HHS Public Access

Author manuscript

Psychiatry Res. Author manuscript; available in PMC 2018 March 30.

Published in final edited form as:

Psychiatry Res. 2018 March 30; 273: 46–53. doi:10.1016/j.pscychresns.2017.12.005.

GABA and glutamate in children with Tourette syndrome: A ^{1}H MR spectroscopy study at 7 T

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Abstract

Tourette syndrome (TS) is characterized by presence of chronic, fluctuating motor and phonic tics. The underlying neurobiological basis for these movements is hypothesized to involve cortical-striatal-thalamo-cortical (CSTC) pathways. Two major neurotransmitters within these circuits are γ-aminobutyric acid (GABA) and glutamate. Seventy-five participants (32 with TS, 43 controls) ages 5–12 years completed ^{1}H MRS at 7 T. GABA and glutamate were measured in dorsolateral prefrontal cortex (DLPFC), ventromedial prefrontal cortex (VMPFC), premotor cortex (PMC), and striatum, and metabolites quantified using LCModel. Participants also completed neuropsychological assessment emphasizing inhibitory control. Scans were well tolerated by participants. Across ROIs combined, glutamate was significantly higher in the TS group, compared to controls, with no significant group differences in GABA observed. ROI analyses revealed significantly increased PMC glutamate in the TS group. Among children with TS, increased PMC glutamate was associated with improved selective motor inhibition; however, no significant associations were identified between levels of glutamate or GABA and tic severity. The dopaminergic system has long been considered to have a dominant role in TS. Accumulating

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Contributors

Study concept and design was done by Drs. Mahone, Puts, Edden, and Singer. Data acquisition and analysis was done by Drs. Mahone, Puts, Edden, Singer, and Mr. Ryan. The manuscript was written by Drs. Mahone, Puts, Edden, and Singer.

Conflict of interest

Dr. Edden receives personal speaking fees from Philips Medical Systems outside of the submitted work. The work of Dr. Singer is partially funded by a grant from a private institution, the Tourette Association of America. Dr. Mahone, Dr. Puts, and Mr. Ryan have no conflicts to report.

evidence, however, suggests involvement of other neurotransmitter systems. Data obtained using ^{1}H MRS at 7 T supports alteration of glutamate within habitual behavior-related CSTC pathways of children with TS.

Keywords

Brain; Corpus Striatum; Putamen; Inhibition; Tics; Neuroimaging; Child

1. Introduction

1.1. Tics as habitual behaviors

Tourette syndrome (TS), a common disorder with onset typically in the developmental period (i.e., by age 18 years), is characterized by presence of chronic, fluctuating, motor and phonic tics. Tics, the hallmark of the TS, are abrupt, rapid, repetitive, non-rhythmic, simple or complex motor movements or phonic productions (Singer, 2011). Tics have been classified as *habits* (Delorme et al., 2016a, 2016b; Singer, 2016) based on their being sudden, rapid, involuntary, non-rhythmic, and repetitive movements or vocalizations that are exacerbated by sensory stimuli, are performed without apparent gain, and are unrelated to a clear outcome. Supporting clinical information includes findings that TS patients have enhanced reward learning (considered to underlie habitual responses), and the observation that unmedicated adults with TS have enhanced habit formation (Delorme et al., 2016a, 2016b).

Available evidence also supports involvement of cortico-striatal-thalamo-cortical (CSTC) circuits and their interconnecting brain regions in the pathophysiology of tics (Felling and Singer, 2011). In human and rodent studies, presence of distinct, cortical-striatal pathways for *habitual actions* (premotor/supplemental motor area to putamen) and flex-ible *goal-directed behaviors* (ventromedial prefrontal to caudate) has been established (Fig. 1) (Balleine and O'Doherty, 2010; de Wit et al., 2012a, 2012b; Tanaka et al., 2008; Tricomi et al., 2009). Several neurotransmitters, including dopamine, GABA and glutamate, play an important role within CSTC circuitry and have been proposed to have roles in both habitual behavior formation and in the pathophysiology of tics (Gasbarri et al., 2014; Singer, 2016).

1.2. GABA in TS

GABA is the primary neurotransmitter of striatal medium sized spiny neurons and interneurons located within both the striatum and cortex. In TS, *cortical* GABAergic involvement is supported by transcranial magnetic stimulation measurements showing reduction of short-interval intra-cortical inhibition (Gilbert, 2006), MRS GABA findings in multiple *cortical* regions (Draper et al., 2014; Freed et al., 2016; Puts et al., 2015), and altered $GABA_A$ receptors quantified by [11C]flumazenil binding (Lerner et al., 2012). Evidence for *striatal* GABAergic involvement includes reduction of parvalbumin containing interneurons in postmortem human studies (Kataoka et al., 2010), and altered $GABA_A$ receptor binding (Lerner et al., 2012).

1.3. Glutamate in TS

Glutamate, the primary excitatory neurotransmitter in mammalian brains, is used by cortical pyramidal neurons, as well as neurons within limbic (anterior cingulate-ventral striatum), and subthalamic nucleus, regions, and within thalamocortical and thalamostriatal pathways (Singer, 2010). Evidence supporting glutamatergic system dysfunction within CSTC pathways in TS patients includes: reduced postmortem glutamate in globus pallidus interna (GPi), externa (GPe), and substantia nigra pars reticulata (SNpr) (Anderson et al., 1992), missense mutation in the glial glutamate transporter gene (SLC1A3) (Adamczyk et al., 2011), and elimination of striatal-injected, picrotoxin-induced, tic-like movements in rodents following striatal injection of a glutamatergic NMDA receptor antagonist (Porgorelev et al., 2015). Additionally, there is an established interaction between glutamatergic and *dopaminergic* neurotransmitter systems via frontal lobe projections to the ventral tegmental area (VTA) and substantia nigra pars compacta (SNpc) (Canales et al., 2002; Singer, 2010; Wu et al., 2000) (See Fig. 1).

1.4. GABA/glutamate interaction

In human primates, rats, and mice, the *striatal* injection of a GABA-A antagonist (bicuculine or picrotoxin) has been shown to cause tic-like behaviors (Bronfeld et al., 2013; Porgorelev et al., 2015; Worbe et al., 2013). Subsequent treatment studies in mice showed that striatal picrotoxin-induced movements could be reduced or abolished by infusing a GABA-A agonist (muscimol) into the striatum, infusing muscimol into the overlying cortex, or by blocking striatal NMDA receptors with (RS)-4-(phosphonomethyl)-piperazine-2-carboxylic acid (PMPA) (Porgorelev et al., 2015). Hence, although tic activity was initially precipitated by blocking striatal GABAergic activity, subsequent “rebalancing” of the striatal GABA/Glu system by increasing striatal GABA or by reducing glutamatergic striatal innervation (diminished cortical input or blocking striatal receptors) served to effectively reduce the tic activity. Similarly, tic-like movements in mice have been elicited by infusing picrotoxin in the *sensorimotor cortex and* reduced by the injection of muscimol into the dorsolateral *striatum* (Porgorelev et al., 2015).

1.5. Mapping GABA and glutamate with MR spectroscopy

MR spectroscopy (MRS) provides an opportunity to measure both GABA and glutamate in vivo and non-invasively. When determined at field strengths of 3 T and lower, glutamate and glutamine are not well resolved and are often reported as Glx. This method may be problematic, since both of these amino acids can influence neuronal functions and there are pathological processes in which one may increase while the other decreases (Novotny et al., 2003). Measurements using ultra-high field spectroscopy (7 T) increase the signal-to-noise ratio and improve separation of metabolite signals including glutamate and glutamine (Dou et al., 2013; Mekle et al., 2009; Pradhan et al., 2015; Tkac et al., 2001).

GABA can be resolved and quantified at both 3 T and 7 T, although methodologies are different (Pradhan et al., 2015). Few studies, however, have employed ultra-high imaging in pediatric populations, and (to our knowledge) only one study has used MRS at 7 T in children under age 10 years (Harris et al., 2016). Among published studies, there is

variability in reported findings within 3 T protocols and differences between 3 T and 7 T MRS investigations of TS (see Table 1).

Two prior MRS studies of GABA in TS conducted at 3 T using the J-edited spin echo difference editing method, Tinaz et al. (2014) reported *no differences* in GABA/Cr ratios in sensorimotor cortex between adult patients and controls. Nevertheless, others (Puts et al., 2015) demonstrated TS related *reductions* in GABA in sensorimotor cortex among 8–12 year olds, with reductions predictive of motor tic severity. Similarly, among two published MRS studies of TS conducted at 7 T (using the LCModel fitting method), Draper et al. (2014) reported *increased* GABA/NAA in supplemental motor cortex, but no changes in GABA/NAA in sensorimotor cortex among adolescents (mean age 15.7 ± 3 years); whereas Freed et al. (2016) showed *reduced* GABA/water ratios in the anterior cingulate among teenagers with TS.

To date, there have been two published studies assessing glutamate in TS patients, both using MRS at 3 T (Kanaan et al., 2017; Naaijen et al., 2016), also with inconsistent findings. Naaijen et al. (2016) reported no TS-related difference in glutamate among 8–12 year olds in dorsal striatum or anterior cingulate; while Kanaan et al. (2017) showed *reduced* Glx in striatum and thalamus among adults with TS. Potential explanations for varying patterns of findings for both GABA and Glu include differences in sample age, sex distribution, presence of existing co-morbidities, current and prior medication use, nuances of voxel placement, and potential differences that may occur when using the J-edited spin echo difference editing method (Rothman et al., 1993) versus fitting methods such as LCModel as means of quantifying GABA.

1.6. TS and inhibitory control

It is essential to acknowledge the presence of both fascillatory and inhibitory cortical-striatal pathways. Recent studies have identified that frontal cortical inputs to the basal ganglia provide both activating, as well as inhibitory proactive and reactive control over behaviors (Aron, 2011; Jahanshahi et al., 2015). *Proactive* inhibition, which subserves individual goal-directed activity, involves DLPFC to caudate pathways, whereas *reactive* inhibition—i.e., stopping a habitual response that is underway (and via motor inhibition tasks such as the Conflicting Motor Response Test) is thought to involve pre-SMA and inferior frontal cortical pathways to the subthalamic region. Additional evidence for a cortical inhibitory involvement in TS includes reduced intracortical inhibition in motor cortex (Gilbert, 2006).

1.7. Hypotheses

Given the aforementioned observations, in the present study, emphasis was placed on measurement of GABA and glutamate within specific brain regions representing the neural substrate (pathways) underlying habitual (PMC and striatum) and goal-directed (VMPFC and striatum) behavior. The DLPFC was also selected because of its role in controlled executive functions, planning, attention, organization, and working memory (Yoon et al., 2016). We hypothesized that GABA and glutamate in these pathways would be altered in TS, with greater alterations observed in habitual behavior circuitry involving premotor and striatal regions. We also hypothesized that anomalies in GABA and/or glutamate in habitual

circuitry would be associated with both tic severity and neuropsychological measures of motor inhibitory control.

2. Methods

2.1. Participants

The Institutional Review Board of the Johns Hopkins Medical Institutions approved the current study. Parents or legal guardians of all participants provided informed, written consent and all participants provided assent prior to the study. Thirty-two children diagnosed with TS, ages 5–12 years, were recruited through the Pediatric Movement Disorders Clinic at Johns Hopkins Hospital, and 43 typically developing children were recruited through community advertisements as control participants.

2.1.1. Inclusion criteria—All children in the TS group included in the study were evaluated by a pediatric neurologist (HSS), who confirmed the diagnosis of TS or Chronic Motor Tic Disorder, which included the following criteria: a) onset before 18 years, multiple involuntary motor tics, one or more vocal tics, a waxing and waning course, the gradual replacement of old symptoms with new ones, the presence of tics for more than one year, the absence of other medical explanations (effects of a substance) or a general medical condition for tics, and observation of tics by a reliable examiner; b) observable tics, achieving a score of 12 or greater on the Total Tic severity score of the modified Yale Global Tic Severity Scale (YGTSS); and, c) drug naïve or not receiving tic-suppressing medication for TS or ADHD at time of enrollment (i.e., off medications for at least three months); and, d) no contraindication to MRI (e.g., metallic emplacements). For Chronic Motor Tic Disorder, criteria were similar to TS except for the absence of vocal tics.

2.1.2. Exclusion criteria—Exclusion criteria for the TS group included the presence of: a) secondary tics; b) significant medical illness (metabolic, endocrine, cardiac, hematological, gastrointestinal, pulmonary, epilepsy); c) OCD, depression, Generalized Anxiety Disorder, Separation Anxiety Disorder, or psychotic symptoms (based on clinical assessment and results of structured psychiatric interview); d) history of autism spectrum disorder, intellectual disability, eating disorder or substance abuse.

Participants in the typically developing control group were included if they were free from all developmental and psychiatric conditions (as noted above as determined by history and structured psychiatric interview), and free from contraindications to MRI.

2.2. Assessment methods

A list of study assessment methods is provided in Table 2. Measures included assessment of tic severity, ADHD symptoms, psychiatric diagnoses, and performance-based measures. All participants completed a focused assessment in order to determine eligibility, characterize the sample, assess symptom severity, and to assess neurocognitive functions associated with inhibitory control (described below). Performance-based neuropsychological testing was completed during a single day as part of a specific research protocol. Concurrent psychiatric diagnoses were assessed using the Diagnostic Interview for Children and Adolescents, 4th

Edition (DICA-IV) (Reich et al., 1997). Modules included: ADHD, Conduct Disorder, Oppositional Defiant Disorder, Major Depressive Disorder, Bipolar Disorder, Dysthymic Disorder, Separation Anxiety Disorder, Panic Disorder, Generalized Anxiety Disorder, Specific Phobia, and OCD. Within the TS group, tic severity was assessed using a modified version of the Yale Global Tic Severity Scale based on parent report (Leckman et al., 1989).

2.3. Neuropsychological measures of inhibitory control

Three performance-based measures of motor inhibitory control were administered to each participant, emphasizing *reactive inhibition* (i.e., withholding a prepotent motor response, or stopping one that is underway—Conflicting Motor Response Test), *selective inhibition* (i.e., unintended, age-inappropriate associated movements—Physical and Neurological Assessment of Subtle Signs-PANESS), and *proactive inhibition* (i.e., involving an active cognitive decision—commission errors from a computerized Go-No/go test). Measures were examined to measure functional impairment in motor control among children with TS, and to assess potential brain-behavior associations with GABA and glutamate.

2.3.1. Conflicting motor response test—Participants were told, "If I show you my finger, you show me your fist; if I show you my fist, you show me your finger." The examiner presented each of the two gestures 24 times in a fixed pseudo-random sequence. Participants were instructed to respond with the preferred hand as quickly as possible. The task therefore required the individual to inhibit the prepotent tendency to mimic the examiner. The variable of interest was total number correct (maximum score=48), with higher scores indicative of better performance.

2.3.2. Motor overflow—PANESS—The revised PANESS (Denckla, 1985) is a quantified motor examination comprised of untimed and timed tasks. Untimed motor tasks include gaits on heels, toes, and sides of feet (and parallel overflow/postures); tandem gait forward/backward; standing/hopping on one foot; standing heel-to-toe with eyes closed; standing both feet together, arms outstretched with eyes closed (and choreiform). Timed tasks include a sequence of 20 toe taps, hand pats, and finger taps; 10 "heel-toe," 10 hand pronate-supinate and tongue side-to-side; and 5 sequences of finger appositions (left and right sides). *Overflow movements*, defined as co-movement of body parts not specifically needed to efficiently complete a task, are considered to represent failure of inhibition of prepotent movement. Overflow is documented during both gaits and timed activities. The total overflow score was used in analyses. Higher total scores indicate greater abnormality.

2.3.3. Commission errors—For this computerized go/no-go test, participants were seated in front of a screen that flashed green and red spaceships and were told to press the spacebar in response to green ships only. Cues appeared on screen for 300 ms. and were presented once every 1800 ms. (fixed 1500 ms. inter-stimulus interval). Cues were weighted towards green spaceships at a ratio of 3:1 and the task lasted 8 min. Commissions were defined as pressing the space bar after the presentation of a red ship. The total number of commission errors was used in analyses.

2.4. MRI and MRS procedures

All participants received mock scan training sessions to improve comfort, decrease anxiety and train them to lie still. No sedation was used. Structural MRI and MRS were performed using a 7 T scanner (Achieva, Philips Healthcare, Best, The Netherlands) equipped with a Nova Medical quadrature transmit head coil and 32-channel receive coil array. A high-quality T1-weighted MPRAGE structural brain image (TE/TR=2.1/4.8 ms.; resolution: 0.6×0.6×0.6 mm^3) was acquired (total scan time: 6 min, 32 s) for planning of the MRS voxel locations and for examining tissue fractions. Spectra were acquired from four voxels: ventromedial prefrontal (VMPFC), dorsolateral prefrontal (DLPFC), premotor (PMC), and striatum. Stimulated echo acquisition mode (STEAM) sequence was used for signal localization with the following parameters: TE/TM/TR/NS = 14 ms./26 ms./3000 ms./96 averages and VAPOR water suppression.

Voxel placement was performed using a documented procedure and images for reference. The ~8 ml PMC voxel was placed in the left hemisphere, with posterior face aligned to the pre-central sulcus, and the inferior face above the level of the corpus callosum. The ~8 ml DLPFC voxel was placed in the left hemisphere, anterior to the pre-motor voxel, angulated so as to maximize GM content whilst avoiding the skull. The ~8 ml VMPFC voxel was placed on the midline, with the posterior face of the voxel aligned with the anterior end of the genu. The ~8 ml striatum voxel was placed in the left hemisphere, aligned in the sagittal plane with the principal axis of the striatum rotated to include predominantly putamen (Fig. 2). Each participant also had an unsuppressed water scan from the same voxels for quantification purposes.

For post-acquisition image analyses, LCModel was used to quantify both GABA and glutamate using an in-house basis set that includes a macromolecular basis spectrum. GABA and glutamate were measured as a ratio to water within each voxel. Based on data from T1 images, the fractions for gray matter (GM), white matter (WM), and CSF were calculated for each voxel, and the measured metabolite was divided by the sum of GM+WM within each voxel. Tissue corrected GABA and glutamate were used in analyses. Only data from voxels with Cramér-Rao lower bound values $\leq 20\%$ were included in analyses.

2.5. Data analyses

First, demographic variables (age, handedness, sex distribution) were compared between groups using ANOVAs for continuous variables and chi-square analyses for categorical variables. Second, data quality metrics (LCModel signal-to-noise ratio, linewidth, Cramér-Rao lower bound measures) were examined for distributions and group differences. Third, voxel locations from all ROIs were pooled and a linear mixed-effects (LME) model was used to examine group differences in metabolite concentrations in the TS group compared to controls. This test benefits from increased statistical power when examining the fixed effects factors, and accounts for missing data. For initial models, age and sex were included as factors. If no significant effects for age or sex or their interaction were observed, these variables were removed from subsequent models. Significant omnibus results were followed by separate LME examinations of regional differences in metabolites for each ROI. Significance level for group comparisons was set at 0.05 for the omnibus test of all ROIs,

and at 0.0125 (0.05/4) for analyses of ROIs and neuropsychological tests. Finally, the associations between regional metabolite concentrations and neuropsychological functions (tic severity, ADHD symptom severity, motor inhibition) were examined within the TS group only for ROIs showing group differences.

3. Results

3.1. Sample demographics

Demographic information is included in Table 2. The total sample included 32 children with TS and 43 controls. Within the TS group, the *mean* age of onset was 5.7 years for motor tics and 7.0 years for vocal tics. *Mean* Total Tic severity score was 18.7 (*range* 12–40; *median*=24). Within the TS group, 2 children met criteria for ADHD on the DICA-IV at the time of assessment, and 1 child had taken tic-suppressing medication (Intuniv) prior to the study, but had stopped at least 3 months before the scan. There were no significant differences between TS and control groups in racial composition status or handedness; however, the TS group was significantly older and had a greater proportion of boys than the control group.

3.2. Neuropsychological assessment

Results of performance-based neuropsychological assessment are also listed in Table 3. The TS group showed significantly reduced performance, relative to controls, for reactive (Conflicting Motor Response Test; p=0.001) and selective motor inhibition (PANESS Total overflow; p=0.005), but not for Full Scale IQ (p=0.03), or the measure of "cognitive" proactive inhibition (Go/No-go commission errors; p=0.15).

3.3. MRS results

Children in the study were given a questionnaire following the scan asking about discomfort, rated the experience on a 1–10 Likert scale, with 1 indicating the "worst experience," and 10 indicating the "best experience they can recall." Fifty-two participants completed the questionnaire. Mean ratings (with standard deviations) were as follows: dizziness=6.5 ± 26; nausea=9.2 ± 1.7; uncomfortable=7.1 ± 2.7; loudness=7.1 ± 2.6; overall experience=8.0 ± 1.8. When asked if they would like to do the MR scan again, responses were as follows: yes=39 (75%), maybe=9 (17%), and no=4 (7%). As such, we concluded that the child experience of the 45–60 min ultra-high field MR scan was generally positive, with no substantial discomfort.

Results of group comparisons for individual GABA and glutamate concentrations across and within ROIs are listed in Table 4, along with group comparisons of quality metrics. There were no significant group differences in signal-to-noise ratio or Cramér-Rao lower bound. Line-width measurement was significantly higher in the TS group (p=0.001), and was significantly associated with GABA (r=0.21, p=0.003), but not glutamate (r=0.02, p=0.79) across regions. As such, linewidth was controlled in subsequent group comparisons for GABA. Across regions, neither sex nor age were significant predictors of GABA (sex p=0.55, age p=0.56) or glutamate (sex p=0.90, age p=0.83), nor were there any significant 2-

or 3-way interactions (GABA: all $p > 0.49$; glutamate: all $p > 0.68$); thus, both variables were removed from subsequent models.

Across regions, glutamate [$F(1219)=4.78$, $p=0.030$, $\eta_p^2=0.021$], but not GABA [$F(1199)=2.08$, $p=0.15$, $\eta_p^2=0.010$] was significantly increased in the TS group, compared to controls. Compared to controls, children with TS showed significantly increased glutamate within the PMC ($p=0.01$). No significant group differences in GABA or glutamate were observed within other individual ROIs.

3.4. Brain/behavior correlations

Within the TS group, increased PMC glutamate was significantly associated with reduced motor overflow on the PANESS ($r=-0.51$, $p=0.009$; Fig. 3). There were no other significant associations between PMC glutamate and either tic severity or neuropsychological function.

4. Discussion

In this study, ultra-high-field ^{1}H MRS at 7 T was used to quantify concentrations of GABA and glutamate within CSTC pathways, with emphasis on the *habitual behavioral pathway*. Several prior ^{1}H MRS studies have been performed at 3 T in children and adults with TS (Kanaan et al., 2017; Naaijen et al., 2016; Puts et al., 2015; Tinaz et al., 2014); however, this report represents the first investigation to obtain ^{1}H MRS measurements of *both* glutamate and GABA at 7 T within the striatum and cortex of young children with TS (including those under age 10 years—an age range not previously studied using 7 T MRS). Overall, the scans were well tolerated by the children in this age range.

Results of the current study suggest that glutamatergic abnormalities may be located within the habitual pathway (i.e., increased glutamate in the premotor cortex) among children with (relatively non-comorbid) TS, as both the PMC and SMA, are contributing cortical sites of origin for the habitual pathway (de Wit et al., 2012a, 2012b). Conversely, the present data do not support prior studies suggesting alterations in GABA among children with TS within habitual pathways (Draper et al., 2014; Freed et al., 2016; Puts et al., 2015). The strength of the evidence supporting the involvement of glutamate and habitual pathway involvement in TS is tempered, however, by the recognition that the habitual, goal-directed, and limbic systems are all interconnected, and that habit development (represented through tics) may involve a shift of influence from the ventral to dorsal striatum, and that disruptions associated with tics can occur at multiple anatomical sites (Graybiel, 2008; Gruber and McDonald, 2012).

In the present study, significant alterations of glutamate were observed in premotor cortex, with no differences identified in the DLPFC—an area involved in the process of calculating action contingencies and outcome/value probabilities (O'Doherty, 2016). Earlier published studies of striatal glutamate or its metabolites, conducted at 3 T in samples including older children or adults with TS, showed no differences from controls (Naaijen et al., 2016), or reduced Glx (Kanaan et al., 2017). While these findings might suggest that the PMC is a primary area of abnormality associated with tics, neither the elevated glutamate levels nor

GABA were significant predictors of tic severity. In prior 7 T studies of TS, elevated levels of GABA in the SMA were significantly associated with cortical excitability in the primary motor cortex and motor tic severity (Draper et al., 2014). It remains unclear why associations between premotor/SMA GABA and tic severity were observed in the Draper study but no associations were seen in the present sample, although in the earlier study, older age (mean age = 15 ± 3 years), presence of more comorbidities, and nuances of voxel placement may have contributed to the results.

It is also possible to speculate that identified glutamatergic alterations in TS are affecting both excitatory and inhibitory actions. Recognizing the complex interplay between the excitatory glutamatergic and inhibitory GABAergic systems in CTSC circuits, pathophysiologically, it is possible that either could be primary, with the other representing a secondary phenomenon. It is also important to recognize a potential impact of glutamate on other neurotransmitters, e.g., a glutamatergic effect on the dopaminergic system. For example, glutamatergic activity, represented by the presence of NMDA receptors on dopaminergic neurons, may affect habit learning (Wang et al., 2011). More specifically, damaging NMDA receptors located on dopamine nigrostriatal neurons prevented habitual, but not goal-directed, learning (Faure et al., 2005, 2010), while amphetamine sensitization (increased dopaminergic activity) enhanced the progression from goal-directed to habitual responses (Nelson and Killcross, 2013). Glutamate may also have its effect on other parts of the circuit, e.g., acting via cortical-basal ganglia (subthalamic nucleus), cortical-substantia nigra pars compacta (SNpc), or cortical-ventral tegmental area (VTA) pathways. Indeed, accumulating evidence from studies employing optogenetic and chemogenetic methods supports the role of glutamatergic output neurons and their targets within the CTSC circuitry, in modulating the expression of tic behaviors (Burton, 2017).

The interpretation of glutamate and GABA measurements in this study relative to prior investigations is also potentially affected by several factors including both technical (magnet strength, the intracellular location of the recorded amino acids) and clinical issues. MRS using ultra high field spectroscopy (7 T) has been shown to improve separation of metabolite signals as compared to 3 T imaging (Dou et al., 2013; Mekle et al., 2009). Glutamate or GABA findings in participants measured using different MR field strengths may not be directly comparable. For example, the published measurement of glutamate within the striatum using 3 T in individuals with TS was either normal (Naaijen et al., 2016) or reduced (Kanaan et al., 2016) whereas with 7 T (this study) levels were elevated in premotor cortex compared to controls.

Despite being relatively free from comorbidities and non-medicated at the time of assessment, our sample of children with TS demonstrated significantly poorer motor control, relative to controls, for both reactive (Conflicting Motor Response) and selective (motor overflow) components of motor inhibition. At the same time, unexpectedly, the observed TS-related increase in premotor glutamate was associated with *improved* (reduced) motor overflow, yet relatively uncorrelated with tic severity. While it is unclear why anomalous (increased) premotor glutamate among children with TS would be associated with improved function, it is possible that glutamate has a range of metabolic roles and that this increase might reffect metabolic rather than neurotransmitter aspects of function. It is also possible

that increase in glutamate is compensatory and acting as a response to correct the issue, rather than a cause, and in doing so, would be associated with other compensatory neuropsychological functions (such as control of unwarranted motor overflow). An additional consideration is that MRS glutamate or GABA measurement in this and other studies reflects the gross concentration of the substance in the entire voxel, including that in neurons and glial, synaptic and extrasynaptic spaces, and cytoplasmic and organellar zones. Thus, while it is often tempting to equate measurements with neurotransmitter function or a physiological activity, it should be recognized that only a fraction of MRS measured glutamate or GABA are neurotransmitter based and concentration may not equate to behavioral activity (Rae, 2014; Chen et al., 2017). Hence, as noted by others (Draper et al., 2015) metabolite levels obtained via MR spectroscopy should be considered to represent a balance of excitatory or inhibitory tone.

This study has several limitations. First, as a function of individual child movement and scan quality, GABA and glutamate measurements for all 4 ROIs were not obtained in all 75 participants, and the number of individuals compared for each ROI differed slightly. Second, in order to maximize the number of individual ROIs obtained for each individual, we chose to obtain representative voxels only from the left hemisphere. As such, the findings may not be fully representative of those differences or associations obtained bilaterally. Third, in order to maximize the likelihood that neuroimaging findings were specific to TS, we screened out participants with some comorbidities common in TS (e.g., OCD), and our sample had proportionally fewer children with comorbid ADHD (6%) than is typically observed in clinic samples, in effect limiting the generalizability of the findings to samples with a wider range of comorbidities.

The present findings do, however, provide guidance for future research investigations. In particular, there is a need for prospective therapeutic studies utilizing agents that modulate glutamatergic activity, and the use of 7 T ^{1}H-MRS to assess neurochemical adaptations. For example, although glutamate-altering medications have demonstrated a beneficial therapeutic effect on obsessive-compulsive symptoms (Kushner et al., 2007; Singer, 2010), their consequences for treatment of individuals with TS remain unclear. In a small study, tic suppression following treatment with a glutamate agonist (D-serine) or a glutamate antagonist (riluzole) did not differ from the placebo control group (Lemmon et al., 2015).

Given the inconsistencies in findings among MRS studies obtained at 7 T versus those obtained at 3 T, future, larger studies are needed to compare and contrast MRS results in children who have been scanned on both 3 T and 7 T scanners. Longitudinal investigations are indicated to determine how the anomalous patterns of GABA and glutamate observed in the habitual brain circuits interact, and how they are related to the onset and persistence of tics. In addition, future longitudinal studies should shed light on whether striatal neurochemical anomalies occur earlier in the course of TS than cortical alterations. Finally, in future studies with larger samples, the impact of comorbidities observed in TS (especially ADHD and OCD) can be explored in greater detail.

Acknowledgments

This study was supported by the Tourette Association of America, 1R21 MH092693, and U54 HD079123. A portion of this paper was presented as a platform session at the Child Neurology Society meeting in Vancouver, October 2016.

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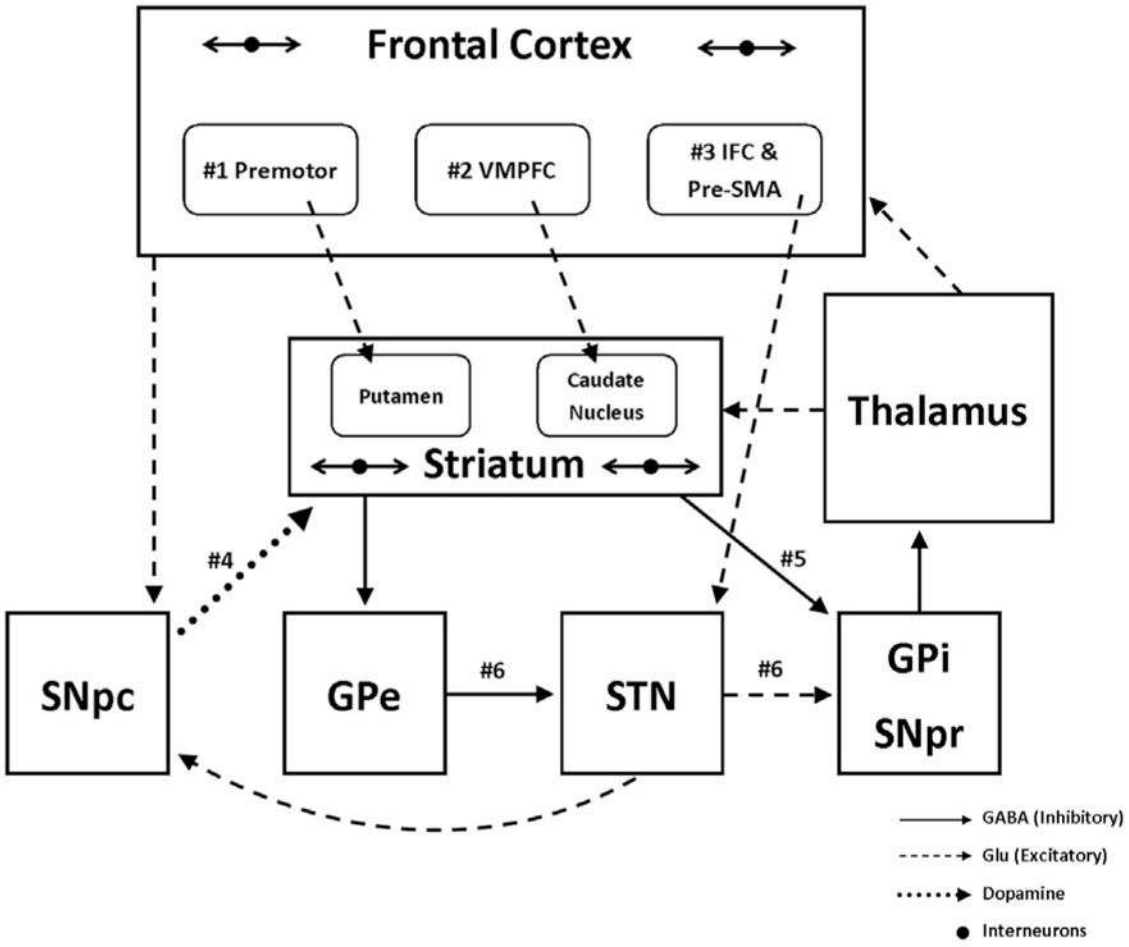

Fig. 1.

Alterations of glutamate and GABA within the cortico-striatal-thalamo-cortical circuit (CSTC) provide the framework for understanding tics in Tourette syndrome. The facillatory component of habitual behavioral pathways originates within excitatory glutamatergic pyramidal cells located in pre-motor/supplementary motor areas (SMA) of the frontal cortex (**#1**); projects to the putamen. The pathway for goal-directed behavior arises from within the associative (cognitive) cortex; more specifically the ventral-medial prefrontal cortex (**#2**); projects to the caudate nucleus. Motor activity is also influenced by the cortical suppression of inappropriate or socially unacceptable behavior. For example, external stimuli triggered reactive inhibition arises from within inferior frontal cortical (IFC) and pre-SMA neurons (**#3**); projects directly to the subthalamic nucleus (Jahanshahi et al., 2015). Cortico-striatal projections from habitual pathways synapse on GABAergic medium sized spiny neurons (MSSN) in the putamen. MSSNs also receive input from GABAergic interneurons, large aspiny cholinergic interneurons, and dopaminergic neurons from the substantia nigra pars compacta (**#4**). Striatal output pathways include a direct pathway that transmits striatal information monosynaptically to the globus pallidus interna (GPi, **#5**) and an indirect pathway that conveys information to the GPi via a disynaptic relay from the globus pallidus externa (GPe, #6) to the subthalamic nucleus (STN). Each pathway has an opposing effect

on GABAergic GPi output neurons; the direct pathway inhibits and the indirect pathway stimulates. Subsequently, these pathways have a reverse effect on excitatory glutamatergic projections from thalamic neurons to the striatum and frontal cortex and, in turn, the facilitation of motor activity. Activation of the direct pathway facilitates motor activity, whereas activation of the indirect pathway reduces motor activity. Substantia nigra pars compacta (SNpc); globus pallidus externa (GPe); subthalamic nucleus (STN); globus pallidus interna (GPi); sub-stantia nigra pars reticulata (SNpr).

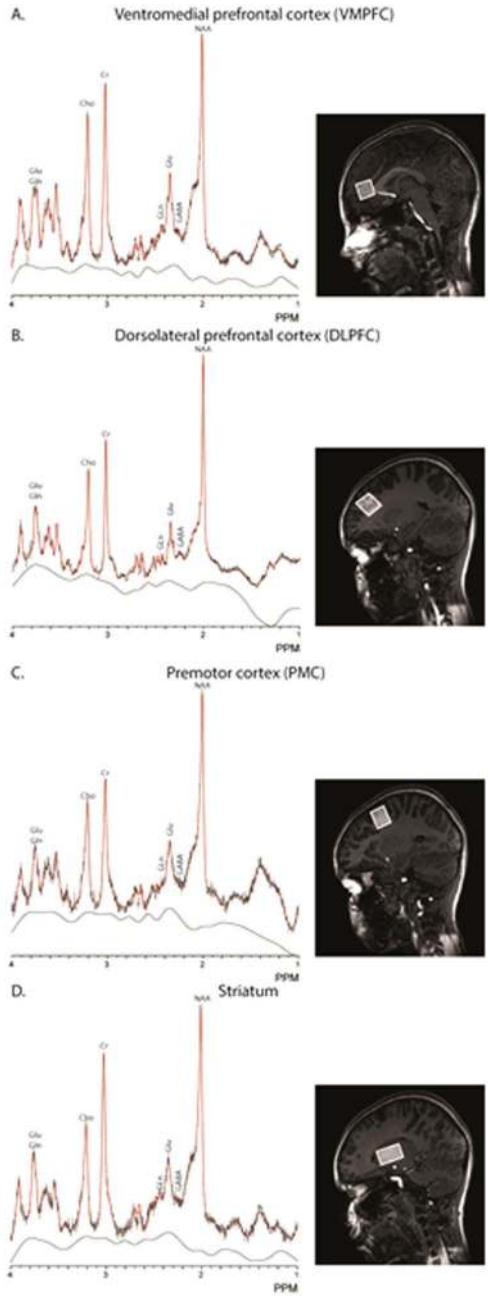

Fig. 2.

Example spectra and LCModel fit from each voxel, A) Ventromedial Prefrontal Cortex (VMPFC); B) Dorsolateral prefontal cortex (DLPFC); C) premotor cortex (PMC), and, D) Striatum.

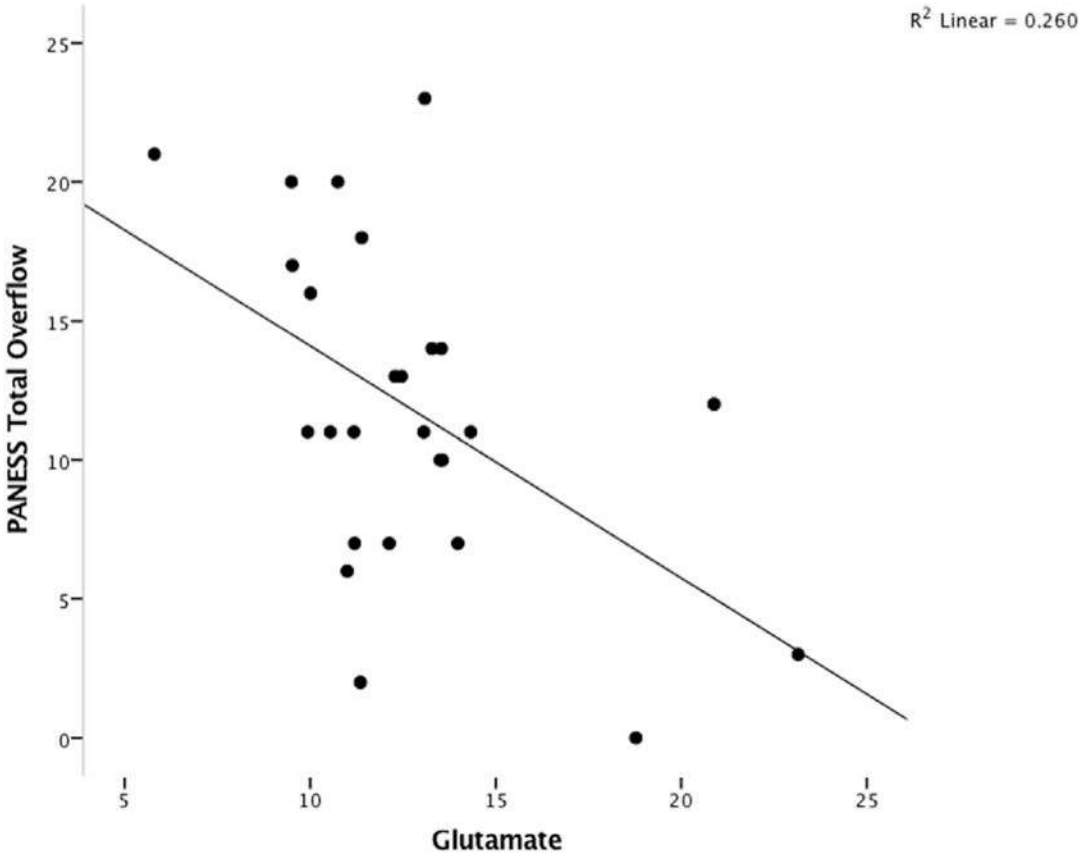

Fig. 3.

Increased glutamate concentration in the left premotor cortex predicts reduced motor overflow total on the Revised Physical and Neurological Assessment of Subtle Signs (PANESS) in children with Tourette syndrome (r=−0.510, p=0.009).

Table 1

MR Spectroscopy Findings in Tourette Syndrome at 3 T and 7 T.

Study	Field Strength	Metabolite	Finding	Region	Age Range
Current study	7 T	GABA	Normal	Striatum, DLPFC, premotor cortex, VMPFC	5–12 years
Draper et al. (2014)	7 T	GABA/NAA	Increased	Supplemental Motor Area (SMA)	Adolescents
Draper et al. (2014)	7 T	GABA/NAA	Normal	Primary Sensory-Motor Region (SM1)	Adolescents
Freed et al. (2016)	7 T	GABA/W	Reduced	ACC	Teenagers
Puts et al. (2015)	3 T	GABA	Reduced	SM1	8–12 years
Tinaz et al. (2014)	3 T	GABA/Cr	Normal	SM1	Adults
Current study	7 T	Glutamate	Increased	PMC	5–12 years
Naaijen et al. (2016)	3 T	Glutamate	Normal	Dorsal striatum (L)	8–12 years
Naaijen et al. (2016)	3 T	Glutamate	Normal	ACC	8–12 years
Kanaan et al. (2017)	3 T	Glx	Reduced	Striatum, Thalamus	Adults
Kanaan et al. (2017)	3 T	Gln/Glu	Normal	Striatum	Adults

Table 2

Behavioral and Neuropsychological Measures.

Test	Type	Measure of	Notes
Hollingshead Index	Parent report	Socioeconomic status	All participants
DICA-IV	Interview	Psychiatric diagnosis	All participants
YGTSS-modified	Parent report	Tic severity	TS group only
Conners' Parent Rating Scale-Revised	Parent rating	ADHD symptoms	T-scores from DSM Inattention and Hyperactivity/ Impulsivity Scales
Full Scale IQ (SB-5)	Performance	Intellectual functioning	All participants
Conflicting Motor Response	Performance	Reactive inhibition	Total correct trials
PANESS - Overflow	Performance	Selective inhibition	Total overflow
Go/No-go Test - Commissions	Performance	Proactive inhibition	Number commission errors

Comments: DICA-IV=Diagnostic Interview for Children and Adolescents-IV; PANESS=Physical and Neurological Assessment of Subtle Signs; SB-5=Stanford Binet Intelligence Test, Fifth Edition. TS=Tourette syndrome; ADHD=Attention-deficit/Hyperactivity Disorder.

Table 3

Participant Demographics and Neuropsychological Test Performance.

	TS (*n* = 32)			Control (*n* = 43)			*p*	η_p^2
	n	**Mean**	**SD**	***n***	**Mean**	**SD**		
Age	32	9.88	1.93	43	8.11	1.93	0.001	0.175
YGTSS Total Tic Severity	28	36.36	18.12				–	–
FSIQ (SB-5)	32	105.56	11.40	40	111.43	10.64	0.028	0.067
Conflicting Motor	18	33.44	9.95	30	41.23	5.08	0.001	0.219
GNG Commission Rate	23	0.39	0.19	28	0.32	0.18	0.153	0.041
PANESS Overflow	27	11.70	5.82	34	7.44	5.60	0.005	0.125
Sex							0.016	
Male	25			22				
Female	7			21				
Handedness							0.717	
Right	28			39				
Left	4			4				
Race							0.456	
Caucasian	28			38				
African-American	2			4				
Other	2			1				

Comments: YGTSS = Modified Yale Global Tic Severity Scale; FSIQ = Full Scale IQ; SB-5=Stanford Binet Intelligence Test, Fifth Edition; GNG = Go/No-go Test; PANESS = Revised Physical and Neurological Assessment of Subtle Signs. Higher scores on GNG and PANESS, and lower scores on Conflicting Motor Exam indicate greater impairment. Significance level for group comparisons of performance data set at 0.05/4 = 0.0125.

Table 4

Regional Means for GABA, Glutamate, Signal-to-Noise Ratio, and Linewidth.

	TS			Control			*p*	η_p^2
	n	**Mean**	**SD**	***n***	**Mean**	**SD**		
GABA *								
VMPFC	27	2.87	1.05	14	2.41	0.37	0.18	0.048
DLPFC	18	2.80	1.35	25	2.49	0.86	0.62	0.006
PMC	30	2.59	1.04	29	2.34	0.65	0.24	0.024
Striatum	30	2.99	0.74	29	2.87	0.60	0.96	0.001
All ROIs	105	2.81	1.02	97	2.54	0.69	0.15	0.010
Glutamate								
VMPFC	29	13.36	3.74	16	11.77	2.99	0.15	0.047
DLPFC	22	19.31	25.78	29	11.94	3.96	0.14	0.045
PMC	31	12.75	3.47	30	10.81	2.69	**0.01**	0.091
Striatum	31	10.81	2.57	33	10.18	2.20	0.30	0.017
All ROIs	113	13.65	11.91	108	11.06	3.05	**0.03**	0.021
Quality Metrics								
LCModel SNR	115	24.73	13.79	134	24.89	9.54	0.92	0.001
Linewidth	115	0.04	0.02	134	0.03	0.02	< **0.01**	0.067
CRLB (GABA)	108	11.37	3.13	120	11.18	3.04	0.65	0.001
CRLB (glutamate)	16	4.29	1.66	134	4.62	1.98	0.12	0.010

Note: GABA and glutamate measurements are tissue corrected for gray matter (GM) and white matter (WM) by dividing metabolite value by the sum of GM+WM in each voxel. GABA measurements require corrected GABA Cramér–Rao lower bound (CRLB) ≤20%; glutamate measurements require corrected glutamate CRLB ≤20%.

* Group comparisons for GABA are controlling for linewidth.

LCModel SNR = signal-to-noise ratio for LCModel (defined as the ratio of the NAA signal to 2× the root-mean-squared residuals). Linewidth = Full width half maximum (FWHM) based on the NAA signal. Significance level for group comparisons of individual ROIs set at 0.05/4 = 0.0125.