Neurobehavioral and neurochemical basis of compulsive behavior: A 7T magnetic resonance spectroscopy study in humans

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Abstract

There has been relatively little analysis of possible neurochemical correlates of compulsive behavior to illuminate its underlying neural mechanisms. We utilised 7-Tesla proton magnetic resonance spectroscopy (\(^1\)H-MRS) to assess the balance of excitatory and inhibitory neurotransmission by measuring glutamate and GABA levels in anterior cingulate cortex and supplementary motor area (SMA) of healthy volunteers and patients with Obsessive-Compulsive Disorder (OCD). Within the SMA, trait and clinical measures of compulsive behavior were related to glutamate levels, whereas a behavioral index of habitual control correlated with the glutamate:GABA ratio. OCD patients additionally exhibited elevated glutamate levels and glutamate:GABA ratios in anterior cingulate cortex which also correlated with habitual control. This study highlights important underlying relationships between SMA mechanisms of habitual control relevant to compulsive behavior, common to the healthy sub-clinical and OCD populations. The results also demonstrate additional involvement of anterior cingulate in the balance between goal-directed and habitual responding in OCD.

Introduction

Compulsivity can be defined as perseverative behavior with potentially maladaptive consequences. The construct of compulsivity – a transdiagnostic psychiatric trait – has no clear boundary between the healthy and pathological parts of the spectrum\(^1\). Individual differences in compulsive behavior have been related to functioning of discrete fronto-striatal 'loops' in individuals with compulsive traits\(^2\) and in psychiatric patients with extreme levels of compulsive symptoms such as those with substance use disorders\(^3\) and obsessive-compulsive disorder (OCD)\(^4\). Changes in fronto-striatal function underpinning compulsive behavior may be influenced by neurochemical dysregulation of cortical networks. Abnormally high levels of glutamate (Glu) within OCD have been suggested by genetic and pharmacological studies and in animal models\(^5,6\), but have not been tested rigorously in humans thus far. Although there is considerable evidence of hyperactivity in certain cortical regions based on metabolic and BOLD neuroimaging\(^4,7,8\), the role of \(\gamma\)-amino butyric acid (GABA) remains more obscure, as well as its relationship with Glu, which may help to set the excitatory/inhibitory balance of cortical networks\(^9\) and thus contributing to their hyperexcitability or hyperactivity.

Evidence for neurochemical dysregulation mediating compulsive behaviour has been hindered by a lack of high resolution quantification of Glu, its metabolite glutamine (Gln), and GABA using proton magnetic resonance spectroscopy (1H-MRS) at field strengths of 3-Tesla or lower. To overcome this limitation, we utilised 7-Tesla 1H-MRS and an optimized MRS sequence to reliably quantify separately Glu, Gln and GABA in both individuals with and without OCD. We further correlated these indices with a range of measures of compulsive behavior. This enabled us to define more accurately a proxy neurochemical index of the balance between excitatory and inhibitory neurotransmission within key regions previously linked to compulsivity and strongly implicated in the pathophysiology of OCD: the anterior cingulate cortex (ACC); and the supplementary motor area (SMA); with the occipital cortex (OCC) as a control.
region. We also tested the hypothesis that compulsive behavior might be linked to a bias towards habitual control rather than being goal-directed\textsuperscript{10}. Competition between habits and goal-directed behavior may require regulation of the former during response preparation\textsuperscript{11}.

The ACC is implicated in error monitoring\textsuperscript{12,13} and reward prediction errors\textsuperscript{14,15}, which are cognitive processes critical for compulsive responses. Moreover, enhanced prediction errors and aberrant activity of the ACC are reported in OCD\textsuperscript{16–18}. The SMA has also been implicated in error processing in OCD\textsuperscript{19} and participates in a sensorimotor circuit with the putamen\textsuperscript{20}, also crucial for habit learning\textsuperscript{10,21,22}. It is an effective target for brain stimulation and improvement of OCD symptoms\textsuperscript{23,24} and is considered a neurocognitive endophenotype of OCD, possibly related to inefficient neural processing, as measured during a response inhibition task\textsuperscript{8}.

We hypothesised in summary that changes in the network properties of two key regions of fronto-striatal circuitry relevant to compulsivity - the SMA and ACC - will be evident in OCD and probably expressed in the healthy population as a function of the transdiagnostic dimension of compulsivity. Specifically, we predicted relationships in the balance between Glu and GABA function in these frontal areas and compulsive and habitual tendencies, as well as clinical symptoms which are an extreme expression of compulsive behavior. MRS and behavioral data were thus collected from 30 healthy subjects and 31 OCD patients (clinical and demographic measures for both groups are shown in Fig. S1, Supplement).

**Results**

*Differential balance between excitatory and inhibitory neurotransmitters in healthy and OCD brain:* Fig. 1 shows the positive relationships between Glu and GABA in the ACC for the OCD and healthy subjects separately (i.e. OCD and healthy subjects; Pearson's $r = 0.51$, $p = 0.00003$, $p$ (FDR-corrected) = 0.0002), whereas only healthy volunteers showed a positive relationship in SMA (Pearson's $r = 0.37$, , $p = 0.04$, $p$ (FDR-corrected) = 0.05) and OCC (Pearson's $r = 0.46$, $p = 0.01$, $p$ (FDR-corrected) = 0.01). However, OCD patients showed this relationship in neither SMA (Pearson's $r = 0.09$, $p = 0.61$, $p$ (FDR-corrected) = 0.71), nor occipital cortex (Pearson's $r = -0.04$, , $p = 0.81$, $p$ (FDR-corrected) = 0.81). Thus this relationship is a likely cortex-wide finding in the healthy group as opposed to the OCD patients who showed an imbalance in the relationship between these metabolites in both SMA and OCC. Fig. 1B depicts these results separately for healthy volunteers and OCD patients.

*Comparing neurometabolite levels between HV and OCD groups:* Within each voxel, the levels of GABA, Glu, Glu/GABA, Gln, Glx (glutamate + glutamine) and NAA (N-acetylaspartate, as a measure of neuronal integrity\textsuperscript{26} were compared between groups (Table 1). OCD patients showed significantly higher levels of Glu (independent sample t-test = 2.08, $p = 0.02$), Glu:GABA ratio (Mann-Whitney U= 618, $p = 0.006$), and Glx (independent sample t-test = 2.13, $p = 0.02$) within the ACC voxel, and enhanced Glu levels within the OCC compared with the healthy group (independent sample t-test = 1.70, $p = 0.04$). There were no
differences between metabolite levels within the SMA between OCD and healthy volunteers. The MRS quality metrics per metabolite and tissue compositions per voxel are presented in Table S1 (Supplement).

**Relationship between compulsivity and SMA brain metabolites:** Fig. 2A shows the SMA voxel and the MRS spectrum for all metabolites and Fig. 2B displays the fitted model for Glu, for one representative individual. There was a positive relationship between compulsive tendencies using the self-administered Obsessive Compulsive Inventory (OCI)\(^{27}\) and Glu levels in SMA for the entire sample (i.e. OCD + healthy subjects: Spearman’s \(r = 0.28\), \(p = 0.02\), \(p\) (FDR-corrected) = 0.03), OCD patients exhibited, as expected, significantly higher OCI scores (\(p < 0.001\)), and as the OCI scores had different distributions within each group, the relationships between the Glu concentrations and OCI were separately analysed per group. Both were significant (see Fig. 2C): OCD (with normally distributed OCI): Pearson’s \(r = 0.40\), \(p = 0.01\), \(p\) (FDR-corrected) = 0.05), and healthy volunteers (with non-parametrically distributed OCI): Spearman’s \(r = 0.44\), \(p = 0.01\), \(p\) (FDR-corrected) = 0.02). In line with these findings, the compulsions subscale of the clinician rated Yale Brown Obsessive Compulsive Scale (YBOCS)\(^{28}\) was also correlated with SMA Glu levels in OCD patients (Pearson’s \(r = 0.41\), \(p = 0.01\), \(p\) (FDR-corrected) = 0.05; Fig. 2D). This measure also correlated positively with the OCI score in patients (Pearson’s \(r = 0.50\), \(p = 0.003\)). Fig. S4 (Supplement) depicts individual spectra for Gln, GABA, and NAA within a representative SMA voxel. With regard to ACC neurometabolites, no correlation was found between ACC Glu:GABA ratio and obsessive compulsive symptoms as measured with OCI and YBOCS in the OCD group (Pearson’s \(r = -0.16\), \(p = 0.39\); \(r = -0.18\), \(p = 0.33\), respectively for YBOCS compulsions and OCI total score).

**Neural correlates of habitual responding:** SMA and ACC Glu:GABA ratios implicated in habitual control: A contingency degradation task\(^{29}\) was used to measure the tendency towards habitual (stimulus-response) as opposed to goal-directed (action-outcome) responding\(^{10}\). The task comprises 3 conditions with varying probabilities (0.60, 0.3, 0.0: see Methods) of gaining rewards of 20 pence (£0.2) by responses of pressing a space bar. These contingencies between instrumental responses and reward outcomes were obtained by varying the provision of “free rewards” where no response was required to earn the monetary rewards. We also required subjects to make regular causal judgements concerning their perception of the relationship between their responses and the reward outcomes, in order to gauge their understanding of the contingencies (see Fig. 3A). As expected (see also Ersche et al., 2021), responding declined significantly (though equivalently) in both groups in the degraded conditions (Fig. S2, Supplement), consistent with the programmed reinforcement contingencies. The differences between the two non-degraded and degraded conditions are assumed to reflect the balance between goal-directed and habitual control, the smaller the decrement being more consistent with the latter. Subjective judgements of contingencies were highly and positively correlated with the behavioral measures for the entire sample (Fig. S3 B, Supplement). An habitual responding index was created by subtracting responses made in the non-degraded condition (i.e. \(p = 0.60\)) condition from those with a low probability (0.3 or 0.0) of actions gaining rewards. This means that increasingly negative values indicate a greater tendency towards goal-directed behavior, whereas less negative and positive values represent bias towards habitual control.
The entire sample showed a significant positive relationship between the habitual responding index and SMA Glu:GABA ratio (Pearson’s $r = 0.26$, $p = 0.02$, $p$ (FDR-corrected) = 0.06; Fig. 3B). This means that higher values of Glu and/or lower values of GABA were associated with a greater habitual tendency. Moreover, OCD patients showed this same relationship within the ACC voxel as well (Pearson’s $r = 0.38$, $p = 0.02$, $p$ (FDR-corrected) = 0.04; Fig. 3C). Note that the habitual responding index was equivalent in both groups (Fig. S3 A, Supplement).

**Discussion**

This study has demonstrated for the first time that compulsivity and clinical compulsive symptoms are related to neurochemical indices in the anterior cingulate cortex and supplementary motor area of the frontal lobes suggestive of an altered excitatory/inhibitory (E/I) balance between the neurometabolites Glu and GABA in these regions. Moreover, this neurochemical imbalance as hypothesized was also related to a measure of habitual responding versus goal-directed control over behavior in these regions, consistent with some theories proposing that this bias underlies compulsive behavior in clinical disorders of obsessive-compulsive disorder and drug addiction\(^3\),\(^30\).

We studied neurochemical correlates of compulsivity in healthy volunteers and in a group of adult patients with OCD, using 7T MRS to measure the proton spectrum with high signal to noise ratios in three brain regions of prior interest (SMA, ACC and OCC), and using clinician-rated (Y-BOCS) and self-rated (OCI) symptom questionnaires, and behavioural performance data on a test of habitual vs goal-directed responding, to define compulsivity and its underlying cognitive traits. We chose to focus on MRS profiling of the ACC and SMA because both have been previously implicated in the neural circuitry of compulsivity.

There were four main sets of findings: Glu and GABA levels were significantly correlated in all regions studied in healthy volunteers, but not in patients with OCD, suggesting that a dysfunctional balance may contribute to pathology. In fact, OCD patients had significantly higher levels of Glu in the anterior cingulate and occipital cortex, with a higher Glu:GABA ratio in the anterior cingulate. This appears to be the first report of significant changes in these neurometabolites in adult OCD to date, possibly reflecting the greater sensitivity of the 7T magnet. These changes were shown to be independent of any structural change in gray and white matter and CSF as well as NAA changes, as a measure of neuronal integrity. Significantly, despite the lack of absolute changes in Glu in the SMA in OCD, we found that clinical symptoms (YBOCS-compulsivity subscale) were significantly positively related to the SMA Glu levels, relevant to recent suggestions of SMA activity providing a neuroendophenotype for OCD\(^8\). In addition, compulsivity trait (OCI) was also related to changes in SMA Glu levels, both in healthy volunteers and OCD patients, consistent with the notion of a transdiagnostic dimension characterising compulsive behavior related to connectivity of frontal regions\(^2\). Finally, the measure of habitual responding was reflected by the Glu:GABA ratio in the SMA in both healthy volunteers and patients with OCD, and additionally in the anterior cingulate in patients with OCD. These findings extend our existing understanding of how fronto-striatal loops control the balance between goal-directed behavior and habits, and how this balance may be related to the generation of compulsive responding.
Previous MRS studies have used the ratio of excitatory (Glu) and inhibitory (GABA) neurotransmitters as a proxy index of E/I balance in cortical networks and recent work found a positive correlation between GABA and Glx (glutamate + glutamine) in medial parietal cortex, consistent with electrophysiological evidence. However, such relationships are controversial and another study, performed at 3T failed to show positive correlations between Glx and GABA in visual and motor cortex. Nevertheless, in this 7T study we were able to demonstrate, at least in healthy volunteers, robust positive relationships between Glu and GABA in anterior cingulate, supplementary motor area and occipital cortex suggesting that these indices may be more stable indices of E/I balance than Glx/GABA. The absence of a positive Glu/GABA relationship in OCD is consistent with other evidence in this study that clinical symptoms are associated with Glu levels in the SMA and with substantial evidence in OCD of BOLD hyperactivity, increased functional connectivity, exaggerated error-related and readiness potentials in the anterior cingulate and SMA.

Naaijen et al. (2017) also found increased Glu in the anterior cingulate in children with OCD or autism spectrum disorder using a 3T scanner, suggesting that these changes may occur early in life. However, the literature overall has hitherto lacked the resolution to quantify metabolites with smaller peaks such as GABA, Glu and Gln with a higher accuracy and a superior signal to noise ratio. Although our hypothesis of elevated Glu in anterior cingulate in OCD was confirmed, we were surprised to find significant increases in the occipital cortex (though not SMA) in this group suggesting that such changes may not be limited to frontal regions in OCD; however, there were no correlations shown for any of the behavioral measures, and so the functional significance of this change is unclear.

In contrast, SMA Glu level was related significantly to the compulsion sub-score for the YBOCS in OCD patients, even though these levels were not different from healthy volunteers. As discussed above, the relevant difference between OCD and healthy volunteers may actually be in the lack of E/I balance. The findings are consistent with other data indicating a significant relationship between right SMA BOLD activity and YBOCS score in OCD. Of course, this does not necessarily indicate that the SMA is the origin of OCD symptoms, especially as other studies have shown similar correlations with fronto-striatal structure and connectivity, but it does place the SMA importantly within that network.

A major developmental study of compulsivity trait using magnetisation transfer (MT) imaging to quantify network connectivity in adolescents found that a composite compulsivity score (OCI plus Padua scale) was related to reduced growth of the MT trajectories in fronto-striatal circuity, most markedly in dorsomedial and dorsolateral prefrontal regions, including the SMA, consistent with our findings in this region, and further supporting a possible causal role of the changes in neural network function in OCD. Although we also found a significant relationship between OCI and SMA (r = 0.28, p < 0.02) Glu for the entire sample (i.e. OCD plus healthy volunteers), separate analyses of two smaller sub-groups in fact show even more significant relationships (Fig. 2C) probably due to OCI scores being normally distributed in patients but not in healthy volunteers, most likely due to our exclusion criteria of > 42 OCI scores in the
latter. In general, the finding is consistent with the hypothesis that compulsive behavior can exist along a continuum and be mediated by similar neural networks.

What has perhaps been lacking in previous studies of the neurocognitive substrates of compulsivity has been a theoretical basis for the psychological mechanisms underlying this form of behavior. Consequently, in this study we also included a behavioral measure of instrumental control over behavior, a modified contingency degradation test, which has previously been shown to be sensitive to deficits in OCD, stimulant drug abusers and in patients with frontal lesions, as well as being related to scores on a subjective habit questionnaire. Our habitual responding index was significantly related to Glu:GABA ratio in the SMA for the entire sample, consistent with a possible role for this premotor cortical region, in conjunction with the putamen to which it projects, as part of the so-called ‘habit system’, being broadly consistent with evidence from both human neuroimaging and animal studies. However, a specific role for the SMA, as distinct from other premotor regions in habitual control, has hitherto not been much researched (e.g., de Wit et al., 2012); although a recent article specifically identified such a relationship for this region. Previous functional neuroimaging studies of action-outcome contingency learning have implicated a ventromedial prefrontal cortex-caudate circuitry, whereas action-independent outcomes were associated with inferior frontal gyrus. The inferior frontal gyrus, in conjunction with the pre-SMA, has also been implicated in behavioral inhibition, including in OCD. This suggests that our findings may relate to the expression of habitual responding, as the SMA (and pre-SMA) are widely believed to mediate the initiation and inhibition of voluntary behavior. Further supporting a cortico-striatal ‘habit’ system, Ersche et al. (2021) found that patients with cocaine use disorder with elevated habitual:goal ratio scores exhibited reduced Glu in the putamen using the same contingency degradation test paradigm and the same 7T scanner with similar sequence parameters.

There was also a relationship between the index of habitual responding and anterior cingulate Glu: GABA ratio in OCD patients alone. This is consistent with recent evidence of effects of pharmacologically stimulating activity of BA 24 (dorsal anterior cingulate) in the marmoset monkey with a Glu reuptake inhibitor, although we also saw deficits when a mixture of GABA agonists (muscimol and baclofen) were infused into this region (the only one of several other prefrontal areas examined to show these effects). Thus, there were differences, as well as similarities, in the neural substrates of habitual control between OCD patients and healthy volunteers. The anterior cingulate cortex may have been recruited in OCD because of its known role in the mediation of action-outcome learning and error prediction especially under uncertainty. These results are more generally consistent with other findings suggesting that the SMA and anterior cingulate have strong reciprocal connections that act to sustain each other’s activity, and that this interaction is mediated during movement preparation according to the readiness potential amplitude, which is enhanced in OCD.

Overall, the findings are at least partially consistent with the hypothesis supported by previous findings that OCD patients exhibit a bias away from goal-directed behavior towards habitual control, although in this particular test of contingency degradation there were actually no significant differences in
performance, contrasting with impairments previously shown in a more parametric and detailed test paradigm\textsuperscript{38}. Other limitations of our analysis include the medication status of the OCD patients, although we assume that this cannot account for the relationships shown with clinical symptoms or in the healthy volunteers. Moreover, SSRIs have generally been shown to reduce Glu\textsuperscript{52} and so are unlikely to have produced the enhanced Glu levels seen here. Sample size is a consideration although we believe our study was adequately powered, especially given the strength of the 7T magnet. We did rigorously control for multiple correlations using FDR. As recommended for clinical studies of MRS we included extreme values as long as they were within 2SD from the group average\textsuperscript{53}.

Finally, we accept that our analysis is entirely correlational and more rigorous testing of causality would probably have to depend on assessing effects of suitable interventions. This could potentially be therapeutically advantageous in considering measures such as pharmacological manipulation of neurotransmitter functions (e.g., regulating Glu activity)\textsuperscript{54} or neuromodulatory interventions such as deep brain stimulation or non-invasive transcranial magnetic stimulation\textsuperscript{23,24} with the same aim of restoring normal E/I balance to specific fronto-striatal or cingulate-striatal circuits.

**Methods**

**Participants**

This study included 30 healthy volunteers and 31 OCD patients who were fluent English speakers and were matched for age, gender and IQ. Fig. S1 (Supplement) shows the demographic and clinical characteristics of both groups.

Healthy individuals were recruited from the community, were all in good health, unmedicated and had no history of neurological or psychiatric conditions. Patients with OCD were recruited through an approved advertisement on the OCD action website (www.ocdaction.org.uk) and local support groups and via clinicians in East Anglia. All patients were screened by a qualified psychiatrist of our team (A.S.) to confirm a primary OCD diagnosis. Additionally, the Mini International Neuropsychiatric Inventory (MINI) was used to confirm the absence of any comorbid psychiatric conditions. OCD symptom severity and characteristics were measured using Yale Brown Obsessive Compulsive Scale (YBOCS)\textsuperscript{28}, mood status was assessed using the Montgomery-Asberg Depression Rating Scale (MADRS)\textsuperscript{55}, anxiety levels were evaluated using the State-Trait Anxiety Inventory (STAI)\textsuperscript{56}, and verbal IQ was quantified using the National Adult Reading Test (NART)\textsuperscript{57}. All patients included had a diagnosis of OCD (as per the DSM-V criteria), and exhibited anxiety and depressive symptoms. Patients with comorbid major depressive disorder or Generalised Anxiety Disorder, or any additional axis-I disorders, were not included in the study. We included only patients who presented a total YBOCS score higher than twelve\textsuperscript{58}. Six patients were
unmedicated, and out of the 25 medicated patients, 2 were additionally on beta blockers, 1 on clomipramine, 1 on Olanzapine and the rest were on SSRIs monotherapy. General exclusion criteria for both groups were substance dependence, neurological or medical illnesses or head injury. All participants had normal or corrected-to-normal vision and hearing.

All participants completed the following additional self-report questionnaires:

1. Obsessive-Compulsive Inventory\textsuperscript{27}: a standardized self-report measure of obsessive-compulsive symptoms

2. Intolerance of Uncertainty Scale\textsuperscript{59}: a standardized self-report measure on the unpleasantness of uncertainty

3. The compulsivity subscale of the Habitual Tendencies Questionnaire (HTQ)\textsuperscript{60}

This study was approved by the East of England - Cambridge South Research Ethics Committee (REC 16/EE/0465). All volunteers gave written informed consent before beginning the testing and received monetary compensation for taking part in the study.

\textit{Contingency Degradation Task}

Habitual versus goal-directed behavior was measured using a contingency degradation task used by Ersche et al. (2021), which consisted of 8 blocks of 120 trials, lasting 1 second each\textsuperscript{29}. Fig. 3A depicts the stimuli used in this task. Participants were presented with a white vase on the screen which could be filled with flowers every time the space bar key was pressed. In 60\% of the trials, the key press was associated with a financial reward of 20 pence and the message “YOU WIN” on the screen for 500 ms. In the first 3 blocks, the association between the action of pressing the key, and winning 20 pence was established (non-degraded action-outcome contingency). This duration has been shown to be enough to induce habits in humans\textsuperscript{61}. In block 4, in addition to the original probability of 60\%, participants also received a free reward with a 30\% probability (partially degraded condition). In block 5, the chance of receiving the free reward was also 60\%, which was equal to the probability of reward after pressing the key/performing an action (fully degraded condition). In the final 3 blocks, the initial contingencies were reinstated (non-
degraded conditions), followed by a partially degraded condition in block 7, and a fully degraded condition in block 8. Table 2 depicts an overview of all blocks and conditions.

To study habitual control, an habitual responding index was created by subtracting the responses during the non-degraded condition (probability of 0.60) from the fully degraded condition (probability of 0.30 or 0) with a lower probability of gaining rewards. The increasingly negative values for this index indicate a greater tendency towards goal-directed behavior, whereas, less negative and positive values point towards a bias towards habitual behavior. Note this index did not take into account overall individual variability in response output. Table 2 depicts an overview of all blocks and conditions.

**MRS data acquisition**

The proton magnetic resonance spectroscopy ($^1$H MRS) took place at the Wolfson Brain Imaging Centre, University of Cambridge (United Kingdom). Participants underwent whole-brain T1-weighted MR and single-voxel proton MRS scans using a 7T Siemens Magnetom-Terra scanner. The scanner was equipped with a Nova single-channel transmit, and 32-channel array head coil for signal reception (Nova Medical). T1-weighted MP2RAGE images were acquired to guide voxel placement and used in the analysis to perform tissue corrections (see below). The following specifications were used: echo time = 4300 ms, repetition time = 1.99 ms, inversion times (1/2) = 840/2370 ms, flip angles = 5/6°, acceleration factor (A >> P) = 3, bandwidth = 250 Hz/px, voxel size = 0.75 mm. To increase the SNR and the amount of GM in each voxel, the spectra were measured bilaterally from one 12 x 20 x 33 mm$^3$ voxel at the anterior cingulate cortex, and two 20 x 20 x 20 mm$^3$ voxels at the supplementary motor area, and occipital cortex (Fig. 1A). All the voxels were located manually by the same researcher (M.B). Clear landmarks were used while placing the voxels to increase the reliability of the voxel placements across subjects. After acquiring the MP2RAGE image, and placing the voxels, a short-echo semi-LASER$^{63,64}$ sequence was used to acquire the spectra, collecting 64 repetitions and time/echo time of 5000/26 ms. For each voxel, the FASTESTMAP$^{65}$ sequence for shimming, and variable radio frequency pulses with optimised relaxation delay (VAPOR) for water suppression calibration$^{66}$ were used.

**MRS data preparation and analysis**

Within subjects, the 64 individual spectra files were corrected for effects of eddy currents, frequency, and phase shifts using MRspa (Dinesh Deelchand, University of Minnesota, www.cmrr.umn.edu/downloads/mrspa) and converted to one single averaged file. Next, LCModel$^{67}$ version 6.2-3 was used with an automated fitting routine, to generate model spectra for GABA, Glu, Gln and NAA, between 0.5 and 4.2 parts per million (ppm), and relative to Creatine and phosphocreatine. The
metabolites were water scaled using 8 unsuppressed water spectra acquired before and after the 64 spectral repetitions (automatically detected by MRspa), and using a simulated basis set that included experimentally acquired macromolecule spectra.

A segmentation analysis was performed using SPM12 and the MP2RAGE images to extract tissue fractions for each subject for Gray Matter (GM), White Matter (WM) and Cerebrospinal fluid (CSF), and performed partial volume corrections within subjects according to Harris et al. (2015)\(^\text{68}\) for GABA, and Provencher (2021)\(^\text{69}\) for the rest of the metabolites. Lastly, in order to avoid exclusion of values that are disorder/group specific and can provide insight into the nature of a disorder, a straight cut-off score (which is usually used) is not recommended\(^\text{53}\). Instead, per metabolite and per group, the average and standard deviation were calculated for Cramér-Rao Lower Bound of each metabolite, and individual metabolite concentrations. Next, values larger than 2SD from the mean of each group were excluded for both measures. The latter were according to Frangou et al. (2019)\(^\text{70}\). According to this criteria, the following data were excluded: within the SMA voxel, GABA in 1 healthy and 1 OCD subjects, and Gln in 2 OCD patients, within the OCC voxel, 2 Glu and Gln in 2 healthy subjects, and 1 GABA in a healthy subject, and 3 GABA in patients. Although the Occipital Cortex was selected as a control region to correct for physiological changes, since groups differed on Glu in this region, the correction was not performed. Lastly, 1 ACC and 1 OCC voxel were excluded for one OCD patient due to error during data collection.

**Statistical Analysis**

An independent sample t-test was used for descriptive data, clinical measures and task performance. When the normality condition was not satisfied, Mann-Whitney U test was used instead. For correlational analysis, when the data was not normally distributed, Spearman rank was used instead of the Pearson correlation coefficient. In addition to p-values, the p-values corrected for False Discovery Rates (FDR) are reported according to the correction suggested by Benjamini & Hochberg (1995)\(^\text{25}\) for each section of the results, with \(p < 0.05\). The source code used for the FDR calculation can be found on GitHub: https://github.com/carbocation/falsediscovery. Python version 3.7.6 was used to perform data analysis.

**Data availability**

All research output data will be placed on Apollo, the institutional repository of Cambridge University, and will be provided upon request.

**Declarations**

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Disclosures

TWR discloses consultancy with Cambridge Cognition and receives research grants from Shionogi inc and Sirgarten. He also has editorial honoraria from Springer Nature and Elsevier. KDE discloses editorial honoraria from Karger Publishers. All other authors report no potential conflicts of interest.

Author Contributions:

MB takes full responsibility for the accuracy of the data analysis and data integrity.

Concept and design: PB, TWR, MB

Data Acquisition: MB, MPH, EK

Data optimisation (7T \(^1\)H-MRS): SJW, MB

Data analysis: MB

Interpretation of the data: MB, PB, TRW

Drafting of the manuscript: MB, TRW, PB
References


**Tables**

Tables 1-2 are available in the supplementary files section.

**Figures**

![Brain images and scatter plots illustrating GABA and glutamate levels in different brain regions.](image-url)
Figure 1

shows the relationship between the main excitatory and inhibitory neurotransmitters, Glu and GABA, in voxels placed in (A) anterior cingulate cortex (12 x 20 x 33 mm$^3$), in yellow, supplementary motor area (20 x 20 x 20 mm$^3$) in purple, and occipital cortex (20 x 20 x 20 mm$^3$) in orange, of (B) healthy participants in green, and patients with OCD in blue. The line of best fit is shown with the confidence intervals for the regression estimate in translucent bands around the regression lines. All metabolites were normalised using (Cr + PCr), corrected for gray and white matter and cerebral spinal fluid of each individual voxel, within subjects. Acronyms: ACC = anterior cingulate cortex, SMA = supplementary motor area, OCC = occipital cortex, GABA = γ-amino-butyric acid, p-FDR = p-value corrected for False Discovery Rates, r = Pearson's r correlation coefficient, HV = Healthy Volunteers, OCD = Obsessive Compulsive Disorder.

Figure 2

demonstrates the relationships between compulsivity and Glu levels in SMA, (A) shows the LCModel analysis of in vivo $^1$H MR spectra acquired from a healthy participant at 7T (semi-LASER, echo
time/repetition time = 1.99/4300 ms, from a 20 x 20 x 20mm voxel placed bilaterally at supplementary and pre-supplementary motor areas, located at medial portion of Brodmann area 6), (B) presents the fitted model for Glu, in red, while the acquired spectrum is plotted in black, (C) demonstrates the relationships between Glu levels in SMA and obsessive compulsive symptoms as measured with the self-administered OCI scale in OCD patients, and in healthy subjects, while (D) depicts the relationship between the clinician rated YBOCS scores and Glu levels in SMA in the OCD group. Acronyms: SMA = supplementary motor area, GABA = γ-amino-butyric acid; Glu = glutamate; Gln = glutamine, NAA = N-acetylaspartate, ppm = parts per million, r = Pearson's r correlation coefficient, p-FDR = p-value corrected for False Discovery Rates, OCI = Obsessive Compulsive Inventory, YBOCS = Yale Brown Obsessive Compulsive Scale, HV = healthy volunteers, OCD = obsessive-compulsive disorder.

Figure 3
shows the relationships between habitual responding as measured with the contingency degradation task and Glu:GABA ratios in ACC and SMA, (A) depicts the stimuli from the contingency degradation task and its task design. Participants are shown a white empty vase on the screen, which fills with flowers when participants press the space bar, and which has a 60% chance of being associated with winning 20 pence. On degraded trials, this 60% positive contingency is reduced to 0% (full degradation) or 30% (partial degradation), by non-response contingent presentations of reward. (B) shows the positive relationship between an index of habitual responding, the difference between full and non-degraded conditions- higher means a bias towards habitual control, and Glu:GABA ratio in SMA of the entire sample (the gray fitted line) of OCD and healthy volunteers. Whereas (C) shows the same relationship in the anterior cingulate cortex for each group separately. Acronyms: SMA = supplementary motor area, ACC = anterior cingulate cortex, GABA = γ-amino-butyric acid; Glu = glutamate, r = Pearson's r correlation coefficient, p-FDR = p-value corrected for False Discovery Rates, O = outcome, P = probability, HV = healthy volunteers, OCD = obsessive-compulsive disorder.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.docx
- floatimage2.jpeg
- floatimage5.jpeg