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Abstract: Pharmacological and anatomical evidence implicates striatal dopamine receptors in Tourette syndrome (TS). Nevertheless, results of positron emission tomography (PET) studies of the dopamine system in TS have been inconsistent. We investigated striatal D2/3 dopamine receptors in TS using the radioligands [11C]raclopride and [11C]-(+)-PHNO, an agonist that binds preferentially to D3 receptors, thus allowing higher sensitivity and measurement of receptors in a high affinity state. Eleven adults with TS and 11 matched healthy control (HC) participants underwent [11C]raclopride and [11C]-(+)-

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PHNO PET scans. General linear model was used for voxelwise contrasts of striatal binding potentials (BP\textsubscript{ND}) between TS and HC participants. Analysis of variance was performed to investigate main effect of radioligand. In addition, BP\textsubscript{ND} values were extracted for ventral, motor, and associative striatum. Finally, we examined the relationship between BP\textsubscript{ND} measures and symptom severity in TS participants. Main effects analyses showed that \[^{[11C]}\text{raclopride}\] BP\textsubscript{ND} was higher in ventral striatum, whereas \[^{[11C]}\text{methylspiperone}\] did not find differences compared with HC. Moreover, there was no significant correlation between BP\textsubscript{ND} and symptom severity. TS and HC participants had similar striatal D2/3 receptor availability measures. These results challenge the assumption that striatal dopamine receptors have a major role in the pathophysiology of TS. Consistent with previous findings, \[^{[11C]}\text{raclopride}\] localized preferentially to ventral striatum, D3 receptor-rich regions, in contrast to \[^{[11C]}\text{methylspiperone}\], which localized preferentially in the dorsal striatum. 

Key words: basal ganglia; corpus striatum; neostriatum; dopamine; receptors, dopamine; receptors, dopamine D2; positron-emission tomography; raclopride; Tourette syndrome

INTRODUCTION

Tourette syndrome (TS) is a developmental neuropsychiatric disorder defined by motor and vocal tics, with an estimated prevalence of up to 1% (Abi-Jaoude et al., 2009; McNaught and Mink, 2011). Common comorbidities include obsessive-compulsive disorder (OCD) and attention deficit/ hyperactivity disorder (ADHD). There has been much interest in the role of dopamine in TS (Buse et al., 2013; Rickards, 2009; Segura and Strafella, 2013). Over half a century ago, high potency dopamine D2 receptor (D2R) blockers were found to be effective in reducing tics (Abi-Jaoude et al., 2009), and these remain as the agents with the most evidence for efficacy in the pharmacological treatment of tics (Pringsheim et al., 2012). Further, cerebrospinal fluid analysis and human postmortem studies have implicated the dopamine system in TS (Buse et al., 2013). In addition, striatal dopamine is known to play a role in habit formation (Graybiel, 2008), and in animal models of TS (Macri et al., 2013). Finally, dopamine is involved in other movement disorders, such as Parkinson’s Disease and Huntington’s Chorea (Buse et al., 2013). In addition, striatal dopamine innervation, dopamine release, presynaptic, and postsynaptic dopamine receptors, but these have yielded inconsistent results. Striatal dopamine receptors are of particular interest, based on their role in habit formation, as well as evidence implicating the striatum in TS (Ganos et al., 2013).

Four single-photon emission computed tomography (SPECT) studies have investigated striatal D2Rs in TS using the D2R antagonist \[^{[123I]}\text{iodobenzamide}\] (\[^{[123I]}\text{IBZM}\]). In the first of these, investigators found decreased ligand binding in the basal ganglia of the seven medicated but no difference in the eight unmedicated TS subjects in comparison to six controls (George et al., 1994). These findings are consistent with those from another \[^{[123I]}\text{IBZM}\] SPECT study which found reduced striatal ligand binding in the seven medicated compared with the ten unmedicated patients and to the seven healthy controls (HCs), but no difference between the unmedicated patients and the controls (Müller-Vahl et al., 2000). The most recent TS report using \[^{[123I]}\text{IBZM}\] found no difference in ligand uptake between TS patients and controls (Hwang et al., 2008). Finally, an interesting \[^{[123I]}\text{IBZM}\] SPECT investigation in five monozygotic twin pairs with TS found higher binding in the caudate of the more severely affected twin; further, the within pair difference in binding correlated positively with within pair differences in tic severity (Wolf et al., 1996).

Several studies investigating striatal dopamine receptors in TS have used PET imaging, which has higher spatial resolution compared with SPECT. In most of these studies, subjects were medication free when they were scanned. An early small PET study using the D2 and D3 receptor antagonist \[^{[11C]}\text{raclopride}\] in five adult patients with TS found no differences in comparison with HCs (Turjanski et al., 1994). A larger study of 29 subjects focusing specifically on the caudate and using the D2R antagonist \[^{[11C]}\text{methylspiperone}\] did not find differences compared with controls (Wong et al., 1997). A subsequent study with \[^{[11C]}\text{raclopride}\] in seven TS patients again showed no baseline difference in D2/D3 striatal receptor availability (Singer et al., 2002). A more recent study investigating various neurotransmitter measures found no baseline differences in \[^{[11C]}\text{raclopride}\] binding potential (BP) between the 12 TS subjects and 3 HCs with complete data (Wong et al., 2008). Interestingly, using high- and low-specific activity \[^{[11C]}\text{raclopride}\] scans, the investigators estimated D2R affinity to be higher in the anterior putamen in TS subjects relative to the controls. Of note, dopamine receptor “supersensitivity” has been hypothesized to play a role
in TS in a biochemical study over 30 years ago (Singer et al., 1982). Finally, in the most recent PET study of its kind, using the radioligand $[11C]$raclopride, Denys et al. found lower D2/D3 striatal receptor availability in the putamen of 12 TS participants compared with 12 HCs (Denys et al., 2013).

Altogether, the findings regarding striatal dopamine receptors in TS have been inconsistent. Moreover, the literature has been characterized by several limitations, including small sample sizes, confounders such as age differences between comparison groups, medication effects, and low spatial resolution in the case of SPECT studies. Furthermore, while dopamine receptor “supersensitivity” has been hypothesized as an underlying pathophysiological mechanism in TS (Buse et al., 2013; Segura and Strafella, 2013; Singer et al., 1982), only one group has actually investigated this question (Wong et al., 2008).

In this article, we report findings from our investigation of striatal dopamine receptors in TS using two different ligands, the D2/3 receptor antagonist $[11C]$raclopride, as well as $[11C]$-(-)-Propyl-Hexahydro-Naphtho-Oxazin ($[11C]$-(-)-PHNO), an agonist with preferential binding to D3 dopamine receptors. This unique binding profile allows the evaluation of differences in D2 versus D3 receptors, which would not be possible with $[11C]$raclopride alone. Furthermore, because $[11C]$-(-)-PHNO is an agonist, it can permit the measurement of dopamine receptors in their high affinity state, thus providing an opportunity to interrogate whether striatal dopamine receptor affinity is involved in the pathophysiology of TS (Sibley, De Lean, and Creese, 1982; Ginovart et al., 2006; Willeit et al., 2006). To our knowledge, this is the first study using the $[11C]$-(-)-PHNO ligand in TS.

**MATERIALS AND METHODS**

**Participants**

A total of 22 adult subjects, 11 with TS and 11 matched HCs participated in the study. The participants with TS were recruited through the TS Neurodevelopmental Clinic at the Toronto Western Hospital, Toronto, Canada. HC participants were recruited through postings and web advertisements. The groups were matched for age and sex. The group mean age and standard deviation was 34.0 ± 9.7 for the HC, and 32.2 ± 10.1 for the TS subjects.

The subject assessments, neuroimaging scans and data analysis were performed at the PET Center, Research Imaging Center at the Center for Addiction and Mental Health, Toronto, Canada. The study was approved by the relevant institutional review boards. The study was conducted in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and the standards established by the relevant Institutional Review Board and granting agencies. All participants received financial reimbursement. After complete description of the study to the subjects, written informed consent was obtained from all study participants prior to any procedures.

**Clinical Measures**

Subjects underwent a neuropsychiatric assessment by a psychiatrist experienced in TS (EA-J). Diagnoses of TS and other comorbidities including OCD and ADHD were made according to criteria in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision (DSM-IV-TR; American Psychiatric Association, 2000). Tic severity scores were measured with the Yale Global Tic Severity Scale-Total Tic Score (YGTSS-TTS; Leckman et al., 1989), and obsessive-compulsive symptoms were measured using the Yale-Brown Obsessive Compulsive Scale (Y-BOCS; Goodman et al., 1989a,b). A detailed medication history was obtained from patient interview and chart review.

**Image Acquisition**

Each study subject underwent two PET scans on separate days, and one magnetic resonance imaging (MRI) scan. PET scans were performed with a high-resolution PET/CT, Siemens-Biograph HiRez XVI (Siemens Molecular Imaging, Knoxville, TN) operating in three-dimensional (3D) mode with an intrinsic in-plane resolution of ~4.6 mm full width at half-maximum (FWHM). To minimize head motion, subjects were fitted with a custom-made thermoplastic facemask that was secured to the scanner platform (Tru-Scan Imaging, Annapolis). Prior to each emission scan, a scout view was used to verify accurate subject head positioning, and a low dose (0.2 mSv) CT scan was acquired to correct for attenuation.

Radioligands were injected into the left antecubital vein. $[11C]$raclopride (mean ± standard deviation; mean dose 10.0 ± 0.7 mCi; specific activity 1,924 ± 582 mCi/µmol; mass 2.0 ± 0.6 µg) emission data were acquired over 60 min and subsequently redefined into 28 frames of progressively increasing duration (five 1-min frames, 20 2-min frames, and three 5-min frames). $[11C]$-(-)-PHNO (mean dose 9.6 ± 1.4 mCi; specific activity 1,286 ± 388 mCi/µmol; mass 2.0 ± 0.4 µg) emission data were acquired over 90 min and subsequently redefined into 30 frames of progressively increasing duration (fifteen 1-min frames and fifteen 5-min frames). The radiosynthesis of $[11C]$-(-)-PHNO has been described in detail elsewhere (Wilson et al., 2005). For each 3D sinogram, data was normalized for attenuation and scatter corrected before applying fourier rebinning to convert the 3D sinograms into two-dimensional (2D) sinograms. The 2D sinograms were then reconstructed into image space using a 2D filtered back projection algorithm, with a ramp filter at Nyquist cut-off frequency. After reconstruction, a Gaussian filter with a 5 mm FWHM was applied.

MRI scans were done to rule out structural brain abnormalities and to provide anatomical reference for the image
<table>
<thead>
<tr>
<th>Participant number</th>
<th>Age</th>
<th>Sex</th>
<th>Education</th>
<th>Comorbid diagnoses</th>
<th>YGTSS-TTS</th>
<th>Y-BOCS</th>
<th>Medications at time of scan</th>
<th>Past medications (listed in reverse chronological order)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>M</td>
<td>College</td>
<td>None</td>
<td>30</td>
<td>12</td>
<td>Nil</td>
<td>6 months prior to scan, 1 month trial of aripiprazole 4 mg daily; &gt;3 years prior to scan: clonazepam, ziprasidone, tetrabenazine, ropinirole, pergolide, risperidone, carbidopa/levodopa, clonidine, bupropion, donepezil, quetiapine, pimozide, haloperidol, nitrazepam</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>M</td>
<td>University</td>
<td>None, OCS</td>
<td>13</td>
<td>13</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>M</td>
<td>High school</td>
<td>OCS, ADHD</td>
<td>38</td>
<td>12</td>
<td>Nil</td>
<td>10 months prior to scan: ziprasidone, aripiprazole, bupropion, methylphenidate, haloperidol</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>F</td>
<td>University</td>
<td>None</td>
<td>8</td>
<td>0</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>M</td>
<td>University</td>
<td>None</td>
<td>26</td>
<td>6</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>M</td>
<td>University</td>
<td>ADHD, LD</td>
<td>9</td>
<td>11</td>
<td>Nil</td>
<td>Methylphenidate brief trial at age 7</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>M</td>
<td>University</td>
<td>None</td>
<td>31</td>
<td>0</td>
<td>Nil</td>
<td>Clonidine × 1 year, discontinued 3 months prior to scan; Clonidine 0.15 mg daily, started 1 month prior to scan</td>
</tr>
<tr>
<td>8</td>
<td>38</td>
<td>M</td>
<td>College</td>
<td>OCD, ADHD, substance abuse (past)</td>
<td>9</td>
<td>19</td>
<td>Clonidine</td>
<td>Nil</td>
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<tr>
<td>9</td>
<td>30</td>
<td>M</td>
<td>University</td>
<td>None, mild OCS</td>
<td>16</td>
<td>0</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>M</td>
<td>University</td>
<td>OCD, MDD (past), substance abuse (past)</td>
<td>23</td>
<td>19</td>
<td>Nil</td>
<td>Dextroamphetamine/amphetamine 20 mg for 3 months, discontinued 3 months prior to scan; paroxetine &gt;2 years prior to scan</td>
</tr>
<tr>
<td>11</td>
<td>28</td>
<td>F</td>
<td>High school</td>
<td>OCD, ADHD, asthma, subclinical hyperthyroidism</td>
<td>16</td>
<td>26</td>
<td>Albuterol, fluticasone, acetaminophen/codeine</td>
<td>4 months prior to scan: escitalopram 10 mg × 3 weeks; prior to this: sertraline, mirtazapine, melatonin, clonidine, methylphenidate, carbidopa/levodopa, trazodone, zopiclone, domperidone, imipramine, risperidone</td>
</tr>
</tbody>
</table>

M, male; F, female; OCS, obsessive-compulsive symptoms; ADHD, attention deficit/hyperactivity disorder; LD, learning disability; OCD, obsessive-compulsive disorder; MDD, major depressive disorder; YGTSS-TTS, Yale Global Tic Severity Scale-Total Tic Score; Y-BOCS, Yale-Brown Obsessive Compulsive Scale.
analyses. A T1-weighted MRI image was obtained for each subject using a high-resolution MRI (GE Discovery MR750 3T, T1-weighted images, FSPGR with repetition time = 6.7 ms, echo time = 3.0 ms, flip angle = 88°, slice thickness = 1 mm, NEX = 1, matrix size = 256 × 192).

Image Analysis

PET imaging analysis was performed in MATLAB version 7.4 (Mathworks, Natick, MA) using an in-house image analysis platform (Gunn et al., 1997; Lammertsma and Hume, 1996). After frame realignment for motion correction in SPM2 (Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm), motion-corrected PET frames were summed, coregistered to the corresponding MRI and transformed into MNI standardized stereotaxic space (Collins et al., 1994) using the transformation parameters of the individual structural MRIs. Voxelwise nondisplaceable parametric binding potentials (BP_{ND}) were calculated using a simplified reference tissue (cerebellum) method (Gunn et al., 1997). Subsequent to calculation of BP_{ND}, parametric BP_{ND} images were smoothed in SPM8 (Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm) with a Gaussian function at 4 mm FWHM. Statistical parametric analysis was performed in SPM8 to obtain voxelwise general linear model contrasts comparing striatal BP_{ND} between the TS and HC groups for [^{11}C]raclopride and [^{11}C](-)-PHNO. In addition, a 2 × 2 repeated measures analysis of variance (ANOVA), with radioligand ([^{11}C]raclopride and [^{11}C](-)-PHNO) and group (HC and TS) included as factors, was used to perform a main effect of radioligand. Statistical map thresholds were set at P < 0.05, familywise error-corrected, with an extent threshold k = 5 voxels. Furthermore, we conducted region of interest (ROI) analysis using probabilistic ROI masks, created manually as previously described (Martinez et al., 2003; Mawlawi et al., 2001), using a histological-based basal ganglia brain atlas (Chakravarty et al., 2006). BP_{ND} values were extracted for ventral, motor, and associative striatum for each subject with the MarsBaR ROI toolbox (Brett et al., 2002). We then used SPSS (Version 16.0) to compare the extracted BP values between HC and TS for each of the striatal regions, using 2-tailed student t-tests and a P-value threshold of 0.05. Moreover, Pearson correlation coefficients were calculated between [^{11}C]raclopride and [^{11}C](-)-PHNO BP_{ND} on the one hand, and YGTSS-TTS and Y-BOCS severity rating total scores on the other. Mean BP images were visualized using MRicrO (Rorden and Brett, 2000), and BP_{ND} voxelwise contrasts were visualized using the xjView toolbox (http://www.alivelearn.net/xjview).

RESULTS

Demographic and Clinical Characteristics

Table I shows demographic and clinical characteristics of TS study participants. Six of the TS subjects had no comorbidities. Three TS subjects had OCD, four had ADHD, and two had a past history of substance abuse. Of the three patients with comorbid OCD, one was female, two had a past history of substance abuse, and their mean age 31.7 years (±5.5), which was not significantly different from that of the rest of the TS participants, or the HC group. Based on YGTSS-TTS, tic symptoms ranged from mild to severe. With the exception of one TS subject who had started a low dose of clonidine one month prior to participating, none of the subjects were on psychotropic medication when they took part in the study. Three patients had been on medication up to 3 months prior to their participation in the study: dextroamphetamine/amphetamine for 3 months (discontinued 3 months prior to scanning); clonidine for 1 year (discontinued 3 months prior to scanning); SSRI for 3 weeks (discontinued 4 months prior to scanning). For three other patients that had been on psychotropic medication, these had been discontinued at least 6 months prior: antipsychotic for 1 month (discontinued 6 months prior to scanning); antipsychotic (discontinued 10 months prior to scanning); remote brief trial of methylphenidate (16 years prior to the study). Four of the 11 TS participants were medication naive. Further, six of the TS participants were naive to dopaminergic medication, seven if the participant with a brief remote trial of methylphenidate is included. As detailed above, one patient had been on dextroamphetamine/amphetamine up to 3 month prior to scanning, and three had been on dopamine antagonist and/or agonist medication at least 6 months prior to scanning (see Table I for further details). The mean age of participants in the TS group was 32.2 years (standard deviation 10.1), and in the HC group 34.0 years (7.9). There were two females and nine males in each of the TS and HC groups. The HC and TS groups each received similar amounts of radioligand for the [^{11}C]raclopride (mean ± standard deviation; HC: mean dose 10.1 ± 0.5 mCi, specific activity 1,843 ± 480 mCi).

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mCi/μmol, mass 2.0 ± 0.6 μg; TS: mean dose 9.9 ± 0.9 mCi, specific activity 2.004 ± 0.684 mCi/μmol, mass 1.9 ± 0.6 μg; P > 0.5) and for the [11C]-(+)-PHNO (HC: mean dose 9.4 ± 1.5 mCi, specific activity 1.262 ± 0.373 mCi/μmol, mass 2.0 ± 0.4 μg; TS: mean dose 9.7 ± 1.5 mCi, specific activity 1.310 ± 0.419 mCi/μmol, mass 2.0 ± 0.5 μg; P > 0.5) scans.

Striatal Dopamine Receptor Radioligand Binding

Mean striatal BP images are displayed in Figure 1, which shows similar radioligand binding for the TS and HC groups.

**TABLE II.** [11C]raclopride and [11C]-(+)-PHNO BP<sub>ND</sub> for TS and HC subjects, with group difference 95% confidence intervals across striatum subregions

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HC N = 11</td>
<td>TS N = 11</td>
</tr>
<tr>
<td>Motor striatum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>2.35</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>(−0.26, 0.16)</td>
<td>(−0.27, 0.26)</td>
</tr>
<tr>
<td>Right</td>
<td>2.48</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td>(−0.31, 0.19)</td>
<td>(−0.27, 0.26)</td>
</tr>
<tr>
<td>Associative striatum</td>
<td>2.18</td>
<td>2.10</td>
</tr>
<tr>
<td></td>
<td>(−0.17, 0.34)</td>
<td>(−0.16, 0.32)</td>
</tr>
<tr>
<td>Right</td>
<td>2.04</td>
<td>2.04</td>
</tr>
<tr>
<td></td>
<td>(−0.27, 0.26)</td>
<td>(−0.27, 0.26)</td>
</tr>
<tr>
<td>Ventral striatum</td>
<td>2.24</td>
<td>2.16</td>
</tr>
<tr>
<td></td>
<td>(−0.16, 0.32)</td>
<td>(−0.16, 0.32)</td>
</tr>
<tr>
<td>Right</td>
<td>2.31</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>(−0.23, 0.24)</td>
<td>(−0.23, 0.24)</td>
</tr>
</tbody>
</table>

BP<sub>ND</sub>, nondisplaceable binding potential; HC, healthy control; TS, Tourette syndrome.

Statistical parametric maps of voxelwise contrasts comparing BP<sub>ND</sub> in the TS and HC groups showed no significant voxels with either [11C]raclopride or [11C]-(+)-PHNO. Individual participant BP<sub>ND</sub> for each of the ligands, across motor, associative and ventral subregions of the striatum are shown in Figure 2. There were no group differences in BP<sub>ND</sub> in any of the striatal subregions with either radioligand, nor was there a trend in either direction. We also extracted group mean [11C]raclopride and [11C]-(+)-PHNO BP<sub>ND</sub> values separately for right and left striatal subregions, and calculated group difference 95% confidence intervals. There were no notable trends and most confidence intervals were fairly symmetrical with respect to zero and showed good precision (Table II). Secondary analyses comparing the three patients with comorbid OCD to the HC group did not reveal any significant differences in BP<sub>ND</sub> in any of the striatal subregions with either of the radioligands. We also tested the correlations between BP<sub>ND</sub> of striatal subregions and YGTSS-TTS for [11C]raclopride (Fig. 3A) and [11C]-(+)-PHNO BP<sub>ND</sub> (Fig. 3B) in TS participants. Although there was a positive correlation between [11C]raclopride BP<sub>ND</sub> and YGTSS-TTS in the ventral striatum (r = 0.62, P-value = 0.044), this would not survive a correction for multiple comparisons. Otherwise, none of the correlations were significant. Likewise, there were no significant correlations between either radioligand BP<sub>ND</sub> and Y-BOCS severity rating total scores (data not shown). Radioligand main effects analysis from the ANOVA showed that [11C]-(+)-PHNO BP<sub>ND</sub> was higher in ventral striatum, whereas [11C]raclopride BP<sub>ND</sub> was higher in motor and associative striatum (Fig. 4; see also Fig. 2).

**DISCUSSION**

This is the first study using [11C]-(+)-PHNO to investigate D2/3 receptors in TS. Consistent with previous findings, we showed that [11C]-(+)-PHNO BP<sub>ND</sub> was higher in
ventral striatum, and \([^{11}C] \text{raclopride} \) was higher in motor and associative striatum. However, we did not find any differences in \([^{11}C]-(+)-\text{PHNO} \) or \([^{11}C] \text{raclopride} \) between TS and HC groups. Further, there was no relationship between symptom severity scores and BP\(_{\text{ND}}\) for either radioligand. In addition, we found no significant differences in the three patients with comorbid OCD, though this is limited by the small number of participants in this subgroup. Our TS and HC groups were well-matched, and most of our TS participants did not have a history of significant exposure to medications that directly influence dopamine transmission. Moreover, our TS participants are a good representation of the TS population in terms of comorbidities and range of symptom severity. These findings do not support a role for changes in striatal D2 or D3 receptor availability or affinity in the pathophysiology of TS.

A number of possible explanations should be considered in interpreting our findings. Radioligand binding in vivo is influenced by the number of available receptors, endogenous dopamine levels, and receptor affinity. It is thus conceivable that opposing processes—for example, increased dopamine receptor affinity but also increased binding competition from endogenous dopamine—may cancel out each other’s effects on radioligand binding such that overall receptor availability as measured by BP\(_{\text{ND}}\) remains unchanged. As we do not have an estimate of endogenous dopamine levels, we cannot rule out this possibility with our study. Moreover, it is possible that striatal dopamine changes in TS are limited to specific micro areas that are beyond the reach of the spatial resolution of current in vivo imaging. In addition, it is likely that the pathophysiology of TS is variable across individuals, and as such, it is possible that striatal dopamine receptors are involved in only a subset of TS patients. Such a possibility cannot be tested in a reliable fashion with typical sample sizes of most PET studies, including ours. One might wonder whether increasing our sample size could result in significant differences between TS and HC groups; however, our data do not suggest any trend, in either direction. Indeed, our effect sizes were all fairly small; further, our confidence intervals were almost all symmetrical around the null (see Table II), and were fairly narrow, comparable to our standard deviations. Our sample size of 11 HC and
11 TS participants was larger or comparable to that of other studies of striatal dopamine receptors in TS. Importantly, based on our effect sizes, P-values and confidence intervals, there is no trend in our data to suggest that increasing sample size would result in a positive finding. This is most clearly illustrated by the individual participant BF\textsubscript{ND} values plotted in Figure 2.

Although striatal dopamine receptors have been believed to be involved in the pathophysiology of TS (Buse et al., 2013), the literature is far from consistent, and studies reported as positive are often limited by methodological issues. The \[^{123}\text{I}]-\text{IBZM}\ SPECT studies reviewed above found decreased binding only in medicated patients (George et al., 1994; Müller-Vahl et al., 2000). This is likely the result of competitive dopamine receptor binding by antipsychotic medications. In one study, five unmedicated patients with a disease duration of 15 years and greater had lower binding compared with the controls, and there was an inverse relationship between ligand uptake and disease duration in the 10 unmedicated patients (Müller-Vahl et al., 2000); however, this finding did not adequately account for age, which differed between the groups and was also inversely related to ligand uptake. Furthermore, there was no relationship between ligand uptake and symptom severity (Müller-Vahl et al., 2000). The study showing higher \[^{123}\text{I}]-\text{IBZM}\ caudate binding in the more severely affected twin among five twin pairs with TS (Wolf et al., 1996) is interesting but has not been replicated in a larger sample. Initial PET investigations did not identify differences in striatal dopamine receptor availability between TS and HC participants (Singer et al., 2002; Turjanski et al., 1994; Wong et al., 1997). The study by Wong et al. (2008) estimated D2R affinity to be higher in the anterior putamen in 12 TS subjects; however, the control group was comprised of only the three subjects with complete data. Moreover, despite numerous tests in that study, there was no correction for multiple comparisons. Of note, consistent with our findings, there were no group differences in their primary outcome measure of striatal D2/3 receptor BP. The \[^{1}\text{C}]-\text{raclopride}\ study by Denys et al. found lower D2/D3 striatal receptor availability in the putamen of 12 TS participants, most of whom were medication naive (Denys et al., 2013). The discrepant results relative to our findings may be related to one or more of the following factors in that study: the groups were not matched for gender; there were higher depression and anxiety scores in the TS group; there was no information about comorbid ADHD.

Some authors have suggested that disturbances in the dopamine system in TS may be more related to changes in striatal dopamine innervation or dopamine release rather than dysfunction of dopamine receptors (Buse et al., 2013; Segura and Strafella, 2013). In the \[^{1}\text{C}]-\text{raclopride}\ study by Singer et al. (2002), there was no baseline difference in D2/D3 striatal receptor availability, but an amphetamine challenge resulted in increased dopamine release in TS subjects in the putamen relative to controls. However, while the result was statistically significant (P-value = 0.04), there were four tests performed (two regions and two analytical methods) without correction, and the study included only seven TS and five HC participants (Singer et al., 2002). In the study by Wong et al. (2008), there was a robust increase in amphetamine-induced dopamine release in the right ventral striatum in the TS group relative to the HC group. However, this result should be interpreted bearing in mind the numerous uncorrected tests in that study including: 10 striatum subdivisions, 7 ligand measures, 2 analysis methods, and various neuropsychiatric and neuropsychological measures used for correlations (only two of which were reported, neither related to tic symptom severity measures). Moreover, the groups were not matched for gender. Showing the opposite effect, the recent study by Denys et al. found amphetamine-induced striatal dopamine release to be decreased in 12 TS participants relative to HC subjects (though the differences disappeared after the investigators controlled for baseline binding; Denys et al., 2013). Conversely, a study of extrastriatal cortical and subcortical D2/3 receptors using the radiotracer \[^{1}\text{C}\text{FBL} 457\] found differences in amphetamine-induced dopamine release between eight medication naive TS and eight HC participants, with some areas being significantly increased in TS, while the opposite was seen for other areas (Steeves et al., 2010). There has not been another study of extrastriatal cortical and subcortical amphetamine-induced dopamine release in TS. Overall, neuroimaging investigations of dopamine in TS have resulted in a heterogeneous literature.

**CONCLUSION**

We have shown similar striatal D2/D3 dopamine receptor availability in adults with TS compared with HC using the radioligands \[^{1}\text{C}]-\text{[(+)-PHNO]}\ and \[^{1}\text{C}]-\text{raclopride}\. Our results challenge the widely assumed role of striatal dopamine receptors in the pathophysiology of TS. Although dopamine has long been believed to underlie the pathophysiology of TS, decades of investigation have yielded inconsistent results. Interestingly, in a case series of four patients with comorbid TS and Parkinson’s disease, there was improvement in parkinsonism without worsening of tics with treatment with levodopa, and there was a lack of a consistent relationship between “on” and “off” states and tic symptom severity (Kumar and Lang, 1997). More recently, the \(\gamma\)-aminobutyric acid-ergic system has been implicated in TS based on two post-mortem histologic studies (Kalaniithi et al., 2005; Kataoka et al., 2010), a PET study (Lerner et al., 2012), a recent magnetic resonance spectroscopy/magnetoencephalography investigation (Tinzal et al., 2014), and a recent basal ganglia transcriptome analysis (Lennington et al., in press). These suggest new avenues that are worth pursuing further as part of investigations to elucidate the underlying pathophysiology of TS. Nevertheless, it is likely that the causes and neural
mechanisms involved in TS are complex, varied, and may involve interactions among different systems. As such, the field would benefit from concerted efforts and collaborations to carry out multimodal studies with large samples and longitudinal design.

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