Levodopa-stimulated dopamine release in Tourette syndrome

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Abstract

BACKGROUND : Several lines of evidence suggest that dopamine (DA)-influenced neuronal pathways may malfunction in Tourette Syndrome (TS). A dopamine-responsive abnormality of brain function in TS could be either presynaptic or postsynaptic. Some PET studies support the hypothesis of presynaptic abnormalities in levodopa uptake, dopamine synthesis, or dopamine release. Alternatively, presynaptic dopaminergic function could be normal in TS but dopamine-sensitive abnormalities could exist in striatum, pallidum, thalamus, or cortex.
METHODS: In this study we directly tested the presynaptic hypothesis using a new approach. We used positron emission tomography (PET) and [^{11}C]raclopride (RAC*) to measure synaptic dopamine release in response to levodopa and placebo infusions (with carbidopa) in 5 neuroleptic-naïve adults with TS and 5 matched control subjects. The primary analysis examined RAC* binding potential (BP_{ND}) in predefined volumes of interest (VOIs). A secondary analysis compared BP_{ND} voxel by voxel over the entire brain.
RESULTS: (1) Overall, baseline RAC* BP_{ND} did not differ significantly between groups, though nucleus accumbens BP_{ND} was higher in TS (16%, p=0.051). (2) Across regions, DA release declined from before to during infusion (p=0.014), including with placebo. (3) This decline was smaller in TS (p=0.080). (4) Levodopa's effect on BP_{ND} differed significantly in

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right midbrain (p=0.002, corrected), where levodopa displaced RAC* by 59% in control subjects but *increased* BP_{ND} by 74% in TS subjects, and in parahippocampal gyrus (p=0.02, corrected). **DISCUSSION:** Our finding that a before/after RAC* design is confounded by time and/or expectation effects has implications for other RAC* PET studies. The smaller magnitude of the decrease with time in TS may be attributable to impaired habituation to the scan environment. Levodopa's opposite effect on RAC* binding in TS dopaminergic midbrain was not predicted, but may signify an abnormal response to dopaminergic stimulation in TS. These findings invite confirmation in a larger sample.

Introduction

Tourette Syndrome is a chronic neuropsychiatric disorder defined by the presence of both vocal and motor tics that begin early in life, fluctuate in phenomenology over time, and are not caused by another illness (American Psychiatric Association 2000; Black 2010b). Tics are brief movements or noises, repeated many times a day in a highly stereotyped fashion, that may look intentional but that serve no useful purpose (Black 2010b). Several lines of evidence suggest that dopamine-influenced neuronal pathways malfunction in Tourette Syndrome (TS) (Albin 2006; Anderson et al. 1999; Black 2008; Hershey et al. 2004; Singer 2013).

One of the earliest clues to the pathophysiology of tics was their clear response to dopamine D_2-like (D2, D3, or D4) receptor antagonists, now confirmed by over 35 randomized controlled trials (Black 2010a; Singer & Wendlandt 2001). Tics also improve with postsynaptic dopaminergic stimulation (Anca et al. 2004; Black & Mink 2000; Carpenter et al. 1999; Feinberg & Carroll 1979; Friedhoff 1982; Gilbert et al. 2003; Gilbert et al. 2000a; Gilbert et al. 2000b; Nomura & Segawa 1982; Nomura & Segawa 2003), but all these treatment studies confirm that in TS, abnormal activity in movement-related brain circuits is sensitive to dopamine. Nonmotor brain circuits also manifest a dopamine-sensitive abnormality of brain function in TS (Hershey et al. 2004).

However, identifying why this occurs has not been easy (for a superb review, see Singer 2013). A dopamine-responsive abnormality of brain function in TS could be either presynaptic or postsynaptic. Studies of TS *in vivo* have examined dopamine D_2-like receptors (D2Rs), dopamine precursor uptake and monoamine transporters (Albin et al. 2009; Anderson et al. 1999; Peterson 2001; Singer & Wendlandt 2001; Wong et al. 2008). Post-mortem data are limited by the small number of adequately studied subjects (Minzer et al. 2004; Swerdlow & Young 2001; Yoon et al. 2007). Most studies suggest that post-synaptic dopamine D2-like receptor binding is similar in TS and control subjects (Albin et al. 2009; Hwang et al. 2008; Singer et al. 2002; Wong et al. 1997), though there are exceptions (de Vries et al. 2010; de Vries et al. 2009; Gilbert et al. 2006; Minzer et al. 2004; Yoon et al. 2007). Even if dopamine D_2-like receptors (D2Rs) are normal in TS, a postsynaptic abnormality in the response to dopamine stimulation could be located downstream in striatum, pallidum, thalamus, or cortex (Mink 2006).

Alternatively, several PET or SPECT studies support the hypothesis of presynaptic abnormalities, *i.e.* dysfunction in levodopa uptake, dopamine synthesis, or dopamine release (Albin et al. 2003; Butler et al. 2006; Ernst et al. 1999; Heinz et al. 1998; Hwang et al. 2008; Malison et al. 1995; Serra-Mestres et al. 2004; Singer et al. 2002; Wong et al. 1994), though some studies do not (Meyer et al. 1999; Singer 2013; Stamenkovic et al. 2001). One widely

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discussed theory is that basal, tonic dopamine release is normal, but that transient, phasic dopamine release is not (Singer 2013; Singer et al. 2002; Wong et al. 2008; Yeh et al. 2007a). Phasic dopamine release is crucial to dopamine's role in changing behavior (Breitenstein et al. 2006), including learning sequences of movements (Badgaiyan et al. 2007). Remarkably, however, little research has been done on phasic dopamine release in TS. Amphetamine-induced striatal dopamine release has been studied, with some support for differences in TS (Singer et al. 2002; Steeves et al. 2010; Wong et al. 2008; Yeh et al. 2007b). However, amphetamine also has some disadvantages—primarily, that it does not really produce *phasic* dopamine release in the usual sense of the word. Rather, it causes prolonged, substantial dopamine release regardless of environmental demands. Amphetamine also induces euphoria (Drevets et al. 2001) and briefly increases tic severity (de Vries et al. 2010; de Vries et al. 2009), clouding interpretation of the results.

Ideally, if a pharmacological challenge drug is used to test phasic dopamine release, it should not produce effects noticed by the subject. Levodopa, the body's natural synthetic precursor to dopamine, is such a drug. Systemic levodopa administration, given with an adequate dose of carbidopa, which prevents conversion to dopamine but does not cross the blood-brain barrier, essentially delivers dopamine only to the brain. Confirming this, with adequate carbidopa levodopa does not alter quantitative whole-brain blood flow (Hershey et al. 2003; Hershey et al. 2000; Hershey et al. 1998). Furthermore, volunteers usually cannot tell whether they are receiving levodopa or a placebo (Black et al. 2003; Gordon et al. 2007).

The present study tests the presynaptic dopaminergic hypothesis in TS using a novel approach. Specifically, the hypothesis tested was that levodopa would stimulate striatal dopamine production differently in people with TS than in people without tics. The radioligand [^{11}C]raclopride (hereinafter RAC*) binds to the dopamine D_2 receptor loosely enough to be displaced by physiological increases of dopamine at the synapse. We used PET and RAC* to measure synaptic dopamine release in response to a standardized levodopa infusion (with carbidopa) in TS and matched control subjects.

Materials & Methods

Regulatory approvals

This study was approved by the Human Studies Committee of Washington University School of Medicine (IRB, protocol # 03-0347, the WUSM Radioactive Drug Research Committee (protocol # 497F), and the U.S. Food and Drug Administration (Investigator IND #69,745 for i.v. levodopa). All subjects provided written confirmation of informed consent before study participation.

Subjects

Diagnostic assessment included psychiatric and neurological examination by a movement-disorders-trained neuropsychiatrist (KJB) and a validated semistandardized psychiatric diagnostic interview (SCID-IV; First et al. 2002). Tic subjects met DSM-IV-TR criteria for Tourette's disorder. Control subjects with no history of tics were matched one-to-one for age, sex and handedness (except one ambidextrous TS subject was matched with a right-handed control).

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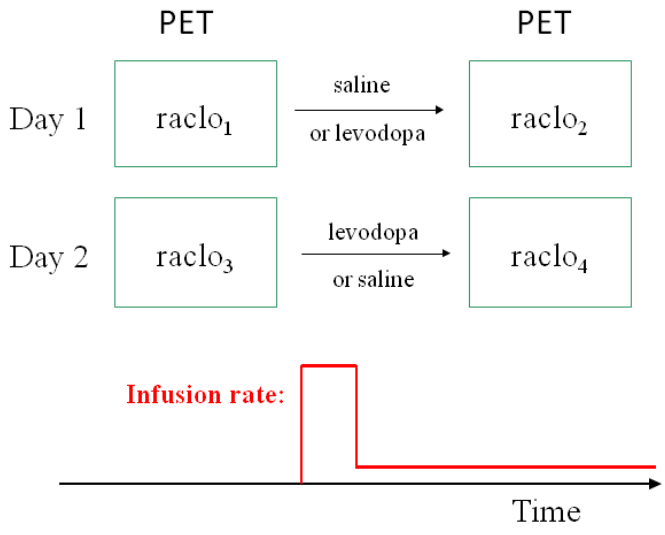
Exclusion criteria included any lifetime neurological or Axis I psychiatric disorder (except TS, ADHD and OCD were allowed in tic subjects, and migraine and specific phobia were allowed in either group), current serious general medical illness, medication history of dopamine antagonists or other drugs likely to affect the dopaminergic system, current use of any neuroactive medication, lactation, possibility of pregnancy, or contraindication to levodopa or MRI.

Clinical features were characterized by the Diagnostic Confidence Index (0=no features of TS; 100=all enumerated features of classic TS; scores in the clinical validation sample ranged from 5 to 100 with mean ± S.D. = 61 ± 20) (Robertson et al. 1999); the YGTSS, an expert-rated measure of tic severity over the previous week (motor tic scale 0-25, vocal tic scale 0-25, impairment scale 0-50, higher scores indicating a higher symptom burden) (Leckman et al. 1989; Walkup et al. 1992); the revised Tic Symptom Self-Report (TSSR) scale, a self-report scale including scores of 0-3 for each of 18 motor tics and 16 vocal tics, with 3 indicating tics were "very frequent and very forceful" over the preceding two weeks (Cohen et al. 1984; Scahill et al. 1999); the ADHD Rating Scale, an expert-rated measure of current severity of Attention-Deficit/ Hyperactivity Disorder (ADHD), based on DSM-IV criteria (range 0-54, higher scores indicating a higher symptom burden) (DuPaul et al. 1998); and the Y-BOCS, an expert-rated measure of current obsessive-compulsive disorder (OCD) severity (range 0-40, higher scores indicating a higher symptom burden) (Goodman et al. 1989a; Goodman et al. 1989b).

Overview of subject participation

Each subject had 4 RAC* PET scans: two scans on each of two days at least a week apart (Figure 1). After oral carbidopa and the baseline PET scan, an infusion of levodopa or saline placebo was begun by vein at an individualized dose intended to produce a steady-state levodopa plasma concentration of 600ng/mL. After allowing 30 minutes to approach steady-state levodopa concentration, a second scan was done while the infusion continued. The order (levodopa on day 1 and placebo on day 2, or the reverse) was assigned randomly to each subject, and subjects and PET staff were blind to drug assignment during all scans.

Figure 1. Study overview.



The room was darkened and subjects were instructed to lie quietly in the scanner with eyes closed throughout each scan. Study staff asked subjects every 5 or 10 minutes if they were comfortable and made sure they were awake.

Levodopa infusion

Subjects took 200mg carbidopa by mouth at least 1 hour before levodopa infusion began. A dose

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of levodopa estimated to fill each subject's volume of distribution at a target concentration of 600ng/mL was infused over 10 minutes, followed until the second PET scan of the day was completed by a maintenance infusion at a rate estimated to compensate for elimination. In prior work, these infusion rates produced a mean blood level across subjects of ~625ng/mL after 25 minutes of infusion (Black et al. 2003). On average, that concentration produces substantial motor benefit in early Parkinson disease (Contin et al. 2001; Harder & Baas 1998). However, this infusion method is well enough tolerated that subjects cannot reliably distinguish the levodopa and saline infusions (Black et al. 2003; Gordon et al. 2007).

Levodopa plasma concentration

Levodopa plasma concentration was measured by a validated method (Karimi et al. 2006).

Image acquisition

RAC* was given i.v. over an interval of 30 seconds. PET images were acquired on a Siemens ECAT 961 camera beginning with arrival of radiotracer in the head and continuing for 60 minutes using image frames of increasing duration.

An MP-RAGE sequence was used to acquire a 3-dimensional T1-weighted image of the brain with acquisition time ~400 sec and voxel dimensions 1.25x1x1mm^3.

Image alignment

The PET images were realigned within each subject and then to the subject's MRI using a rigid-body alignment method with low measured error, optimized for dynamic PET images (Black et al. 2001; Black et al. [submitted]; Eisenstein et al. 2012; Perlmutter et al. 1998).

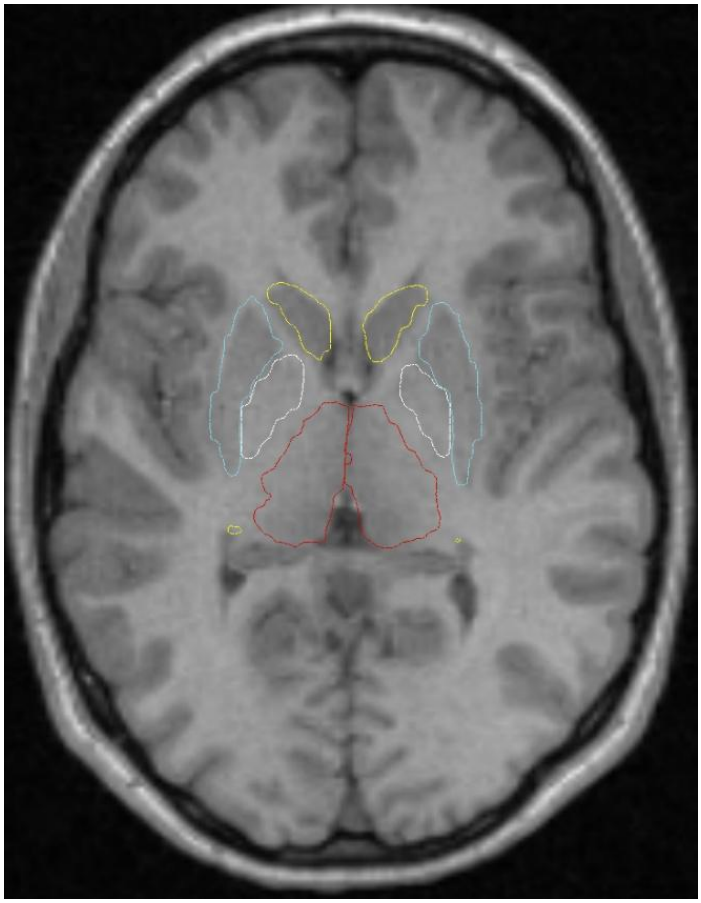

Figure 2. Automated striatal VOIs.

VOI analysis

Nine subcortical volumes of interest (VOIs) were defined for each subject from that subject's MRI by a high-dimensional semi-automated method of known high test-retest reliability (Wang et al. 2007) (Figure 2). These VOIs corresponded to thalamus (Th) and to left and right putamen (Pu), caudate (Cd), nucleus accumbens (NA), and globus pallidus (GP). A tenth VOI was created from the average (weighted by region volume) of 22 FreeSurfer-labeled gray matter regions comprising frontal cortex (11 left- and 11 right-hemisphere VOIs). This large frontal VOI

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produced adequate counting statistics for modest noise in the time-activity curve (Figure 3, lower panel). A cerebellum VOI was traced on each subject's MR image. All VOIs were transferred to each subject's realigned PET images using the optimized MRI-to-PET transformation matrix computed in the alignment step. The cerebellar VOI was trimmed if needed so that no voxel in

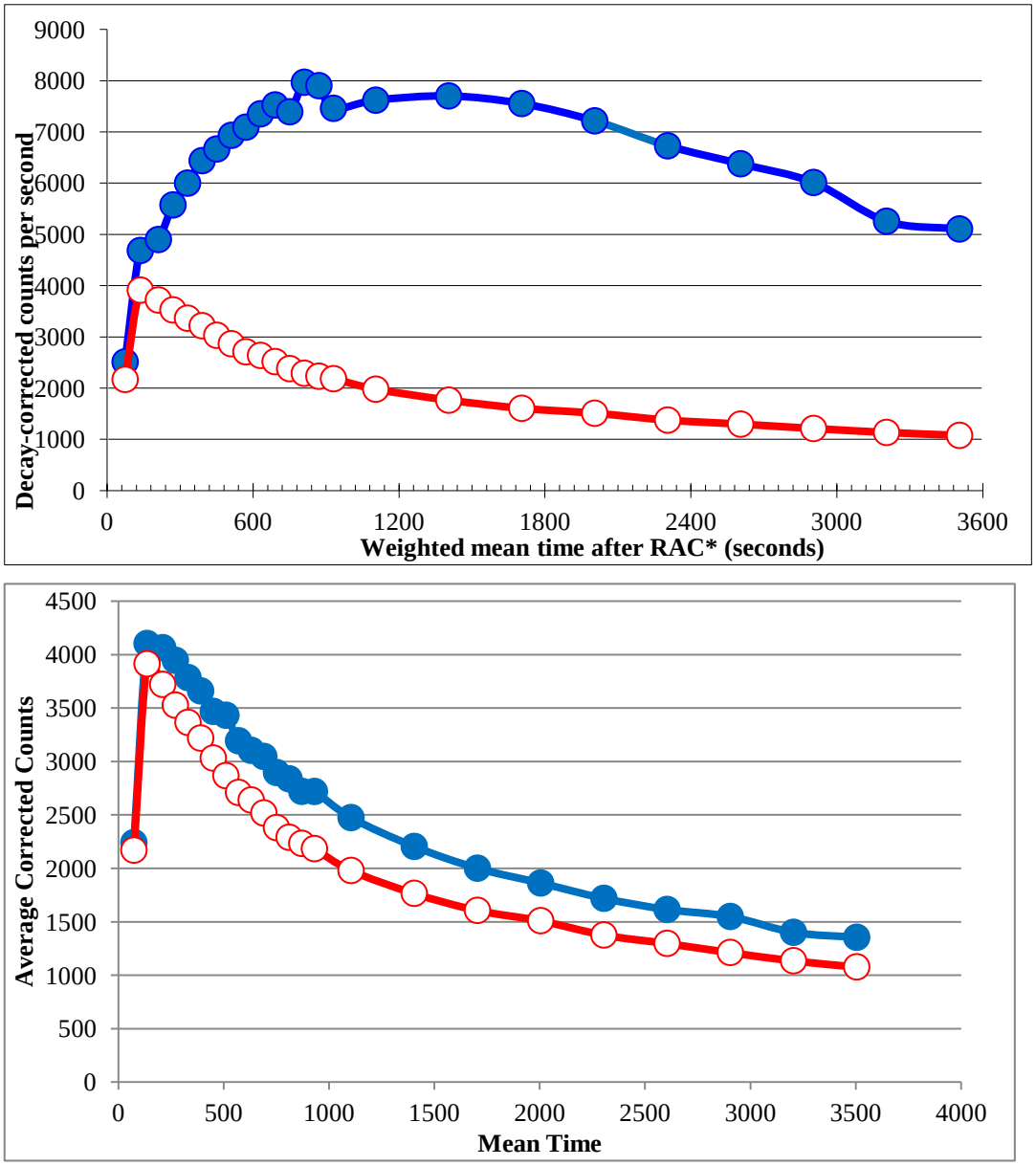

Figure 3. Decay-corrected time-activity curves (filled circles) for the putamen VOI (upper panel) and the frontal lobe VOI (lower panel) from one subject's pre-levodopa PET scan. Hollow circles mark the TAC in the cerebellar reference region.

the VOI corresponded to any of the inferior-most 4 slices in any frame of that subject's original PET images. Thus in each subject the VOI corresponding to a given region was identical for all 4 PET scans.

The binding potential BP_{ND} (Innis et al. 2007; Mintun et al. 1984), an estimate of the quotient B_{max}/K_D, was computed as one less than the distribution volume ratio (DVR), which was derived

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for each of the nine subcortical VOIs and the frontal lobe VOI using the cerebellar reference region (Logan et al. 1996). As we had no *a priori* hypothesis about laterality of results in any of the paired basal ganglia nuclei, we averaged corresponding left and right BP_{ND}s (weighted by VOI volume) to produce for each PET scan 6 final BP_{ND} values, one each for frontal lobe cortex (FL), thalamus (Th), putamen (Pu), caudate (Cd), nucleus accumbens (NA), and globus pallidus (Pl).

The primary statistical analysis used a repeated-measures analysis of variance (rmANOVA) with BP_{ND} as dependent variable, diagnosis (tic or control) as a between-group variable, time (before or during the infusion) and day (placebo or levodopa) as within-subject variables, and region (the 6 VOIs) as a repeated measure. Exploratory analyses used a rmANOVA for each of the 6 VOIs.

Whole-brain analysis

For each subject, a DVR image was computed using at each voxel in the brain the Logan graphical method with the cerebellar VOI described in the preceding section as reference region (Logan et al. 1996). As a methods check, the mean across striatal VOIs of the voxelwise DVR value was essentially identical to the regional DVR computed using the standard methods described above. Analysis was limited to voxels in atlas space at which every subject contributed data from all frames of the dynamic PET acquisition.

Whole-brain comparisons used voxelwise *t* tests corrected by FDR for multiple comparisons in SPM 8, as follows. A *t* test compared DVR images between the TS and the control group, and clusters of contiguous voxels with *t* exceeding the threshold corresponding to $p<0.001$ were accepted as significantly different between groups if cluster volume exceeded the threshold required to control False Discovery Rate for the entire dataset at $p<0.05$.

Two comparisons were made, one based on mean baseline DVR images and the other based on levodopa effect ΔDVR images. Each subject's two pre-infusion RAC* PET scans, one from each scan day, were averaged to create that subject's mean baseline DVR image. The difference of the during-levodopa DVR image and the during-placebo DVR image in a subject was used to create that subject's levodopa effect ΔDVR image.

Results

Subjects

Subject characteristics and adequacy of matching are reported in Table 1, and clinical characteristics of the Tourette syndrome group are reported in Table 2.

Table 1.

Measure	Tic Subjects (N=5)	Controls (N=5)
Age (years; mean ± S.D.)	33.8 ± 12.9	32.8 ± 11.1
Sex, male (N)	4	4
Race, Caucasian (N)	4	4

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Table 1.		
Measure	**Tic Subjects (N=5)**	**Controls (N=5)**
Handedness, right (N)	4	3
OCD dx (N)	1	0
ADHD dx (N)	2	0

Table 2.		
Scale		**Scores (mean ± S.D.)**
DCI score		36.8 ± 22.0
YGTSS	Motor tic score	10.6 ± 3.4
	Vocal tic score	7.8 ± 4.0
	Impairment score	9.4 ± 9.8
TSSR score	Motor	9.3 ± 5.9
	Vocal	3.2 ± 2.3
	Total	12.5 ± 7.9
ADHD Rating Scale		11.6 ± 10.7

*Abbreviations: DCI=Tourette Syndrome Diagnostic Confidence Index, YGTSS=Yale Global Tic Severity Scale, Y-BOCS=Yale-Brown Obsessive Compulsive Scale, ADHD=Attention Deficit Hyperactivity Disorder, TSSR=Tic Symptom Self Report
**The Y-BOCS was completed for only 1 tic subject; the score was 9 on day 1 and 14 on day 2.

Levodopa levels

Levodopa plasma concentrations were ~800-1000ng/ml before the RAC* scan and ~500-700ng/ml after the RAC* scan. in ng/mL, and did not differ significantly between groups (Table 3 or Figure 4).

Table 3. Levodopa plasma concentrations, ng/ml, mean ± SD			
Time	**Controls**	**Tic subjects**	***p* (*t* test)**
Peak (10' into infusion)	1591.5 ± 232.5	1938.8 ± 726.3	0.36
Just before RAC* scan	788.0 ± 152.4	992.4 ± 322.9	0.26
Just after RAC* scan	529.5 ± 149.2	662.8 ± 136.1	0.21

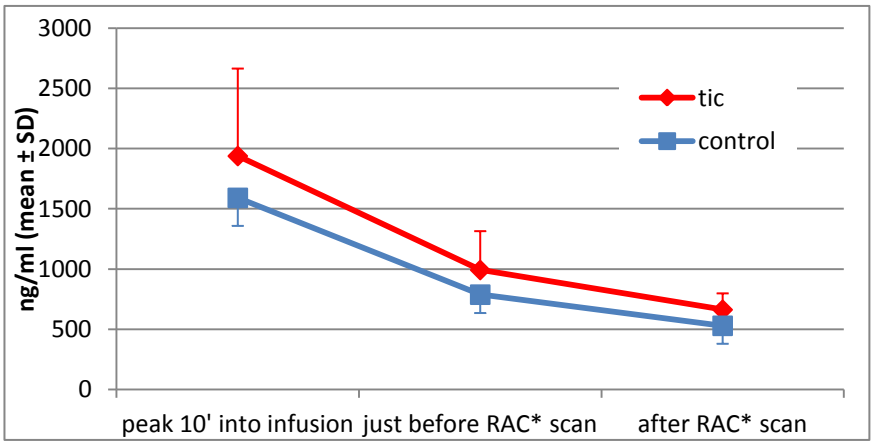

Figure 4. Levodopa plasma concentrations.

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Stability of RAC* binding between days and with time

This study includes a before- and after-infusion scan on each of two days. On one day the infusion contains levodopa, and on the other day the solution is a saline placebo. Thus each subject has three non-levodopa scans (the first scan of each day plus the scan during the placebo infusion). As expected, BP_{ND} was similar in the two pre-levodopa scans (correlated at $r = 0.99$ across VOI and subject).

BP_{ND} changed between the 1st and 2nd scan of the day (main effect of time, F=10.605, df=1,8, p=0.012), but to our surprise this change did not differ significantly between the levodopa and placebo days (time x day interaction, F=0.014, df=5,4, p=0.909). In other words, the two scans on the placebo day were *not* identical. Mean BP_{ND} was 2.7% to 24.0% higher during the *placebo* infusion, indicating decreased dopamine release compared to earlier on the same day. The change from the first to the second scan of each day was significant in most individual region analyses: main effect of time, thalamus p=0.002, frontal lobe p=0.032, caudate p=0.039, pallidum p=0.048, and nucleus accumbens p=0.052 (Figure 5; multivariate time x region interaction F=4.173, df=5,4, p=0.096).

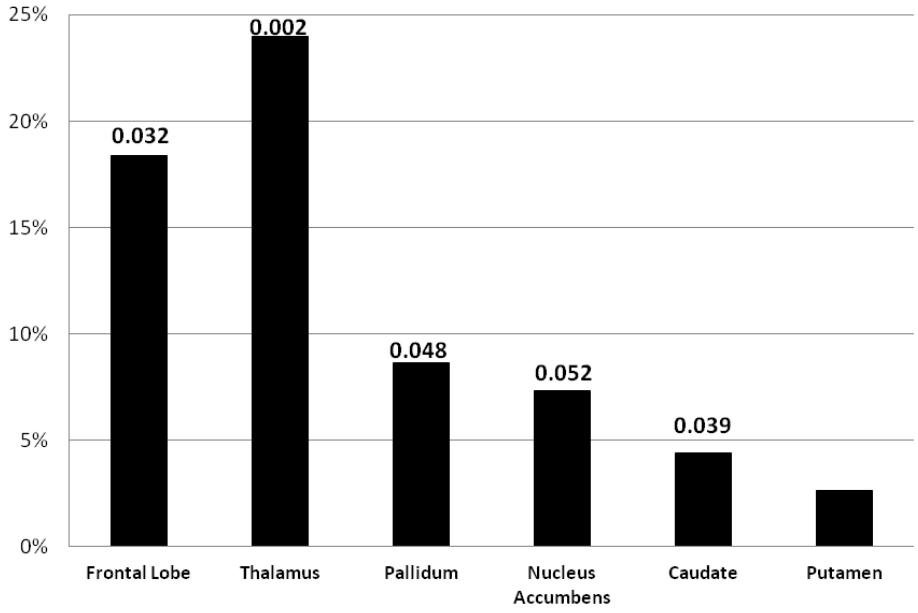

Figure 5. Change in BP_{ND} with *placebo* infusion.

There was a trend for the change in BP_{ND} during the infusion to be smaller in tic subjects (time x diagnosis interaction F=4.211, df=1,8, p=0.074; in individual regions, $0.05 < p < 0.10$ for NA, Pu, and Cd VOIs). The change in BP_{ND} on the placebo day is shown in Figure 6).

Baseline RAC* binding

Across VOIs, RAC* binding did not differ significantly between tic and control subjects (multivariate main effect

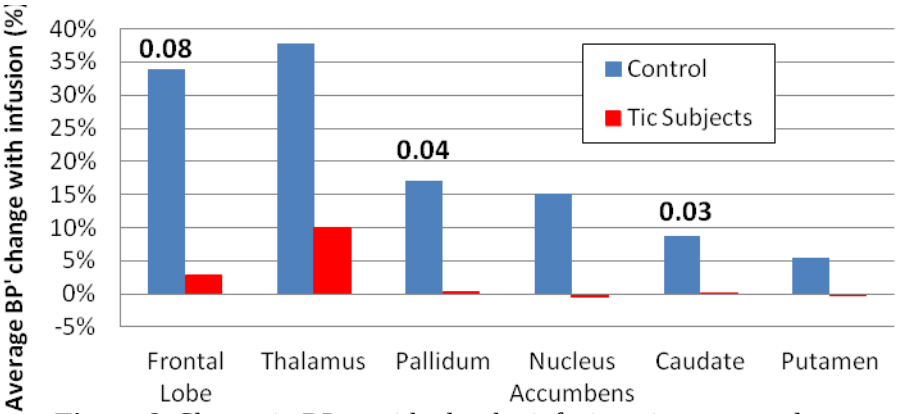

Figure 6. Change in BP_{ND} with placebo infusion: tic vs. control (*p* values for difference between groups, from *t* tests for each region).

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of diagnosis, F=0.744, df=1,8, p=0.413; tic vs control). Nevertheless, baseline RAC* binding was numerically higher in TS by 13-17% in the three striatal VOIs and by 5-7% in the FL and Th VOIs. The whole-brain analysis identified no significant differences in baseline RAC* binding between TS and control subjects.

Effect of levodopa on RAC* binding

Since the pre- and on-placebo scans differed, the only appropriate comparison for the on-levodopa *RAC scan is the on-placebo scan. Therefore we assessed the effect of levodopa by comparing the BP_{ND} in the post-LD and post-placebo scans.

In the VOI analysis, there was not a significant effect of LD (day x time interaction, F=0.014, df=1,8, p=0.909, the effect of LD did not differ overall in tic subjects (day x time x diagnosis interaction, F=1.308, df=1,8, p=0.286), and the 4-way interaction (diagnosis x day x time x region) was not significant (F=1.577, df=5,4, p=0.340). However, the diagnosis x day x time interaction was significant for pallidum (p=0.050) with a trend in thalamus (p=0.098; **Error! Reference source not found.**). In these regions BP_{ND} decreased in control subjects, consistent with an increase in dopamine release during the levodopa infusion, whereas the mean effect in the tic subjects was in the opposite direction.

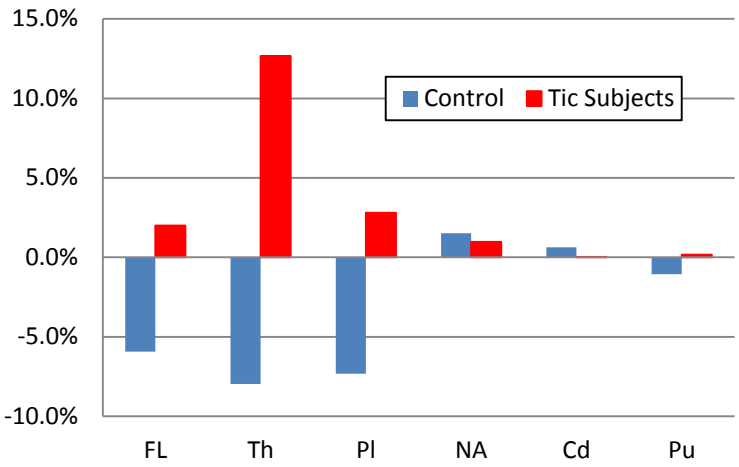

Figure 7. Levodopa-induced change in BP_{ND}, tic vs. control. Mean difference in BPND during levodopa vs. placebo infusion is shown for each group.

The whole-brain analysis identified a similar effect (decreased RAC* binding with levodopa in controls, increased in TS) in a cluster of 38 midbrain voxels (1.0 ml) with peak *t* at atlas coordinate (1.5, −21, −15) and extending laterally, in the right substantia nigra (peak *t*(_df) = 9.0, FDR corrected *p*=0.002; Figure 9, upper panel). A second significant cluster of 19 voxels (0.5 ml) was seen in parahippocampal gyrus (peak *t*=7.92 at (22.5,−39,−6), corrected *p*=0.023; Figure 9, lower panel). The mean regional change in BP_{ND} with levodopa is shown in Figure 8. In both

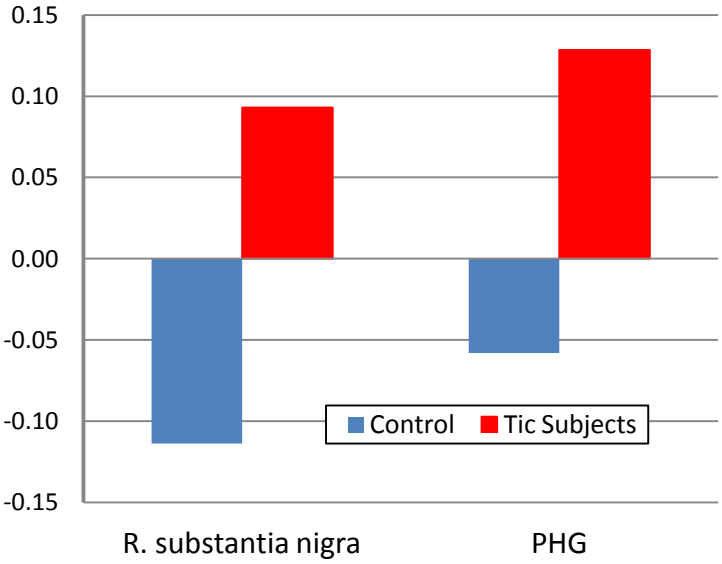

Figure 8. Levodopa-induced change in BP_{ND}, TS vs. control, in the clusters identified in the whole-brain analysis. Same conventions as in the previous figure.

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these clusters, the BP_{ND} on placebo was positive in all subjects ($p < 0.001$, binomial distribution), consistent with nontrivial RAC* binding. The highest *t* value in the whole-brain comparison, 11.62, occurred at (−31.5, 6, −15) in Brodmann's area 13 (uncorrected $p = 1.37 \times 10^{-6}$; Bonferroni threshold 1.17×10^{-6}), but the cluster volume was only 0.1 ml, not significant by FDR correction (Figure 10). A third statistically significant cluster was centered at the posterior edge of the occipital lobe and in this cluster the BP_{ND} on placebo was negative in half the subjects; this cluster likely does not reflect D2R binding.

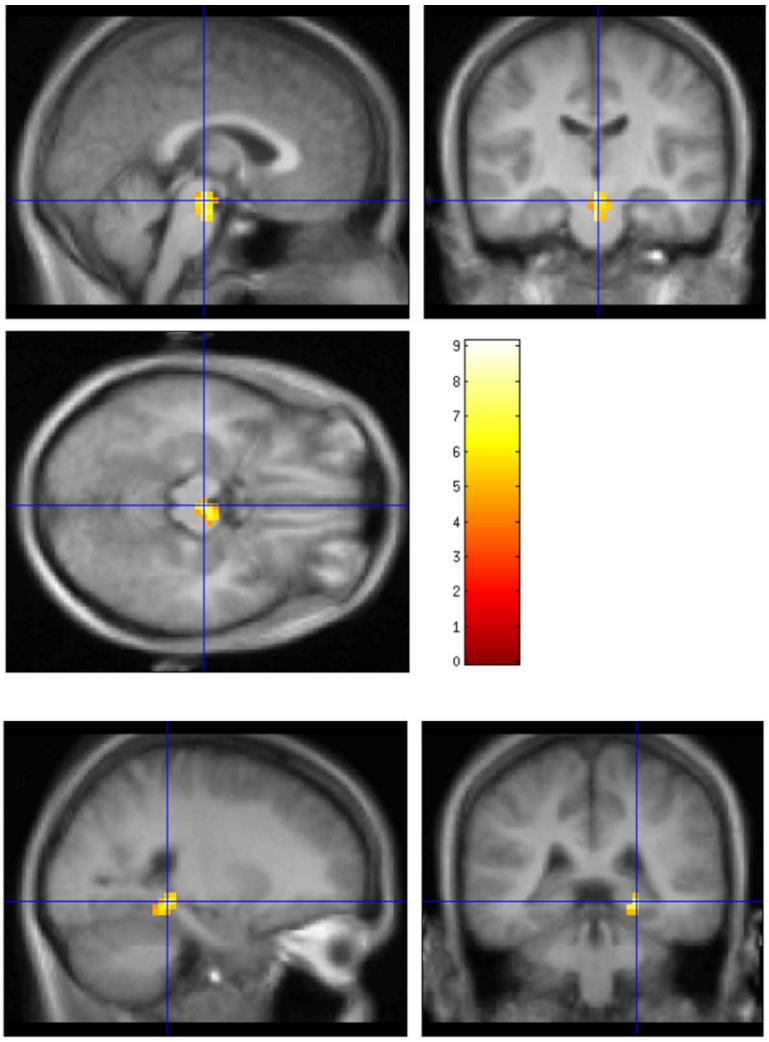

Figure 9. Significant clusters in which the RAC* binding response to levodopa differed between TS and control subjects. Upper 3 sections, substantia nigra. Lower 2 sections, parahippocampal gyrus. Color bar indicates *t* statistic.

Discussion

Baseline striatal RAC* binding

We found no difference in RAC* binding between subjects with or without TS. Previous RAC* PET studies (Singer et al. 2002; Turjanski et al. 1994) or IBZM SPECT studies in TS (George et al. 1994; Muller-Vahl et al. 2000) similarly found no difference. However, an unpublished study by De Vries and colleagues reported decreased RAC* binding at baseline in the putamen and right caudate nucleus (de Vries et al. 2010; de Vries et al. 2009). Outside the striatum, two PET studies using higher affinity D2R radioligands indicated decreased binding in thalamus and frontal cortex (Gilbert et al. 2006; Steeves et al. 2010). *In vivo* studies with these radioligands are sensitive to synaptic dopamine concentration as well as to receptor number and affinity. A postmortem study found increased cortical dopamine receptor binding in TS (Yoon et al. 2007), though such studies are

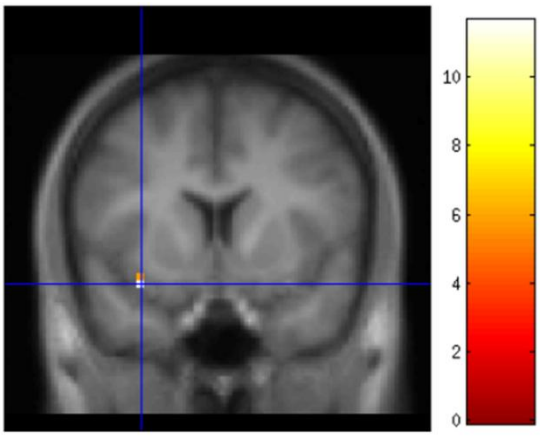

Figure 10. Peak voxel for difference in RAC* binding response to levodopa between TS and control subjects. Color bar indicates *t* statistic.

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necessarily limited in sample size.

Change in striatal BP_{ND} with placebo

Implications for other RAC challenge studies*

BP_{ND} increased during the placebo infusion in the striatum, thalamus and frontal lobe VOIs, especially in control subjects. Most published information on the stability of RAC* binding over time reflects time intervals of days to months (Hietala et al. 1999; Volkow et al. 1993; Volkow et al. 1994; Yoder et al. 2011). Mawlawi et al. (2001) scanned 10 subjects twice each on the same day using a bolus-plus-constant-infusion method, and found no significant mean change from the first to the second scan. However, Alakurtti and colleagues (2011) found that mean BP_{ND} increased from the first to the second scan of the day in striatal and thalamic regions, with the change (about +5%) reaching statistical significance in medial and lateral thalamus.

With this background, the observation in the present study of increased BP_{ND} from the first to second scan of the day has implications for RAC* challenge PET studies in general, essentially all of which use a before- *vs.* after-intervention design. If the results in our sample are typical, the before-after design is flawed in that BP_{ND} increases from the first to the second scan even without active intervention. This does not invalidate the results of methylphenidate challenge RAC* studies, since that challenge *decreases* striatal RAC* BP_{ND} by a large fraction, but it may mean that before-after RAC* studies are less sensitive to manipulations that would decrease dopamine release.

Possible pathophysiological interpretation

The increase in BP_{ND} during the placebo infusion is most likely associated with passage of time rather than a placebo effect *per se*, especially as placebo administration is more likely to increase dopamine release (de la Fuente-Fernandez et al. 2001b; de la Fuente-Fernandez & Stoessl 2002). The presumed decrease in dopamine release during the placebo infusion could indicate that control subjects accommodate to the scanner environment after a while.

The fact that TS subjects do this less may correspond to more persistent alertness/arousal. Greater arousal would correspond to the observation of Chappell and colleagues that TS subjects release more ACTH and norepinephrine with lumbar puncture, which the authors interpreted to indicate a higher level of arousal/anxiety in TS (Anderson et al. 1999; Chappell et al. 1994). Additionally, many people with TS report hypersensitivity to mild unchanging sensations, which can be seen as a failure of habituation to an unchanging sensory environment (Belluscio et al. 2011; Panagopoulos et al. [submitted]).

Alternatively, a smaller change in dopamine release may indicate a more steady level of boredom in TS subjects. Decreased dopamine release with boredom would fit with the observation that at baseline the TS group had (nonsignificantly) higher RAC* than controls in the striatal and thalamic VOIs. Boredom, or its complement novelty seeking, have been related to dopamine; in Cloninger's model of temperament, the Novelty Seeking trait was designed with the intent to reflect central dopaminergic status, and some experimental data have supported that connection (Cloninger 1987; Keltikangas-Järvinen & Jokela 2012). Boredom is also a typical clinical manifestation of ADHD, which can be diagnosed in about half of TS subjects, and is

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influenced by dopamine. Adults and children with TS showed improvement in ADHD rating scale scores when treated with levodopa (Gordon et al. 2007 and unpublished data).

Effect of levodopa infusion on RAC* binding

Levodopa effect on RAC binding in striatum*

Striatal RAC* binding was not substantially changed by levodopa. Initially this result came as a surprise to the authors, because levodopa was given expressly with the expectation that it would increase synaptic dopamine levels. Briefly, support for this expectation includes the following. First, in Parkinson disease there is overwhelming evidence both by clinical observations and by RAC* PET imaging that exogenous levodopa substantially increases striatal dopamine release (Antonini et al. 1997; de la Fuente-Fernandez et al. 2001a; Pavese et al. 2006). In subjects without dopamine deficiency, the evidence is somewhat less direct, but still supportive: intravenous levodopa is rapidly taken up from the bloodstream into the brain and converted into dopamine, and several studies provide evidence that in healthy subjects it then boosts synaptic dopamine release (reviewed in Gordon et al. 2007). For instance, exogenous levodopa produces has clear sedative and cognitive effects in healthy people (Andreu et al. 1999; Kelly et al. 2009; Weis et al. 2012).

Thus the authors originally expected that exogenous levodopa would decrease striatal RAC* binding. However, further reflection and reading have motivated a different view whereby the results support the original goal of choosing a pharmacological challenge agent that would stimulate phasic dopamine release, but under endogenous control. Recall that the concern with stimulants as challenge agents was that they cause a substantial release of dopamine at the striatal synapse regardless of current environmental demands; it may produce a ceiling effect for dopamine release that does not reflect typical endogenous control. A sensible hypothesis to explain the results of the present study would be that a research subject lying awake in a quiet, darkened room without specific cognitive demands has no need for a substantial release of dopamine, and thus even if exogenous levodopa has added dopamine to presynaptic vesicles, they are not released at a substantial rate at the synapse. A levodopa-raclopride study of a motor task in healthy individuals provides direct experimental support of this hypothesis (Floel et al. 2008). The study was properly designed with two sessions, placebo on one day and levodopa on another, with randomized order. Levodopa increased striatal dopamine release during performance of a motor task, but not at rest! Since in the present study all subjects were at rest during all scans, the results are consistent with those of Floel and colleagues (2008).

Levodopa effect on RAC binding in midbrain, cortex, and thalamus*

Levodopa stimulated dopamine release in controls but reduced it in TS subjects in midbrain (approximately VTA/substantia nigra) and in parahippocampal gyrus. Similar effects, though not statistically significant, were observed in orbital cortex (Brodmann's area 13) and in thalamus.

One expects exogenous levodopa to increase dopamine release in the substantia nigra, and this occurred in the control subjects. D_2 and D_3 dopamine receptors are present in the substantia nigra and their activation inhibits spike firing, dopamine synthesis and dopamine release by nigral dopaminergic cells (Grace 2002). We hypothesize that levodopa increased dopamine stimulation

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of these inhibitory D2-like receptors in control subjects, and this may have prevented levodopa from stimulating nigrostriatal dopamine release into the striatum.

Subjects with TS, however, showed an increase in substantia nigra RAC* binding with levodopa, consistent with a decrease in nigral dopamine release. Nigral dopamine release has been related to reward and novelty in humans. Healthy adults with higher novelty seeking scores had lower D2-like binding ([^{18}F]fallypride) in SN, consistent with greater dopamine release (Zald et al. 2008). Functional MRI studies have also demonstrated substantia nigra signal related to stimulus novelty or to the Novelty Seeking trait (Bunzeck & Duzel 2006; Krebs et al. 2011; Krebs et al. 2009). Healthy adults receiving a sweet vs salty taste had BOLD activation in this region (O'Doherty et al. 2002). Despite this information, it is not clear how to relate a decrease in levodopa-stimulated dopamine release in substantia nigra to the pathophysiology of TS. Explaining the similar difference in nigral levodopa response in TS in parahippocampal gyrus and orbital cortex is no easier. Nevertheless, these results document an abnormality of presynaptic dopaminergic pharmacology in TS.

There was a trend for a similar effect in thalamus; dopamine release increased with levodopa infusion in control thalamus but decreased in TS subjects. A [^{11}C]FLB-457 PET study found a similar result, in that amphetamine provoked thalamic dopamine release in control subjects but not in TS (Steeves et al. 2010).

Limitations

Higher affinity radioligands, such as [^{18}F]fallypride or [^{11}C]FLB457, have advantages for measuring cortical D2Rs, *e.g.* in the frontal lobe where D2Rs appear at much lower concentrations than in the striatum. There are two primary concerns with RAC* outside the striatum (reviewed thoroughly in Egerton et al. 2009). The first is a reliability issue: since the concentration of D2-like receptors is low in cortex compared to striatum, the counting statistics are poor for cortical VOIs of similar volume, and this renders the computed BP_{ND}s suspect. For instance, some regional RAC* BP_{ND}s are negative or close enough to zero that displacement studies produce results that are hard to interpret. In the present study, FreeSurfer-defined cortical regions allowed the creation of a large, reliably defined frontal lobe VOI, in which PET time-activity curves were low in noise (Figure 3, lower panel), allowing a statistically reliable estimate of BP_{ND} that was uniformly positive.

The second concern with RAC* in extrastriatal regions is one of validity or interpretation. RAC* binding in cortex occurs at low levels, only some of which is attributable to specific binding (Farde et al. 1988). The concern is whether specific binding in cortex represents dopamine D2-like receptors. D2 and D4 receptors are expressed in human prefrontal cortex, though at relatively low concentrations compared to striatum (Meador-Woodruff et al. 1996). Raclopride may even have superior sensitivity to fallypride for measuring dopamine release in some cortical regions (Slifstein et al. 2010). Human thalamus contains predominantly D3 rather than D2 receptors (Sun et al. 2012). The validity concern is less worrisome in substantia nigra, where D_2 and D_3 receptors are well characterized. There are precedents for interpreting substantia nigra RAC* displacement in terms of synaptic dopamine release (Egerton et al. 2009).

Finally, the limited sample size likely prevented identifying some significant findings (type II

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error). Nevertheless, the sample size was adequate to find the significant group differences described above.

Future directions

These results suggest a natural next step for research in TS: testing whether dopamine release in TS differs during a dopamine-releasing cognitive (or other) task. Levodopa may augment the task-evoked release or interact with it differently in people with versus without tics. Along these lines, a cognitive-pharmacological interaction fMRI study found that LD changed the BOLD responses to a working memory task (Hershey et al. 2004). A newer levodopa infusion produces roughly twice as high a levodopa plasma concentration as the infusion used in this study (Gordon et al. 2007), and may produce greater dopamine release.

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