by 17.0% and 17.8%, respectively, with p-values of 0.010 and 0.014, respectively. Overall, these analyses indicate that the influence of the lowest GSH/Cr in the OCD group was modest, and indeed was even slightly less than the magnitude of the influence of the lowest ratio in the non-OCD group. Combined with the findings from the analysis using ranked data above, the sensitivity analyses collectively provide reassurance that the observed findings are not unduly influenced by a single low GSH/Cr value in the OCD group.

Exploratory analyses revealed no significant group differences in any other PCC metabolites of interest (Table 3). We found no significant differences in the Cr/total signal ratio ($P = 0.57$) between groups.

¹H-MRS/Clinical Correlations

We found no significant association between PCC GSH/Cr and total Y-BOCS score in the OCD group ($\beta = 5.7 \times 10^{-4}$ [95% CI: -4.8×10^{-3} to 5.9×10^{-3}]; $P = 0.83$; $d = 0.09$).

Effects of Potential Confounding Variables

Comparing the 6 OCD participants who were not taking medications with the 23 OCD participants taking medications, we found no significant effect of medication status on PCC GSH/Cr ($\beta = -5.5 \times 10^{-3}$ [95% CI: -0.032 to 0.021]; $P = 0.68$; $d = 0.18$; see Figure 2). We also found no significant association between MADRS score and PCC GSH/Cr in the OCD group ($\beta = -3.8 \times 10^{-4}$ [95% CI: -2.0×10^{-3} to 1.2×10^{-3}]; $P = 0.62$; $d = 0.21$).

DISCUSSION

To our knowledge, this is the first study to examine cerebral GSH levels in OCD. We found significantly lower PCC GSH/Cr in individuals with OCD compared to individuals without OCD. Decreased PCC GSH/Cr in people with OCD suggest that oxidative stress in this brain region, and possibly other brain regions implicated in OCD, could contribute to the underlying pathophysiology of this disorder.

ROS are endogenous by-products of cellular metabolism, and lead to impairments in cellular reduction-oxidation status – so-called “oxidative stress” (44). Unchecked oxidative stress can impair

normal cellular functioning and lead to cell death (45). GSH, the primary antioxidant in brain, plays a critical role in the defense of brain cells against oxidative stress. Therefore, decreased levels of GSH could significantly impact brain function. Indeed, inborn errors of GSH metabolism result in a variety of neurologic symptoms including ataxia, mental retardation, seizures, spasticity, and tremor (46), and brain GSH depletion has been hypothesized to precede the clinical progression of age-related neurodegenerative disorders (47-49).

The mechanism for the observed decrease in GSH/Cr in OCD individuals is unclear, but several possible explanations exist. Prior neuroimaging studies of individuals with OCD have demonstrated PCC hypermetabolism at rest (31) and hyperactivity during cognitive challenge (28, 29). Such a hypermetabolic state would result in increased production of ROS via mitochondrial oxidative phosphorylation, potentially depleting GSH stores. While exposure to ROS and subsequent GSH depletion have been shown to result in short-term increases in GSH synthesis (50), longer-term GSH overconsumption may overwhelm this regulatory system, leading to increased oxidative stress. Moreover, oxidative stress has been shown to inhibit the neuronal glutamate transporter, excitatory amino acid carrier-1 (EAAC1), which is critical for neuronal uptake of cysteine – a rate-limiting substrate for neuronal GSH metabolism (46). This process could initiate a vicious cycle of increased oxidative stress precipitating further decline in GSH levels.

The PCC has been hypothesized to play a role in assessing the self-relevance of emotional stimuli (39, 51), and is a key component of the default mode network – a system of brain regions that are collectively activated during internally focused thought processes and deactivated during externally focused cognitive tasks (52). As such, hypermetabolism in this region could lead to the uncontrollable focus on internal thoughts observed in individuals with OCD (53). However, in addition to the PCC, studies using positron emission tomography have consistently identified

hypermetabolism at rest in brain regions comprising a cortico-striatal-thalamo-cortical circuit in OCD patients – most notably orbitofrontal cortex, (54-57) anterior cingulate cortex, (31, 57) caudate nuclei, (54, 55) putamen, (31) and thalamus (31) – suggesting that decreased GSH/Cr, and increased oxidative stress, may be present in some of these brain regions as well. However, in our prior study (25), we did not observe lower pgACC GSH/Cr in a largely overlapping group of OCD participants, suggesting that hypermetabolism is not always accompanied by GSH depletion. To further investigate this question, future MRS studies should examine GSH levels in several cortico-striato-thalamo-cortical brain regions in OCD.

Decreased GSH/Cr in our OCD group could also reflect decreased GSH synthesis. Neuronal GSH synthesis is dependent on cysteine uptake via the neuronal glutamate transporter EAAC1, as evidenced by significant reductions in neuronal GSH levels in genetically altered mice deficient in EAAC1 (5). Genetic studies in humans have repeatedly demonstrated an association between the SLC1A1 gene, which encodes the EAAC1 transporter, and individuals with OCD (58-64), suggesting a potential genetic predisposition to GSH deficiency in a subgroup of OCD individuals.

Consistent with our findings of decreased brain GSH/Cr, prior studies of both children and adults with OCD demonstrate elevations in peripheral markers of oxidative stress (8-13), which in one study strongly correlated with clinical severity (9). Moreover, genetic variants in the nuclear-encoded mitochondrial proteins superoxide dismutase and uncouple-2, which buffer mitochondrial oxidative stress, have been associated with OCD (6). These additional findings suggest that peripheral oxidative stress in people with OCD could be associated with, or related to, central abnormalities. Our present human findings are also consistent with our report of concurrent striatal GSH depletion and metabolic stress in the *Sapap3* knockout mouse model of OCD (34). Future studies examining both brain GSH and peripheral markers of oxidative stress in people with OCD and other OCD would help

to further elucidate the relationship between central and peripheral oxidative stress in people with these disorders.

Given our findings of decreased brain GSH/Cr in OCD, interventions that increase GSH levels may represent novel treatments for OCD and possibly other OCD. Indeed, the GSH-precursor, NAC, has demonstrated preliminary efficacy in the treatment of OCD (65-67) and other OCD such as trichotillomania (68-70), excoriation disorder (69, 71) and nail biting (69, 72). While others have theorized that the beneficial effects of NAC in OCD result from its ability to modulate glutamatergic neurotransmission (68), we propose that NAC's capacity to increase GSH synthesis may also play a significant role. Thus, future studies examining the impact of NAC treatment on brain GSH levels and the association with clinical response in individuals with OCD would be valuable to better understand NAC's primary mechanism of action and to determine whether brain GSH levels and/or peripheral measures of oxidative stress may represent predictive biomarkers of treatment response to NAC and other GSH-enhancing treatments.

We acknowledge several study limitations. First, the data presented here were initially collected as part of a study focused on glutamate/Cr, glutamine/Cr, and glutamine/glutamate in the pgACC in individuals with OCD (25). As noted above, this study also acquired data in the PCC as a comparison region, but we decided not to examine these data in our primary analysis, due to the large amount of data to be analyzed and reported. However, after we published the results of this primary analysis, a study in our center found decreased striatal GSH levels in the *Sapap3* knockout mouse model of OCD (34). Accordingly, we returned to analyze our PCC data with a specific a priori hypothesis of lower GSH levels in the OCD group. Since our hypothesis involved a single metabolite, we did not correct for multiple comparisons. We would note parenthetically that we show results for metabolites other than GSH in Table 3 for the reader's interest, but these analyses were exploratory

and not related to our primary GSH analysis.

Second, we observed one very low GSH/Cr value in the OCD group, which potentially could have impacted our results. However, extensive sensitivity analyses provided reassurance that this one point did not unduly influence our findings – suggesting that our finding of decreased PCC GSH/Cr in the OCD group is statistically robust. Third, we accepted participants with OCD who were on a stable medication regimen. Overall, animal studies examining the impact of medications on brain GSH levels have been inconsistent. For example, antidepressants and benzodiazepines have been associated with both unchanged (73) and increased (74-76) GSH levels while antipsychotic medications have been associated with increased (77) and decreased (78, 79) GSH levels. To our knowledge, only two studies have used MRS to assess the impact of medication effects on brain GSH levels in humans. One (73) found no change in individuals with depression following a six-week course of treatment with the SSRI escitalopram and the other (80) demonstrated an acute GSH *increase* following intravenous NAC administration in individuals with Gaucher and Parkinson's diseases. These findings, together with our post-hoc analyses showing no significant influence of medications on PCC GSH/Cr, argue against a medication effect in our sample. However, the subgroup of unmedicated OCD participants was small, and thus our analyses cannot exclude a medication effect. Fourth, we included participants with co-morbid mental disorders, including depression and anxiety disorders, which may have impacted our results. Fifth, we expressed GSH levels as ratios to total creatine – a method that presumes no between-group differences in total creatine (81). This presumption seems reasonable given that prior MRS studies of OCD patients have suggested no significant total creatine abnormalities (24) and we found no between-group differences in the total creatine/total signal ratio in our sample. Sixth, since it is not possible to quantify absolute brain tissue GSH levels in living humans, our MRS method cannot be directly validated in vivo, so we cannot be certain that the GSH values we report reflect

actual brain GSH levels. Seventh, we did not acquire separate water-unsuppressed acquisitions at each echo-time and therefore did not correct for eddy currents in our data, which could have impacted our results. Eighth, we did not collect data on cigarette smoking habits in our sample. Given the possibility that cerebral GSH levels may be impacted by nicotine use (82), we cannot exclude a potential confounding effect of nicotine use on our findings. Finally, our OCD patients were recruited from an intensive residential treatment program, and thus likely represented a severe, treatment-refractory subgroup (83), potentially not representative of the broader OCD population.

In summary, using 2D J-resolved proton MRS, we found lower GSH/Cr in the PCC of individuals with OCD. This may be indicative of increased oxidative stress secondary to hypermetabolism in this brain region. Peripheral oxidative stress has been reported in children and adults with OCD, suggesting that both peripheral and brain oxidative stress could contribute to the disorder. Interventions that enhance brain GSH levels, such as NAC, may have utility as novel treatments for OCD and other OCD. Future MRS studies are warranted to: 1) examine GSH levels in unmedicated individuals with OCD; 2) investigate GSH levels in other parts of the cortico-striato-thalamo-cortical circuit thought to be abnormal in OCD; 3) determine whether lower cerebral GSH levels may represent a neurobiological diagnostic marker across other OCD; 4) examine GSH levels in pediatric OCD to determine whether decreased GSH levels could be used to expedite diagnosis and treatment; and 5) explore whether lower GSH levels may represent a predictive biomarker of response to NAC treatment for OCD and other OCD.

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TABLE/FIGURE LEGENDS

Figure 1. Sagittal MRI showing placement of magnetic resonance spectroscopy (MRS) voxel in the posterior cingulate cortex and a sample 3 Tesla proton MRS spectrum from the posterior cingulate cortex extracted from $J = 0.0\text{Hz}$. Spectrum is displayed with LCModel fit and residual. Cho, choline; Cr, creatine; Glu, glutamate; GSH, glutathione; ml, myo-inositol; NAA, N-acetylaspartate.

Figure 2. Comparison of the glutathione (GSH)/total creatine (Cr) ratios in the posterior cingulate cortex (PCC) in participants without obsessive-compulsive disorder (non-OCD) and in participants with OCD. Open circles represent unmedicated participants with OCD. Horizontal bars indicate means.

Table 1. Demographic and Clinical Characteristics

	Participants with OCD		Participants without OCD	
Characteristic	(<i>N</i> = 29)		(<i>N</i> = 25)	<i>P</i>
Age, years, mean (SD)	32.2 (11.7)		34.0 (12.3)	0.59 ^a
Range	18-54		18-55	
Sex				0.59 ^b
Male, <i>N</i> (%)	17 (59)		12 (48)	
Female, <i>N</i> (%)	12 (41)		13 (52)	
Yale-Brown Obsessive-Compulsive Scale, mean (SD)	27.6 (2.2)		0	
Montgomery-Asberg Depression Rating Scale, mean (SD)	16.8 (7.4)		0.60 (0.96)	

OCD, obsessive-compulsive disorder;

^aBy t-test (2-tailed).

^bBy Fisher's exact test (2-tailed).

Table 2. Additional Demographic and Clinical Characteristics of Participants with OCD, (*N* = 29)

Characteristic	Participants with OCD
Age of OCD onset, years, mean (SD)	12.9 (6.4)
Duration of OCD, years, mean (SD)	19.5 (12.8)
Predominant OCD Symptom Dimension, <i>N</i> (%)	
Contamination	18 (62)
Aggression	4 (14)
Sexual/religious	3 (10)
Symmetry/exactness	4 (14)
DSM-IV Axis I Psychiatric Comorbidity at Time of Study, <i>N</i> (%)	
None	10 (34)
Major depressive disorder	11 (38)
Dysthymic disorder	3 (10)
Panic disorder	3 (10)
Social anxiety disorder	2 (7)
Anorexia nervosa	1 (3)
Bulimia nervosa	1 (3)
Body dysmorphic disorder	2 (7)
Trichotillomania	1 (3)
Post-traumatic stress disorder	1 (3)
Medications at Time of Study, <i>N</i> (%)	
None	6 (21)
SSRI ^a	18 (62)
SNRI ^b	1 (3)
Clomipramine	3 (10)
Second generation antipsychotic ^c	6 (21)
Benzodiazepine ^d	14 (48)
Other	4 (14)
Bupropion	2 (7)
Lithium	1 (3)
Trazodone ^e	1 (3)
Zolpidem ^e	1 (3)
Naltrexone ^e	1 (3)
Number of Weeks on Stable Dose of Medication, mean (SD, range)	
SSRI/SNRI/Clomipramine	51 (109, 4-520)
Second generation antipsychotic	35 (37, 12-108)
Benzodiazepine	91 (133, 8-520)
Other	175 (232, 36-520)

OCD, obsessive-compulsive disorder; SNRI, serotonin-norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor.

^afluoxetine [*N*=8], sertraline [*N*=7], escitalopram [*N*=2], citalopram [*N*=1].

^bvenlafaxine [*N*=1].

^crisperidone [*N*=3], aripiprazole [*N*=3].

^dclonazepam [*N*=7], lorazepam [*N*=5], alprazolam [*N*=1], clorazepate [*N*=1].

^eUsed by the same participant.

Table 3. Mean ($\pm$ SD) Posterior Cingulate Cortex Metabolite Levels for Participants with Obsessive-Compulsive Disorder (OCD) and Participants Without OCD^a

Metabolite	OCD (<i>N</i> = 29)	Without OCD (<i>N</i> = 25)	<i>P</i> ^b
GSH/Cr	0.236 (0.038)	0.254 (0.035)	0.014
Glu/Cr	0.994 (0.152)	0.960 (0.151)	0.55
Gln/Cr	0.201 (0.063)	0.196 (0.056)	0.96
Gln/Glu	0.211 (.088)	0.214 (.081)	0.84
GABA/Cr	0.168 (0.045)	0.176 (0.057)	0.4
NAA/Cr	1.37 (0.147)	1.41 (0.160)	0.27
Cho/Cr	0.638 (.109)	0.624 (.071)	0.98
mI/Cr	0.718 (.175)	0.678 (.072)	0.28

Cho, choline; Cr, total creatine; GABA, gamma-aminobutyric acid; Gln, glutamine; Glu, glutamate; GSH, glutathione; mI, myo-inositol; NAA, N-acetylaspartate.

^aMetabolite levels are represented in arbitrary units.

^bBy linear regression adjusted for age, sex, and race.

Figure 1

Figure 2