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Frequency and Intensity of Premonitory Urges-to-tic in Tourette Syndrome is associated with SMA GABA+ levels

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Abstract

Background. Individuals with Tourette syndrome (TS) often report that they express tics as a means of alleviating the experience of unpleasant sensations. These sensations are perceived as an urge to act and are referred to as premonitory urges. Premonitory urges have been the focus of recent efforts to develop interventions to reduce tic expression in those with TS.

Objectives. To examine the contribution of brain GABA and glutamate levels of the right primary sensorimotor cortex (SM1), supplementary motor area (SMA), and insular cortex (insula) to tic and urge severity in children with TS.

Methods. Edited magnetic resonance spectroscopy was used to assess GABA+ (GABA + macromolecules) and Glx (glutamate + glutamine) of the right SM1, SMA and insula in 68 children with TS (M_{Age} = 10.59, SD_{Age} = 1.33) and 41 typically developing controls (M_{Age} = 10.26, SD_{Age} = 2.21). We first compared GABA+ and Glx levels of these brain regions between groups. We then explored the association between regional GABA+ and Glx levels with urge and tic severity.

Results. GABA+ and Glx of the right SM1, SMA and insula were comparable between the children with TS and typically developing controls. In children with TS, lower levels of SMA GABA+ was associated with more severe and more frequent premonitory urges. Neither GABA+ nor Glx levels were associated with tic severity.

Conclusions. These results broadly support the role of GABAergic neurotransmission within the SMA in the experience of premonitory urges in children with TS.

Introduction

Individuals with Tourette syndrome (TS) report that their tics are often preceded by a feeling of mounting inner tension that can only be relieved through the expression of a tic[1]. This sensory phenomenon is referred to as a "premonitory urge". Premonitory urges are experienced as either generalized or localized tension and are sometimes described as a tactile sensation or a 'tickle'[2,3]. While premonitory urges are related to tics, the nature of their relationship has not been fully established. Some have argued that since not all individuals with TS experience premonitory urges, tics cannot be caused by premonitory urges and there is some evidence suggesting that the experience of premonitory urges is heightened when tics are suppressed[8]. Indeed, only ~24% of children aged 8-10 years[4] and 57% of adolescents aged 15-19[4] report experiencing premonitory urges. However, by adulthood, up to 98% individuals with TS report experiencing premonitory urges[5–7], suggesting that low estimates of premonitory urges experience in TS may be partially due to difficulties describing premonitory urges in early childhood and adolesence[8]. Importantly, if premonitory urges do indeed cause tics, understanding the underlying neurobiology of premonitory urges may help inform the development of interventions for alleviating tics in TS.

Much of the existing work towards understanding the neurobiology of premonitory urges has come from functional neuroimaging studies. Functional magnetic resonance imaging (fMRI) studies have shown that prior to the expression of tics, at a time that would be concurrent with the experience of a premonitory urge, there is an increase in the activation of the anterior cingulate cortex, primary sensorimotor cortex (SM1), supplementary motor area (SMA), insular cortex (insula), and parietal operculum in individuals with TS[9–13]. Studies using magnetoencephalography[14,15] and position emission topography[16,17] report similar findings. While these studies suggest premonitory urges to be associated with these brain regions, premonitory urges could not be directly measured in these studies, meaning

that activation of these brain regions could be due to other behaviours such as tic suppression. However, these findings are still sufficiently robust to warrant investigation of these neocortical and paralimbic brain regions using other modalities.

While functional neuroimaging studies can localize the brain regions likely to be involved in the experience of premonitory urges, a deeper mechanistic understanding of how these brain regions contribute to the experience of premonitory urges and tics requires the consideration of metabolite profiles and neurotransmission. Historically, investigations into the pathophysiology of TS have focused on the dopaminergic system[18]. Despite there being many individual accounts of altered dopaminergic functioning in TS, the evidence in support of dopamine hypothesis of TS is mixed[19]. An alternative hypothesis proposes that the symptoms of TS may be explained by an imbalance of inhibitory and excitatory neurotransmission. Specifically, altered phasic GABAergic neurotransmission and/or reduced tonic levels of GABA in higher-order motor areas has been suggested to result in a failure of control over the gain of motor excitability and tic expression in TS[20]. In support of this alternative hypothesis, reduced short-interval intracortical inhibition (a marker of inhibitory motor control and $GABA_A$ergic neurotransmission) in left motor cortex (M1) has been identified in both children and adults with TS through paired-pulse transcranial magnetic stimulation (TMS) studies, although the stronger associations in children are with co-occurring ADHD[21–24]. A recent pilot study using double-coil-TMS reported adolescents with TS have diminished inhibitory signalling from right SMA to left M1[25]. Reduced SM1[26] and ACC[27] GABA levels have also been identified in individuals with TS through the application of magnetic resonance spectroscopy (MRS). These findings align well with the body of animal studies showing that striatal injection of $GABA_A$ antagonists such as bicuculine and picrotoxin can produce tic-like behaviors in both rodents[28,29] and primates[30]. Interestingly, reducing glutamatergic striatal innervation appears to reduce tic-like behaviors in rodents[29],

supporting the idea that the symptoms of TS may manifest out of an imbalance of excitatory and inhibitory signalling.

In the current study, we used edited proton MRS to measure and compare both GABA and Glx (the combination of glutamate + glutamine since glutamate cannot be fully resolved at 3 Tesla (T)) levels as markers of inhibition and excitation in right SMA, right SM1, and right insula in children with and without TS. These three brain regions were selected based on their associations in prior TS imaging research with urges and tics. Concurrent with the time period in which the present study took place, other investigators have published studies GABA and glutamate, with heterogeneous findings (see Mahone and colleagues[31]). Our specific objective was to characterize group differences in neurotransmitter levels in affected children and to quantify associations with tic and premonitory urge severity.

Methods

Participants

Children with TS were recruited from specialty clinics at the Kennedy Krieger Institute (KKI) and Johns Hopkins Hospital (the Pediatric Neurology Movement Disorders, OCD, and TS Specialty Clinics), and at Cincinnati Children's Hospital Medical Center (CCHMC) (Pediatric Neurology TS Clinic). Typically-developing children (TDC) were recruited through community advertisements as previously described[32]. The final sample consisted of 116 children (44 KKI, 72 CCHMC). With 74 children in the TS group and 42 children in the typically developing controls (TDC) group.

Diagnosis of TS, ADHD, and OCD were based on DSM-5[33] criteria. After a standard telephone screen, the diagnostic process began with a structured diagnostic interview using the KSADS[34] to screen for excluded diagnoses such as major depression, and a review of all available electronic health record clinical information including, if available, any

psychological testing. Symptom severity was assessed with standard clinical rating scales for tics (Yale Global Tic Severity Scale – YGTSS[35]), tic-urges (Individualized Premonitory Urges in Tourette Scale – I-PUTS[36], ADHD (Conners ADHD scale 3rd edition[37] and DuPaul's ADHD rating scale[38] – ADHD-RS), and OCD (Child Yale-Brown Obsessive Compulsive Scale[39] – CYBOCS). Assessment of TDC involved the same process, except that scales for tics and OCD were not performed. Board certified pediatric neurologists (SHM, DLG) with over 20 years of clinical and research experience with TS and co-occurring conditions confirmed all diagnoses and clinical ratings.

Children were excluded if they had a presence or history of a) a neurological disorder other than TS, including seizures, tumor, head injury or stroke, b) a severe chronic medical disorder or c) presence of a major visual impairment, d) a history of substance abuse or dependency or e) any psychiatric (including ASD) or developmental disorders other than ADHD, OCD. Finally, both TS and TDC children had a full-scale IQ (FSIQ) of > 80 as measured by the Wechsler Intelligence Scale for Children – V[40] (WISC-V).

Descriptive statistics for relevant demographic variables, including age, sex, handedness, prescribed medication, scores on the I-PUTS, YGTSS, CYBOCS and SRS-R, as well as GABA+ and Glx levels for each of the regions of interest (see below) are available in Table 1.

[TABLE 1 HERE]

MRS

Prior to MRI scanning, children underwent a mock scan with a research assistant in order to improve their comfort and decrease their anxiety. To motivate children to lie still during the scan, the children were allowed to watch movies during the acquisition and

received ‘points’ for lying still. These points could be exchanged for prizes after the scan. MRI and MRS scans were conducted using Phillips 3T Achieva scanners (Best, The Netherlands; 32-channel head coil for receive and body coil for transmit) at both sites using identical exam cards for the pulse sequences. One person (NP) travelled to both sites to ensure cross-site consistency and train in voxel placement. For voxel placement and segmentation, a 1-mm^3 isotropic T1-weighted image (MP-RAGE) was first acquired (TR = 7.99 ms, TE = 3.76 ms, flip angle = 8°). $3 \cdot 3 \cdot 3$ cm^3 voxels were then positioned in three regions of interest (ROIs): The right SMA, right SM1 and right insula. See Fig. 1. We chose to examine right hemisphere voxels due to previous work having suggested that the right rather than left insula was specifically involved in the experience of the premonitory urge to tic and to correspond with a separate study of tactile function, which has traditionally been examined in the left hand. Full details of the MRS data acquisition can be found in Supplementary Materials (section 1.1.). Results presented in main text are referenced to water and are presented in institutional units[1]. There were no significant group differences on any of the quality assurance metrics (see Supplementary Table 1).

[FIGURE 1 HERE]

Statistical analyses. All statistical analyses were conducted using the R programming language (v4.0.3) in Rstudio (v1.3.1093). The code used to generate the results and figures of this manuscript are available online through the Open Science Framework (https://osf.io/e7yw9/). The alpha level for all analyses was set to 0.05. For group

[1] When MRS is quantified relative to unsuppressed water signal it is typically accounted for metabolite and compartment specific relaxation values, as well as editing efficiency, approximating concentration values in the mM range. However, as this is not a direct comparison to MM values it is typically presented as institutional units or “(IU)”.

comparisons using analysis of variance (ANOVA), partial eta-squared (η^2_p) was estimated using the 'effect size' package[41] and Bayes factors were estimated using the 'BayesFactor' package[42]. BF_{10} was estimated for each variable of interest by comparing the BF_{10} of the model with the variable of interest included against the model without the variable of interest included. Similarly, to determine the BF_{10} of interaction effects, the BF_{10} of the model with the interaction term included was compared against the model without the interaction term included.

First, after controlling for age and sex, a 3 × 2 ANOVA was conducted to determine whether there were differences with regard to GABA+ and Glx levels between our ROIs (SMA, SM1 and insula), and Groups (TS and TDC). Where there was evidence of an interaction effect, which was determined through joint consideration of p-values, η^2_p and Bayes factors, simple main effect analyses were conducted. If there was evidence of a main effect, post-hoc comparisons were conducted, and Tukey's HSD was used to control for inflation of Type I Error. Second, to determine whether individual differences in GABA+ and Glx levels were related to urge and tic severity, Pearson's correlation analyses were conducted between metabolite concentration levels of each ROI and scores on the PUTS and YGTSS. Due to the large number correlations being conducted here, the more conservative Bonferroni's method was used to correct for inflation of Type I error. Additional analyses where co-occurring ADHD and/or OCD are considered are presented in Supplementary Materials (section 1.5.).

Results

Comparison of GABA+ and Glx levels between regions and groups

GABA. After controlling for age ($F(1, 299) = 0.22$, $p = 0.639$; $\eta^2p = 0.00$; $BF10 = 0.14$) and sex ($F(1, 308) = 0.17$, $p = 0.681$; $\eta^2p = 0.00$; $BF10 = 0.16$), there was a significant

main effect of Region ($F(2, 299) = 9.16$, $p < 0.001$; $\eta^2p = 0.06$; BF10 = 164.30) but not Group on GABA+ levels ($F(1, 299) = 0.13$, $p = 0.724$; $\eta^2p = 0.00$; BF10 = 0.13). There was no significant Region by Group interaction effect ($F(2, 299) = 0.35$, $p = 0.705$; $\eta^2p = 0.00$; BF10 = 0.09). Together, the results of these analyses suggests that while GABA+ levels were different across the three regions, GABA+ levels across these regions were comparable between children in the TS and TDC groups. Subsequent post hoc comparisons revealed that GABA+ levels were higher in the SM than SMA ($p_{Tukey} < 0.001$) and that GABA+ levels were otherwise comparable between SM and insula ($p_{Tukey} = 0.089$), and SMA and insula ($p_{Tukey} = 0.088$). See Fig. 2a. While there was no significant main effect of Group, we visualized the post hoc group comparisons across the regions in Fig. 2b to d.

Glx. After controlling for age ($F(1, 299) = 4.95$, $p = 0.027$; $\eta^2p = 0.02$; $BF_{10} = 1.24$) and sex ($F(1, 299) = 3.83$, $p = 0.051$; $\eta^2p = 0.01$; BF10 = 0.89), there was a significant main effect of Region ($F(2, 299) = 5.24$, $p = 0.006$; $\eta^2p = 0.03$; BF10 =4.31), but no significant main effect of Group on Glx levels ($F(1, 299) = 0.03$, $p = 0.973$; $\eta^2p = 0.00$; BF10 = 0.15). There was no significant Region by Group interaction either ($F(2, 299) = 0.03$, $p = 0.973$; $\eta^2p = 0.00$; BF10 = 0.06). Post hoc comparison revealed that Glx levels were higher in SMA than in SM1, though this effect fell shy of significance ($p_{Tukey} = 0.074$). There were no significant differences in Glx levels between insula and SMA ($p_{Tukey} = 0.575$), but was Glx levels were higher in insula than SM ($p_{Tukey} = 0.005$). See Fig. 2e. Again, while there was no significant main effect of Group, we visualized the post hoc group comparisons across the regions in Fig. 2g to h.

[FIGURE 2 HERE]

Associations between brain metabolite levels and TS symptom severity

A heatmap of the associations between brain metabolite levels (i.e., GABA+ and Glx) and TS symptom severity (i.e., urges and tics as measured using the I-PUTS and YGTSS respectively) is presented in Fig. 3a.

Urges. Among children with TS, lower levels of SMA GABA+ levels were correlated with Number of Urges ($r = –0.40$, $p_{Bonferroni} = 0.020$; Fig. 3b), Urge Frequency ($r = –0.39$, $p_{Bonferroni} = 0.022$; Fig. 3c) and Urge Intensity ($r = –0.42$, $p_{Bonferroni} = 0.011$; Fig. 3d) scores of the I-PUTS. There were no correlations between GABA+ levels in insula or SM cortex with any index of urges (all $p_{Bonferroni} > 0.999$). Glx levels were also not associated with any index of urges (all $p_{Bonferroni} > 0.150$)

Tics. Neither GABA+ or Glx levels were associated with YGTSS scores or number of tics on the I-PUTS (all $p_{Bonferroni} > 0.140$).

[FIGURE 3 HERE]

Parallel analyses with creatine rather than water referenced metabolites

As with the water-referenced metabolites, group differences of GABA+/Cr and Glx/Cr levels were comparable between groups. With regard to the associations between brain metabolites and TS symptom severity, the effects were even stronger for the associations between SMA GABA+/Cr and urge severity. For example, the Pearson's r value for the associations between SMA GABA+/Cr and the Number of Urges ($r = –0.47$, $p_{Bonferroni} = 0.003$), Urge Frequency ($r = –0.48$, $p_{Bonferroni} = 0.002$) and Urge Intensity ($r = –0.48$, $p_{Bonferroni} = 0.003$) were ~10-20% stronger than those shown in Fig. 3a (see Supplementary Fig. 2). Interestingly, insula GABA+/Cr levels also showed significant associations with the Number of Urges ($r = –0.40$, $p_{Bonferroni} = 0.032$), Urge Frequency ($r = –0.46$, $p_{Bonferroni} = 0.008$) and Urge Intensity ($r = –0.45$, $p_{Bonferroni} = 0.011$). Given that the associations between

SMA GABA+ and urge severity remained significant across reference variables while insula GABA+ did not, these results suggest that the SMA GABA+ associations are not being driven by the reference variable (and are hence reliable) while the insula GABA+ associations might be spurious. We discuss these reference variable discrepancies and the robustness of our correlations in more detail in Supplementary Materials.

Effects of co-occurring ADHD and OCD

The statistical analyses used to investigate the effects of co-occurring ADHD and OCD, as well as their results, are described comprehensively in Supplementary Materials (section 1.5.). Broadly, TS individuals with co-occurring ADHD appeared to have significantly higher Glx levels than those without co-occurring ADHD (see Supplementary Figure 10). Co-occurring ADHD also appeared to moderate associations between brain metabolite levels and TS symptom severity (Supplementary Figures 11 and 12). For instance, higher insula GABA+ levels were associated with a higher number of urges in TS individuals without co-occurring ADHD, but a lower number of urges in those with co-occurring ADHD, suggesting ADHD as an important diagnostic moderator of TS. Regarding OCD, there was no difference in GABA or Glx levels between TS individuals with and without co-occurring OCD (Supplementary Figures 13 and 14). While co-occurring OCD also appeared to moderate some of the associations between brain metabolite levels and TS symptom severity (Supplementary Figures 15 and 16), none of the interaction effects would have survived correction for multiple comparisons.

Discussion

We measured, in 8 to 12 year old children with TS and TDC, GABA+ and Glx in right SM1, SMA, and insula. We found that although both groups have comparable GABA and Glx

profiles, lower SMA GABA+ levels were associated with more frequent and more intense premonitory urges to tic in TS. In contrast, GABA+ of SM1 and insula did not show any significant association to urge severity. GABA+ was also not associated with tic severity. We also did not identify any associations between Glx and urge or tic symptom severity.

SMA GABA+ levels are associated with premonitory urge to tic severity in children with TS.

Our most consistent finding were the associations between SMA GABA+ levels with urge severity, which were present regardless of whether GABA+ was referenced to water or creatine. Based on the putative role of the insula (e.g., its role in interoceptive awareness[43] and awareness of the urge-for-action in other behavioral domains[8]), it is likely that premonitory urges do have their origins in the insula (specifically the anterior-dorsal region of the right Ins, which has been demonstrated to be positively associated with premonitory urge scores but uncorrelated with motor tic severity[8]). However, since the posterior of the insular is anatomo-functionally connected to the middle cingulate around the area of the SMA[44–47], we speculate that although premonitory urges may be initially generated within the insula, awareness of these urges may be partially mediated by tonic GABA levels of the SMA. This would explain our results showing that children with TS who had lower SMA GABA+ levels reported more urges, more frequent urges, and more severe urges. Consistent with our findings of a role for SMA, Tinaz and colleagues found that, in 13 adults, functional connectivity between the right dorsal anterior insula and bilateral SMA was positively associated with urge severity, assessed using the PUTS[13]. Still, our interpretation of the results here assumes that urges tics are caused by urges (i.e., premonitory urges generated in the insular drive tics). However, if premonitory urges are only experienced when tics are suppressed, a reasonable alternative interpretation could be that lower SMA GABA+ levels

results in less efficient tic suppression, which in turn results in an increase in premonitory urges-to-tic.

Draper and colleagues[48] had previously reported higher GABA levels in SMA in TS ($N = 8$, $M_{Age} = 18.30$, $SD_{Age} = 2.7$) compared to TDC ($N = 9$, $M_{Age} = 17.20$, $SD_{Age} = 3.30$). GABA levels in the SMA also predicted the amount of change in blood-oxygen level dependent (BOLD) activity in the same voxel prior to voluntary action, with an increase in SMA GABA being interpreted as a possible mechanism by which tic suppression is gained with increasing age[20]. While we did not identify increased SMA GABA+ levels in our sample, our sample contained an appreciably larger and younger sample than that of Draper and colleagues, thus the results are not necessarily in conflict. However, since our results suggest that GABA+ levels of the SMA mediates urge rather than tic severity in younger children with TS, it is possible that the mechanism of gaining control over tics first occurs through the suppression of urges, rather than simply through control over tics.

Comparable GABA and Glx levels between children with TS and TDCs

Perhaps to our surprise, we were unable to reproduce our prior finding of reduced SM1 GABA+ is reduced in children with TS[26]. We do not believe that the discrepancy between our prior and current findings are due to the differences in MRS acquisition, as both studies utilized methods to minimize motion artifact. Similarly, the demographic characteristics of both studies were similar (children aged 8 to 12 years of age, with comparable mean age and sex ratios). Instead, we believe the discrepancy to be better explained by the larger sample size ($n = 68$ in the Tourette syndrome group for the SM1 group comparison vs. $n = 17$ in our pilot study). While small samples are not necessarily an issue in the presence of large effects, this larger study likely generated more valid and generalizable results. When taken with the work of others assessing GABA of SM1 in

adolescent[48] and adult[15] TS populations (which were conducted at 7T and 3T respectively), our results suggest that SM1 GABA+ levels are comparable between children with TS and TDC.

With regard to the lack of group differences and associations identified for Glx, few studies have assessed glutamate levels in TS. Of the studies which have compared Glx (or glutamate at 7T, where glutamate and glutamine can be resolved) levels between individuals with TS and TDC, the findings are mixed. At 7T, Mahone and colleagues[31] found that glutamate levels were increased in the primary motor cortex of young children with TS (TS: N = 32, M_{Age} = 9.88, SD_{Age} = 1.93; TDC: N = 43, M_{Age} = 8.11, SD_{Age} = 1.93). For studies at 3T, Naaijen and colleagues found normal Glx levels in the dorsal striatum and anterior cingulate cortex of children with TS (N = 15, M_{Age} = 10.40, SD_{Age} = 1.20; TDC: N = 53 , M_{Age} = 10.00, SD_{Age} = 1.00) and TS+ADHD (N = 28, M_{Age} = 10.70, SD_{Age} = 1.60). Kanaan and colleagues[49] found reduced striatal and thalamic Glx in adults with TS (N = 37, M_{Age} = 38.30, SD_{Age} = 11.10; TDC: N = 36, M_{Age} = 38.40, SD_{Age} = 11.10). Given the variability in the sample size, MRS acquisition and ROIs across these studies, it is perhaps too premature to try and draw any firm conclusions regarding the status of glutamate levels in TS based on these studies.

Limitations

GABA concentrations in the brain are low, necessitating measurement and averaging of GABA+ over relatively large brain areas. This could limit the ability to detect important differences within smaller regions. Additionally, over and above the limitations of using 3T rather than 7T, and the degree to which MRS-derived measures of GABA and glutamate can be used as markers of inhibition and excitation (both of which we discuss in the Supplementary Materials), many of the correlations identified in this study are influenced by

outliers. To be more confident that the associations identified in our study were true associations rather than spurious, we tested how robust the significant associations were to various stages of outlier removal. We also used additional measures of association such as Spearman's Rho and Percentage-bend[50,51]. The results of those analyses are presented in Supplementary Materials. Broadly, while removal of outliers did reduce the strength of the associations, the significant associations between SMA GABA+ and urge often remained significant, making us confident that the associations presented here are not simply driven by outliers. Importantly, in comparing our results to those of others, it is important to note differences not only in imaging techniques but also that most other studies focus on adults with TS, whose anatomy and physiology may reflect compensatory processes. Finally, with respect to urge assessment, we used the I-PUTS, whereas many other studies used the PUTS. Correlations between these two scale scores are modest, particularly in children with comorbid diagnoses (McGuire et al, J Psychiatric Res, 2016).

Future Directions

If SMA GABA levels mediate the conscious experience of premonitory urges generated by the insula, it follows that targeting SMA GABA levels (or GABAergic functioning within the SMA) may be a plausible way of treating the symptoms of TS. Suppression of the SMA using high-frequency repetitive TMS has already been shown to temporarily reduce tic severity in individuals with TS, though the findings have been mixed[55–57]. Increasing tonic GABA levels or improving GABAergic functioning in the right dorsal anterior insula may also help dampen the limbic drive it imposes on the SMA. However, there have only been a few studies that have demonstrated direct stimulation of the insula with TMS[52,53], with some questioning its feasibility[53]. Further discussion of future

directions and additional considerations of our interpretation of the results can be found in Supplementary Materials.

Conclusion

Using edited MRS, we found that although children with TS had comparable GABA+ and Glx levels as their typically developing peers, SMA GABA+ levels were specifically associated with the number, frequency and intensity of premonitory urges in children with TS. That is, the children with TS who had higher SMA GABA+ levels were also those who had less severe premonitory urges. When taken with existing work, our results suggest that targeting SMA GABA levels may reduce both urge and, thereby, tic severity in children with TS.

Author roles

JLH contributed to manuscript preparation, statistical analysis, and interpretation of the results. MM contributed to statistical analysis and contributed to the quality appraisal and processing of the magnetic resonance spectroscopy (MRS) data. MM also contributed to the organization of the data. DC and DAH contributed to the organization and execution of the study. KMC and HSS provided guidance with the analysis and interpretation of the data. HSS also provided expert clinical insight when interpreting the findings and the preparation of the manuscript. RAEE contributed to the execution, processing, and analysis of the MRS data. DLG and SHM contributed to the conception, organization and interpretation of the data and results. NAJP contributed to supervision of the manuscript preparation, as well as to the interpretation of the results. NAJP also helped organize the study across the two testing sites, ensuring standardisation of data acquisition. All authors contributed to the review and critique of the manuscript.

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Figure Captions:

Figure 1. Voxel placement and resultant spectra. (a) Voxel placement in SM1 and (b) accompanying spectra. (c) Voxel placement in SMA and (d) accompanying spectra. (e) Voxel placement in insula and (f) accompanying spectra. *SM1 = primary sensorimotor cortex, SMA = supplementary motor area, Insula = insular cortex. GABA+ = GABA + macromolecules, Glx = glutamate + glutamine.*

Figure 2. Comparing GABA+ and Glx Across Regions and Between Groups. The top row depicts analyses conducted on GABA+, while the bottom row depicts analyses conducted on Glx. (a). Group x Region on GABA+ analysis. There were differences in GABA+ between regions, but no differences of GABA+ between groups. The latter was confirmed by subsequent post hoc comparisons showing comparable GABA+ between TDC and TS in the (b) SM1, (c) SMA and (d) Insula. (d) Group x Region on Glx analysis. For Glx, again, there were significant differences between the regions (e), but no significant differences between the groups in terms of (f) SM1, (g) SMA and (h) Insula Glx. *GABA+ = GABA + macromolecules, Glx = glutamate + glutamine, SM1 = primary sensorimotor cortex, SMA = supplementary motor area, Insula = insular cortex, TDC = typically developing controls, TS = Tourette*

Syndrome, IU = institutional units. Error bars in panels (a) and (e) represent standard error of the mean. Note, values on the y-axis have been corrected for age and sex.

Figure 3. Associations of Urge and Tic with Metabolite Levels (a) Heatmap depicting the associations between GABA+ and Glx across the ROIs on the x-axis, with urge and tic severity on the y-axis. Scatterplots depicting the associations between SMA GABA+ and the number (b), frequency (c) and intensity (d) of urges as assessed by the I-PUTS. Note that while we corrected for multiple comparisons using the Bonferroni method in the main text, the p-values presented in panels b to d are uncorrected. *GABA+ = GABA + macromolecules, Glx = glutamate + glutamine, I-PUTS = individualised premonitory urge to tic scale, YGTSS = Yale global tic severity scale, SM1 = primary sensorimotor cortex, SMA = supplementary motor area, Insula = insular cortex, IU = institutional units. Note, values on the y-axis have been corrected for age and sex.*