Structural and neurochemical correlates of Tourette’s disorder and attention-deficit hyperactivity disorder
Forde, Natalie

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Chapter 4

Cortico-Striatal-Thalamo-Cortical White Matter Microstructure in TS and/or ADHD

Submitted as:
Abstract

Background: Dysregulation within cortico-striatal-thalamo-cortical (CSTC) networks is hypothesised to underlie Tourette syndrome (TS) and attention-deficit/hyperactivity disorder (ADHD). However, evidence from neuroimaging studies implicating the white matter of the CSTC networks has been inconsistent. Here we aimed to determine the common and unique CSTC white matter correlates of TS, ADHD-severity and their interaction.

Methods: We analysed 82 magnetic resonance imaging (MRI) data sets of children with TS (n=22, age M=10.7 [SD=1.2] of whom n=8 also had ADHD), ADHD (n=19, age M=11.1 [SD 1.3]) and of healthy controls (n=41, age M=11.0 [SD=1.1]). The MRI data included diffusion-weighted (DW, B§=2, diffusion gradients=64 [b=1500s/mm²]) and T1-weighted (MPRAGE) datasets that survived quality assessment. DW images were corrected for motion and cardiac artefacts. The constrained spherical deconvolution algorithm was applied followed by deterministic tractography in Dipy. Tractography seeds (striatum and thalamus) and targets (frontal cortex regions) were derived from subject specific Freesurfer reconstructions. Fractional anisotropy (FA) and mean diffusivity (MD) were extracted from each isolated tract and analysed in relation to TS (categorical), ADHD severity (continuous across all subjects) and their interaction for each connection of interest, with age and sex as covariates.

Results: Analyses revealed no association between CSTC white matter metrics and TS, ADHD severity or their interaction (p>0.05 in all cases following multiple comparison correction).

Conclusion: In conclusion our findings do not show large CSTC white matter involvement in TS, ADHD-severity or their interaction in children. Subtle abnormalities may be present and detectable with larger samples.
Introduction

Dysregulation within cortico-striatal-thalamo-cortical (CSTC) networks is hypothesised to underlie both Tourette syndrome (TS) and attention-deficit/hyperactivity Disorder (ADHD; Leisman and Melillo 2013). Individual CSTC loops are thought to be primarily, although not exclusively, dysfunctional in TS and ADHD with aberrant integrative interplay between the loops accounting for the high rate of comorbidity between the disorders (Mink 2006). TS is characterised by the presence of motor and vocal tics and approximately 40% of those with TS also present with ADHD (Robertson 2000; Roessner et al. 2007; Rickards 2011), showing age-inappropriate symptoms of inattention, hyperactivity and/or impulsivity (American Psychiatric Association 2013). Both TS and ADHD are common childhood onset disorders affecting approximately 1% (Robertson 2003; Robertson 2008) and 5% (Polanczyk et al. 2007) of school aged children, respectively.

Several CSTC loops, originally hypothesised by Alexander and colleagues (Alexander et al. 1986), have been described and were initially thought to be functionally and anatomically segregated. However, more recent research suggests that CSTC loops are highly integrated (Haber and Knutson 2009; Milad and Rauch 2012). Dysfunction of individual loops is supposedly responsible for specific symptoms such as tics, obsessions, compulsions, impulsions, hyperactivity and irregular reward processing (Mink 2006; Makki et al. 2009; Langen et al. 2011a; Lapidus et al. 2014). Studies have suggested that the sensorimotor, associative and limbic loops may be primarily associated with tics, ADHD symptoms and obsessive/compulsive behaviours, respectively (Langen et al. 2011a; Langen et al. 2011b). Interaction between these loops, rather than (or as well as) the anatomical proximity of affected areas in the basal ganglia, may account for the high level of comorbidity between disorders like TS and ADHD.

The white matter between distant structures constitutes the biological substrate through which regions connect. The organisation of the white matter may influence the functional connectivity within and between the loops. A useful method for the non-invasive in vivo analysis of white matter microstructure is diffusion-weighted magnetic resonance imaging (DWI). This technique is sensitive to the movement of water molecules which varies according to tissue type. A model of the major white matter tracts in the brain can be constructed from DWI data (tractography) as water molecules diffuse more easily along white matter fibres than across them. Metrics derived from DWI data, such as fractional anisotropy (FA) and mean diffusivity (MD), are proposed to relate to the microstructure of the white matter fibres. These metrics may relate to numerous biological variables such as the degree of myelination of fibres, their coherence or packing density (Beaulieu 2002).

Evidence implicating the structure of white matter in the CSTC networks in the respective disorders has been inconsistent. TS in adults has been associated with both increased (Worbe et al. 2014) and decreased (Cheng et al. 2014) CSTC structural connectivity, while in children with TS one study reported decreases (Makki et al. 2009). Structural connectivity has been inferred from various metrics and methods of analysing DWI data and in itself is an ambiguous term which should
be used with caution (Jones et al. 2013). Structural abnormalities are indexed by FA, MD and apparent diffusion coefficient (ADC) amongst others. Increased MD (adult study; Draganski et al. 2010) and ADC (pediatric and adolescent study: Govindan et al. 2010) in orbitofrontal regions have been reported while other DWI studies found no evidence for CSTC involvement in TS in either child or adult populations (Thomalla et al. 2009; Neuner et al. 2010; Jackson et al. 2011). ADHD too has been associated with alterations in the white matter tracts of the CSTC networks although again not always consistently, (for reviews see; Konrad and Eickhoff 2010; Cubillo et al. 2012; van Ewijk et al. 2012). More recently, a large familial study of ADHD from van Ewijk and colleagues (2014) reported widespread reduced FA in ADHD compared to controls. However, paradoxically within the ADHD group FA increased with increasing symptom count in various white matter tracts including sections of the CSTC network (partially overlapping with categorical findings of reduced FA in ADHD).

The heterogeneity of previous findings is likely related to factors such as small sample sizes, the presence of comorbidities and potentially different neurobiological presentation of the disorders in child and adult patients. Finally, various methodological approaches have been employed. The differences relate to two main choices (1) the type of algorithm used to model the diffusion of water within voxels; tensor (diffusion tensor imaging [DTI]) or non-tensor based and (2) whether analyses are conducted in a voxel-wise (e.g. voxel-based analysis [VBA] and tract-based spatial statistics [TBSS]) or tract-wise manner, where metrics are extracted from a reconstructed tract (tractography). Unfortunately, no gold standard has been established as the field is still progressing rapidly and each method has its own merits and limitations as has been widely noted (Jones 2010; Jones and Cercignani 2010; Jones et al. 2013; Maier-Hein et al. 2016). However, the superiority of non-tensor based methods over DTI and the advantages of tract-based measures over voxel-based are clear (Cercignani 2010; Tournier et al. 2011; Auriat et al. 2015).

In the current study we attempted to address the limitations faced by previous studies by investigating a relatively large sample of children (n=82, 8-12 years) with TS and/or ADHD and healthy children to determine what white matter structural variation within the CSTC networks relates to the respective disorders or their co-occurrence. Furthermore, we applied an advanced pre-processing technique to enhance image correction for motion artefacts (PATCH; Zwiers 2010), a tractography algorithm (Constrained Spherical Deconvolution [CSD]; Tournier et al. 2008) that allows the tracking of fibres in areas of crossing fibres and extracted density weighted FA from a priori selected CSTC tracts. We hypothesised that CSTC white matter structure would vary with ADHD-symptom severity (across all participants) and differ between those with and without TS. Moreover, we expected that these findings would be tract specific. The limited age range was meant to reduce the confound of developmental changes and focus on an age where both TS and ADHD are commonly seen (Bloch and Leckman 2009; Hirschtritt et al. 2015). We modelled ADHD severity across all participants as ADHD symptoms are commonly seen in children with TS and can also be found to an extent within typically developing children.
Methodology

Participants

Patients with TS and/or ADHD were recruited via child and adolescent psychiatry/neurology departments and patient associations throughout the Netherlands. Control participants were recruited via schools. Investigations of fronto-striatal glutamate concentrations (Naaijen et al. 2017) and basal ganglia volume and shape analyses (Forde et al. 2016) of this cohort have previously been reported.

Eighty-two participants with good quality magnetic resonance imaging (MRI; see Pre-processing section) and full phenotypic data were included in this study. Written informed consent was provided by parents or guardians of all participants. Additionally, participants of 12 years of age gave written assent. The study was approved by the regional ethics board (CMO Regio Arnhem Nijmegen).

Inclusion criteria for all participants included being aged 8-12 years on day of testing, IQ>70, Caucasian decent, no previous head injuries or neurological disorders, no contra indications for MRI assessment and no major physical illness. Participants of the TS group met DSM-5 criteria for Tourette’s Disorder or Persistent Motor or Vocal Tic Disorder (Motor type), assessed with the Yale Global Tic Severity Scale (YGTSS; Leckman et al. 1989). Psychiatric comorbidities (e.g. ADHD and obsessive-compulsive disorder [OCD]) were not excluded. Participants of the ADHD group were required to have a diagnosis of ADHD or subthreshold ADHD (4 or 5 symptoms of either inattention or hyperactivity-impulsivity), assessed with the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS; Kaufman et al., 1997). Participants with a sub-threshold diagnosis were included for two reasons; first, to match the high level of subthreshold ADHD in the TS group and second, to allow the use of continuous measures for ADHD severity across all participants within the study. Those with tics (who did not meet inclusion criteria for the TS group) and/or OCD were excluded from the ADHD group. Healthy controls had no mental disorders, according to the Child Behaviour Checklist (CBCL) and Teacher Report Form (TRF; Bordin et al. 2013), in addition to meeting common inclusion criteria.

Phenotypic information

Tic severity was rated by diagnostic interview with both parent(s) and child present using the YGTSS. The K-SADS screening semi-structured interview was administered to a parent of each participant, followed if needed by appropriate modules, to determine the presence of ADHD and/or other psychiatric disorders. The Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS; Scahill et al. 1997) was administered in participants of the TS group to determine OCD comorbidity.

Phenotypic traits were assessed using questionnaires. The Conners’ Parent Rating Scale - Revised Long version (CPRS-RL; Conners et al. 1997) and Children’s Social Behavioural Questionnaire (CSBQ; Luteijn et al. 2000) were used to rate ADHD severity and assess symptoms of autism, respectively. Compulsive behaviours were
rated with the compulsivity subscale of the Repetitive Behaviour Scale - revised (RBS-R; Lam and Aman 2007). Full-scale IQ was estimated by four subtests of the Wechsler Intelligence Scale for Children-III (WISC-III; Wechsler, 2002): Vocabulary, Similarities, Block design and Picture completion. Medication history was collected from parental report of current and previous treatment, this method of data collection has previously been shown to correspond well with pharmacy records (Kuriyan et al. 2014).

MR image acquisition

MRI datasets were acquired on a 3T Siemens Prisma scanner (Siemens, Erlangen, Germany) at the Donders Institute for Brain, Cognition and Behaviour, Nijmegen, the Netherlands. Before scanning participants were first familiarised with the MRI procedure and instrument sounds in a practice scanner where the importance of lying still was explained. During scanning participants had their heads stabilised with cushions and had a piece of tape across their foreheads to help awareness of possible movement while scanning.

Diffusion-weighted datasets were acquired with a pulse gradient spin echo EPI sequence. Two non-diffusion weighted reference images ($B_0$) and 64 images with a diffusion gradient ($b = 1500 \text{s/mm}^2$) were acquired at each of 72 transversal slices with the following parameters: TE = 103 ms, TR = 12,000 ms, slice thickness = 2 mm, in-plane resolution = 2 x 2 mm and acquisition time = 13.48 minutes.

T1-weighted anatomical images were acquired with a sagittal, 3D magnetization prepared rapid gradient echo (MPRAGE) parallel imaging sequence with the following parameters: TE = 2.98 ms, TI = 900 ms, TR = 2300 ms, slice thickness = 1.2 mm, flip angle = 9 degrees, in-plane resolution of 1 x 1 mm and acquisition time = 5.30 minutes.

Pre-processing

Diffusion data were de-noised with a local principal component analysis (LPCA) noise filter as well as affine transformed to correct for motion and eddy current distortions in SPM8 (London, UK). Susceptibility distortions were non-linearly corrected along the phase encode direction to optimally match the T1-weighted image (Visser et al. 2010). Finally, diffusion tensors were robustly estimated using the PATCH algorithm (Zwiers 2010) to eliminate artefacts in the data from cardiac and head motion.

T1-weighted data were processed using the ‘recon-all’ FreeSurfer v5.3 pipeline to generate reconstructions of the pial and white/grey matter surfaces as well as subcortical segmentations (Fischl et al. 1999b; Dale et al. 1999; Fischl et al. 1999a; Fischl and Dale 2000).

Image quality and metrics of image quality were inspected at each stage of pre-processing and reconstruction. 4D DW images were also looped and viewed in each
Seeds, targets and stop mask

Frontal cortical labels (caudalmiddlefrontal, lateralorbitofrontal, precentral, rostralmiddlefrontal, superiorfrontal, frontalpole, medialorbitofrontal, paracentral, parsopercularis, parsorbitalis and parstriangularis) from the Desikan-Killiany Atlas (Desikan et al. 2006) were converted to volumes in FreeSurfer (mri_label2vol), while the FreeSurfer subcortical segmentations were converted to nifti format with mri_convert before individual nuclei (left and right; thalamus, caudate and putamen) were isolated and binarised with fslmaths. Caudate and putamen structures were combined to produce left and right striatal seeds. Seeds and targets were transformed from FreeSurfer space to diffusion space by applying the sum of the transformation matrices from FreeSurfer to native T1 space and T1 to diffusion space, respectively generated by linear registration of the FreeSurfer T1 image to the T1 native space image and the T1 native space image to the average B₀ diffusion image with FMRIB’s Linear Image Registration Tool (FLIRT; Jenkinson and Smith 2001; Jenkinson et al. 2002). Next, targets and seeds were binarised to create masks for tractography.

A stop mask was defined on the MNI152 T1 2mm brain image provided with the FSL package. Regions of interest (ROIs) consisting of an axial plane inferior to the thalamus, the mid sagittal slice and a coronal plane posterior to the thalamus were combined to produce a stop mask. This mask was similarly transformed to diffusion space for each individual by applying the transform acquired from registering the MNI image to each individual subjects average B₀ image. Targets were also eroded slightly using the fslmaths erode function and all combined together with the stop mask (consisting of one axial, sagittal and coronal slice) to generate one master stop mask that would restrict tractography to frontal intra-hemispheric tracks only and not allow tracking to continue through targets.

Tractography of CSTC tracts

The CSD algorithm was applied to the data within the Dipy program (Garyfallidis et al. 2014). This is a non-tensor based method for robustly determining the fibre orientations within a voxel. A liberal (FA>0.15) white matter mask was used to reduce computing time and limit reconstruction of the ODF to within white matter regions. A deterministic tracking approach was undertaken (DeterministicMaximumDirectionGetter and LocalTracking) which incorporated the stopping mask, described above (8 tracks were propagated per seed voxel, step size = 0.5, stop threshold FA=0.15). Tracking was seeded separately from the striatum and thalamus in each hemisphere. Generated streamlines for each seed were then filtered using select frontal lobe targets (caudalmiddlefrontal, lateralorbitofrontal, precentral, rostralmiddlefrontal and superiorfrontal) from the same hemisphere. Individual tracts were then explored in TrackVis where the manual removal of
spurious streamlines was conducted if necessary before density weighted mean FA and MD for the tract bundle were extracted. This mean is the average of values from the points along the streamlines in a bundle. If less than 20 streamlines were reconstructed for a tract then these metrics were excluded from analysis.

Statistics

Statistical analyses were conducted in the R statistics program (R Core Team 2013). Group (TS vs ADHD vs HC) differences in sex and handedness were tested with Pearson’s chi-squared test while differences in age and IQ were assessed with a one-way analysis of variance (ANOVA). If assumptions of homogeneity of variance and normality of distributions were not met ($p>0.05$ in Bartlett’s test of homogeneity of variance and Shapiro-Wilk normality test) a non-parametric Kruskal-Wallis rank sum test was used instead. See Table 1 for group characteristics.

Indices (mean FA and MD) extracted from tracks connecting seeds to targets were analysed separately using linear mixed-effects models (lmer function of lme4 package [Bates et al. 2015]). To determine their association with the metrics TS (yes/no), ADHD severity (CPRS-RL DSM combined T-score) and the interaction were modelled as fixed factors. Age and sex were also included. Hemisphere was treated as a repeated measure and modelled as a fixed factor with a per-participant random intercept. Interactions with hemisphere were included in the initial model with higher order interactions removed in a stepwise manner if they were not significantly adding to the model. Statistical significance of factors was determined using conditional F tests with Kenward-Roger correction of degrees of freedom as implemented with the Anova function of the car package (Fox and Sanford, 2011).

In total there were 20 models (5 targets x 2 seeds x 2 measures). To adjust for multiple comparisons we calculated the number of effective tests ($M_{eff}$ Nyholt 2004) that were run given the relatedness of metrics/tracts and adjusted our alpha level accordingly to 0.003. Later the influence of IQ, autism symptoms (CSBQ core score [sum of subscales 2, 4, 5 and 6]) and compulsive behaviours (RBS-R compulsivity subscale) on the metrics were examined. Finally, within the patient groups similar models, with age, sex, ADHD-severity and hemisphere, were used to investigate the influence of tic severity (TS group only) and medication use (all patients, model additionally included TS) on the metrics.

Results

Demographics

Following exclusion based on MRI image quality, as outlined in the Pre-processing section, 82 participants were included for analysis, see Table 1 for demographic details. Both patient groups has lower IQ compared to the control group ($t=3.4$, $p=0.04$). There was also a sex imbalance across groups where girls were under-represented in the TS group, this is in line with studies that have shown TS to be much more common in boys (Hirschtritt et al. 2015). Of the twenty-two participants
in the TS group n=21 had a diagnosis of Tourette’s Disorder while n=1 had a diagnosis of Persistent Motor or Vocal Tic Disorder (Motor type). Moreover, n=8 met criteria for co-occurring ADHD (6 or more symptoms of either inattention or hyperactivity-impulsivity on the K-SADS) and n=4 for co-occurring OCD (≥16 total score on the CYBOCS). This is in line with the previously reported high levels of comorbidity in TS (Hirschtritt et al. 2015) and makes it a representative sample of participants with TS. Total tic severity ranged from minimal to moderate or marked in participants the week before scanning (7-33 [out of a possible 50] on the YGTSS). Symptoms of inattention and hyperactivity-impulsivity in the ADHD group ranged from mild to severe (CPRS-RL DSM T-scores; inattentive 52-88 and hyperactive-impulsive 44-90).

**Table 1** Demographic details

<table>
<thead>
<tr>
<th></th>
<th>TS</th>
<th>ADHD</th>
<th>Control</th>
<th>Test statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>19</td>
<td>41</td>
<td>0.77</td>
<td>0.47</td>
</tr>
<tr>
<td>Age years, mean (SD)</td>
<td>10.7 (1.2)</td>
<td>11.1 (1.3)</td>
<td>11.0 (1.1)</td>
<td>13.3</td>
<td>0.001**</td>
</tr>
<tr>
<td>Sex, m/f</td>
<td>20/2</td>
<td>7/12</td>
<td>27/14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aIQ, mean (SD)</td>
<td>104 (12)</td>
<td>104 (9)</td>
<td>110 (11)</td>
<td>3.4</td>
<td>0.04*</td>
</tr>
<tr>
<td>Handed, r/l</td>
<td>20/2</td>
<td>19/0</td>
<td>37/4</td>
<td>2.0</td>
<td>0.38</td>
</tr>
<tr>
<td>aADHD severity, mean (SD)</td>
<td>60.9 (11.2)</td>
<td>72.5 (10.9)</td>
<td>45.5 (5.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aTic severity, mean (SD)</td>
<td>22.5 (8.0)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aAge tic onset years, mean (SD)</td>
<td>5.4 (1.9)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aDuration since tic onset years, mean (SD)</td>
<td>5.3 (1.9)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aOCD, n</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antipsychotic</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulant</td>
<td>6</td>
<td>13</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clonidine</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aEstimated from a subtest of the Wechsler Intelligence Scale for Children-III (Wechsler 2002) rating. bT-scores from the DSM combined subscale of the Conners Parent Rating Scale Revised Long version (Conners et al. 1997). cDetermined with the Yale Global Tic Severity Scale (Leckman et al. 1989). dTotal-score ≥16 on the Children’s Yale-Brown Obsessive Compulsive Scale (Scahill et al. 1997). eCurrent medication status, determined from parental report. *p<0.05, **p<0.01. ADHD - Attention-Deficit/Hyperactivity Disorder, OCD - Obsessive Compulsive Disorder, SD - standard deviation, TS - Tourette Syndrome.
Tractography

Analyses of tracts connecting the striatum to the frontal targets included all 82 subjects with the number of observations ranging from 161-164. Analyses of tracts from the thalamus to the caudal middle frontal region included 75 subjects (n=37 HC, n=16 ADHD and n=22 TS) with only 105 observations (n=61 left and n=44 right). The other tracts seeded from the thalamus included all 82 subjects with the number of observations ranging from 154-164. Given the low number of observations and that metrics from the left and right hemisphere were not always available for the same subjects the thalamus to the caudal middle frontal tracts were analysed separately for left and right.

Linear mixed effects model analyses revealed no significant associations between the DWI metrics and TS, ADHD-severity or their interaction in any of the CSTC tracts investigated (all \(p\)-values > 0.01, adjusted alpha level = 0.003, Table 2, Figure 1). IQ (all \(p\)-values > 0.02), autism symptom severity (all \(p\)-values > 0.04) and compulsive behaviours (all \(p\)-values > 0.03) had no influence on the model. Current stimulant and antipsychotic medication use within patients was also not significantly associated with FA or MD (all \(p\)-values > 0.006, adjusted alpha = 0.003) and tic severity did not significantly predict either metric within the TS group (all \(p\)-values > 0.03, adjusted alpha = 0.003).

Analysis of the right thalamus to caudal middle frontal connection indicated lower FA in those with TS (\(F=14.78, p=0.0004\)) and an association with TS-by-ADHD-severity (\(F=16.32, p=0.0003\)). However, this analysis was based on very few participants (n=10 with TS) and the figure (Figure 1) contradictorily suggests a higher median FA in the TS group compared to those without TS. Further investigation suggests the effect is driven by 3 of the 10 individuals with a low FA. Given the small number of participants, the large variance and the higher median FA we strongly caution against over interpretation of this finding before its confirmation in a larger sample. No other significant associations were seen between the DWI metrics and TS, ADHD-severity or their interaction in the left or right connection of the thalamus to the caudal middle frontal region (all \(p\)-values < 0.09).
Table 2 Association of TS, ADHD-severity and their interaction with CSTC white matter metrics

<table>
<thead>
<tr>
<th>Superior Frontal</th>
<th>Rostral Middle Frontal</th>
<th>Precentral</th>
<th>Lateral Orbital Frontal</th>
<th>Caudal Middle Frontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coef</td>
<td>F</td>
<td>Coef</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>p</td>
<td>SE</td>
<td>p</td>
</tr>
<tr>
<td><strong>Striatum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS</td>
<td>-0.047</td>
<td>0.06</td>
<td>-0.018</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>0.023</td>
<td>0.05</td>
<td>0.025</td>
<td>0.08</td>
</tr>
<tr>
<td>ADHD severity</td>
<td>8.8E-6</td>
<td>0.00</td>
<td>-4.5E-7</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>1.7E-4</td>
<td>0.96</td>
<td>1.8E-4</td>
<td>0.99</td>
</tr>
<tr>
<td>TS x ADHD severity</td>
<td>7.7E-4</td>
<td>4.02</td>
<td>3.0E-4</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>3.8E-4</td>
<td>0.05</td>
<td>4.1E-4</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Thalamus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS</td>
<td>-0.069</td>
<td>4.98</td>
<td>-0.064</td>
<td>4.05</td>
</tr>
<tr>
<td></td>
<td>0.031</td>
<td>0.03</td>
<td>0.032</td>
<td>0.05</td>
</tr>
<tr>
<td>ADHD severity</td>
<td>-1.1E-4</td>
<td>0.23</td>
<td>-5.9E-5</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>2.3E-4</td>
<td>0.63</td>
<td>2.3E-4</td>
<td>0.80</td>
</tr>
<tr>
<td>TS x ADHD severity</td>
<td>1.2E-3</td>
<td>5.12</td>
<td>1.0E-3</td>
<td>3.86</td>
</tr>
<tr>
<td></td>
<td>5.1E-4</td>
<td>0.03</td>
<td>5.3E-4</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>MD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS</td>
<td>2.3E-5</td>
<td>0.74</td>
<td>4.4E-5</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
<td>2.7E-5</td>
<td>0.39</td>
<td>3.1E-5</td>
<td>0.15</td>
</tr>
<tr>
<td>ADHD severity</td>
<td>-2.4E-7</td>
<td>1.44</td>
<td>-9.1E-8</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>2.0E-7</td>
<td>0.23</td>
<td>2.2E-7</td>
<td>0.69</td>
</tr>
<tr>
<td>TS x ADHD severity</td>
<td>-2.9E-7</td>
<td>0.42</td>
<td>-6.7E-7</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td>4.4E-7</td>
<td>0.52</td>
<td>5.1E-7</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Results from linear mixed effects models where hemisphere was treated as repeated measure with age and sex as covariates. Adjusted alpha level following correction for multiple tests was 0.003. ADHD - Attention-Deficit/Hyperactivity Disorder, coef - coefficient, FA - fractional anisotropy, MD - mean diffusivity, SE - standard error, TS - Tourette Syndrome. No associations survived correction for multiple comparisons.
Figure 1 For illustrative purposes FA and MD are depicted per group for each tract investigated. The two left most columns represent tracts derived from the striatal seeds while the two right most columns correspond to those from the thalamic seeds. ADHD - Attention-Deficit/Hyperactivity Disorder, FA - fractional anisotropy, HC - healthy control, MD - mean diffusivity, TS - Tourette Syndrome.
Discussion

This study investigated white matter structure of the CSTC networks in children with TS, ADHD or both and healthy controls. FA and MD were used as metrics of white matter structure and were extracted from tracts connecting the thalamus and striatum to various intra-hemispheric frontal cortex targets. Following multiple comparison correction no associations were seen between CSTC network white matter and TS, ADHD-severity or their interaction. Although not significant anymore following multiple comparison correction a subtle pattern in the results appears to indicate lower FA in TS in various CSTC white matter tracts. However, this requires confirmation. In a small subset of participants metrics were available for the tract between the thalamus and caudal middle frontal region, analysis of this tract from the right hemisphere revealed lower FA in TS, however, as mentioned in the Results section this effect appears to be driven by only three individuals with TS while the TS group median is higher than in those without TS. We therefore suspect this is a false positive finding resulting from a combination of a small sample size and large variance. No associations were seen between CSTC white matter and autism symptom severity or compulsive behaviours across all participants or between CSTC white matter and tic severity within the TS group. Our findings suggest that proposed abnormal dysfunction of the CSTC loops in TS and ADHD is unrelated to any large variation in the connecting white matter tracts between the frontal cortex and striatal or thalamic regions.

Few previous studies have focussed on CSTC white matter in TS. Of those that have, two investigated adult patients, one concluding there to be enhanced connectivity between basal ganglia nuclei and the cortex including the supplementary motor area (Worbe et al. 2014) and the other reduced connectivity between basal ganglia nuclei and frontal cortical regions again including the supplementary motor area (Cheng et al. 2014). Finally one study of children with TS (n=18) found reduced connectivity between the caudate nucleus and left anterior dorsolateral frontal cortex but no differences in the white matter connecting the basal ganglia nuclei to any of the other frontal regions (Makki et al. 2009). Results of the current study fit with the mainly negative previous findings of this former study (Makki et al. 2009). The small discrepancy in findings may relate to methodological differences; for instance probabilistic versus deterministic tractography or seed choice – caudate nucleus versus dorsal striatum (caudate nucleus + putamen). Multiple whole brain voxel-based studies corroborate the current findings of no large CSTC white matter involvement in TS (Thomalla et al. 2009; Neuner et al. 2010; Jackson et al. 2011), however, only the last of these included children with TS (n=14, 10-18 years). Another small study in children with TS (n=15, 8-17 years) again found no association of FA with TS but did see increases in ADC in discrete regions of the CSTC circuitry (Govindan et al. 2010). Finally, adult studies have reported no association between FA and TS in CSTC white matter but increased MD in the orbitofrontal cortex (Draganski et al. 2010) and decreased FA and increased ADC in various frontal clusters (Müller-Vahl et al. 2014). Thus the literature is heterogeneous and the various methodological approaches that have been employed make comparisons and drawing conclusions troublesome. However, the current study and previous
studies of children with TS indicate that there are no large track-wise alterations in the CSTC white matter. Alterations reported in adults with TS may occur later as a consequence of the disorder or in response to it, alternatively adult patients may constitute a subset of patients with more severe biological abnormalities and whose tics persist into adulthood.

Most studies of ADHD, in contrast to the current study, have used a case-control design. However, we were interested in the cross disorder impact of ADHD symptoms and thus utilised a dimensional approach as advocated previously (Robbins et al. 2012). Numerous previous studies found reduced FA of CSTC tracts in children with ADHD (Konrad and Eickhoff 2010; Xia et al. 2012; Cubillo et al. 2012; Wu et al. 2014; Gau et al. 2015) although there have also been some reports of increased FA (Silk et al. 2009; Davenport et al. 2010; Li et al. 2010). Despite this there is little evidence linking white matter variation to ADHD symptoms, consistent with the current study. One previous study did find an association between (generalised) FA of the tract connecting the left caudate nucleus to the orbitofrontal region and inattentive symptoms within the ADHD group but this association was not reflected across all participants (Wu et al. 2014). Similarly in a large study of adolescents, FA within the participants with ADHD was positively associated with symptom count (van Ewijk et al. 2014), unfortunately, symptom count data on healthy participants was not analysed. The former of these two studies along with the current study show that severity of ADHD symptoms across disorders and healthy variation are not related to CSTC white matter. Although within those with a diagnosis of ADHD and therefore higher severity there is some, albeit limited, evidence to suggest there is an association between white matter structure and symptom severity or count.

Our study included children with TS and/or ADHD allowing the in-depth study of these highly comorbid conditions together for the first time. Furthermore, the use of continuous measures - for hyperactivity/impulsivity, inattention, autism and compulsive behaviours - across all subjects enabled the modelling of these various cross-disorders symptoms without applying arbitrary thresholds as recommended (Robbins et al. 2012; Cuthbert 2014). However, this study also has some limitations. First, only a small number of girls with TS were included. This reflects the higher population prevalence in boys (Hirschtritt et al. 2015). Analyses were repeated in the male sample only (data not shown) with similar findings but caution is warranted if extrapolating these results to girls with TS. Second, although we find no large CSTC white matter variation associated with TS or ADHD-severity the possibility remains that subtle differences may be present but undetectable given our current sample size. While results were not significant following multiple comparison correction in this study a subtle pattern was present potentially implicating various CSTC white matter tracts in TS which warrants clarification in a larger sample. Third, this study focussed solely on the CSTC loops and while we deem this was appropriate, the CSTC model of TS, ADHD (and OCD) may be an over simplification of complex disorders (Milad and Rauch 2012; Castellanos and Proal 2012). Interaction with multiple other regions (i.e. amygdala and hippocampus) can modulate CSTC loops (Haber and Knutson 2009) as well as other brain regions having been implicated in the disorders (Menzies et al. 2008; Felling and Singer 2011; Greene et al. 2015). Finally,
and as is the case with all studies of DWI data, there are methodological limitations to consider. For instance the use of tensor based metrics (i.e. FA and MD) of the white matter microstructure is problematic as it is severely affected by the presence of crossing fibres in the brain (which is estimated to occur in approximately 90% of voxels (Jeurissen et al. 2013) at the standard resolution that dMRI scans are acquired [~2x2x2 mm]) and partial volume effects. Here although we continue to utilise tensor metrics we address partial volume effects by using a weighted mean of the metrics rather than averaging the value of the voxels that streamlines pass through.

In conclusion our findings do not show large CSTC white matter involvement in TS, ADHD-severity or their interaction in children. Subtle abnormalities may be present and detectable with larger samples. Furthermore, functional connectivity and the relationship between structural and functional connectivity within the CSTC circuitry warrants further investigation.

References


Nyholt DR (2004) A simple correction for multiple testing for single-nucleotide polymorphisms in linkage


R Core Team (2013) R: A language and environment for Statistical computing.


I’m freezing and losing my way
I don’t need another map of your head