Title
Levodopa-stimulated dopamine release in Tourette syndrome

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Abstract

RATIONALE: Several lines of evidence suggest that dopamine (DA)-influenced neuronal pathways may malfunction in Tourette Syndrome (TS). Some PET studies support the hypothesis of presynaptic abnormalities in levodopa uptake, dopamine synthesis, or dopamine release.

OBJECTIVE: Directly test the presynaptic hypothesis using a new approach.

METHODS: We used positron emission tomography (PET) and [11C]raclopride (RAC*) to measure synaptic dopamine release before and during 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 (BPND) in predefined volumes of interest (VOIs). A secondary analysis compared BPND voxel by voxel over the entire brain.

RESULTS: (1) Baseline RAC* BPND did not differ significantly between groups, though nucleus accumbens BPND was higher in TS (16%, p=0.051). (2) DA release declined from before to during infusions (p=0.014), including with placebo. (3) This decline was smaller in TS (p=0.080). (4) Levodopa’s effect on BPND differed significantly in right midbrain (p=0.002, corrected), where levodopa displaced RAC* by 59% in control subjects but increased BPND 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 may have implications for other RAC* PET studies. The smaller decrease of BPND 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 but may signify an abnormal response to dopaminergic stimulation in TS.

Keywords
dopamine D2 receptor; raclopride; positron emission tomography; PET; levodopa; dopamine; Tourette syndrome; nucleus accumbens; substantia nigra; midbrain
Introduction

Tourette Syndrome (TS) 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). Tics are brief movements or noises, repeated many times a day in a 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 TS (Albin 2006; Anderson et al. 1999; Black 2009; Hershey et al. 2004; Singer 2013).

One of the earliest clues to the pathophysiology of tics was their clear response to dopamine D2-like (D2, D3, or D4) receptor antagonists, now confirmed by over 35 randomized controlled trials (Black 2010a: Singer and Wendlandt 2001). Tics also improve with dopaminergic stimulation (Anca et al. 2004; Black and Mink 2000; Carpenter et al. 1999; Feinberg and Carroll 1979; Friedhoff 1982; Gilbert et al. 2003; Gilbert et al. 2000b; Nomura and Segawa 1982; Nomura and Segawa 2003), and such stimulation is primarily postsynaptic (Gilbert et al. 2000a; Gilbert et al. 2000b). 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 D2-like receptors (D2Rs), dopamine precursor uptake and monoamine transporters (Albin et al. 2009; Anderson et al. 1999; Peterson 2001; Singer and Wendlandt 2001; Wong et al. 2008). Post-mortem data are limited by the small number of adequately studied subjects (Kalanithi et al. 2005; Kataoka et al. 2010; Minzer et al. 2004; Swerdlow and Young 2001; Yoon et al. 2007). Most studies suggest that (baseline) 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 (Denys et al. 2013; Gilbert et al. 2006; Minzer et al. 2004; Yoon et al. 2007). Even if dopamine D2-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 detected no such abnormality (Meyer et al. 1999; Singer 2013; Stamenkovic et al. 2001). One widely discussed theory is that basal, tonic dopamine release is normal in TS, but that transient, phasic dopamine release is not (Singer 2013; Singer et al. 2002; Wong et al. 2008; Yeh et al. 2007). 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. 2007).

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 transiently increases tic severity (Denys et al. 2013), 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. When given with an adequate dose of carbidopa, which prevents conversion to dopamine but does not cross the blood-brain barrier, systemic levodopa administration essentially delivers dopamine selectively to the brain, boosting dopamine synthesis almost immediately in both parkinsonian and healthy brains (reviewed in Gordon et al. 2007). Confirming this, when given 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

Participants

All human studies were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. 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.

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 (with one ambidextrous TS subject matched to a right-handed control). Exclusion criteria included any lifetime neurological or Axis I psychiatric disorder (except that 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 original clinical validation sample ranged from 5 to 100, mean=61, S.D.=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 (Fig. 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 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.

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 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 and Baas 1998), yet 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).

Radiotracer preparation

[11C]raclopride was prepared by O-[11C]methylation of (S)-O-desmethylraclopride HBr (ABX Advanced Biochemical Compounds, Radeberg, Germany) using a modification of previously
reported procedures (Ehrin et al. 1986; Farde et al. 1988). Carbon-11 was produced as $^{11}$CO$_2$
using the Washington University JSW BC 16/8 cyclotron and the $^{14}$N(p,α)$^{11}$C nuclear reaction.
The $^{11}$CO$_2$ was converted to $^{11}$CH$_3$I using the microprocessor-controlled PETtrace MeI
MicroLab (GE Medical Systems, Milwaukee, WI), and immediately used for $^{[11]}$Cmethylation of
(S)-O-desmethylraclopride. Product $^{[11]}$Craclopride was purified via semipreparative HPLC, and
reformulated in a 10\% ethanol/normal saline solution. The radiochemical purity exceeded 95\%,
and the specific activity exceeded 500 Ci/mmol, as determined by analytical HPLC. The mass of
raclopride was ≤13.9 μg per injected dose.

Image acquisition

RAC* 14.5 ± 4.79mCi (mean ± S.D.) 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

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) (Fig. 2). These VOIs corresponded to the thalamus (Th) and the left and right
putamen (Pu), caudate (Cd), nucleus accumbens (NA), and globus pallidus (Pl). An additional
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 produced adequate counting statistics for modest noise in the time-activity curve
(Fig. 3). 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
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 each VOI was identical for all 4 PET scans.

The binding potential $B_{ND}$ (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
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 $B_{ND}$s (weighted by
VOI volume) to produce for each PET scan 6 final $B_{ND}$ 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 BPND 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 VOI-based BPNDs) as a repeated measure. Exploratory analyses used a rmANOVA for each region.

**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.

**Levodopa levels**

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

**Baseline RAC* binding**

Across VOIs, RAC* binding did not differ significantly between tic and control subjects (multivariate main effect 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.
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, BPND was similar in the two pre-levodopa scans (correlated at r = 0.99 across VOI and subject).

BPND increased between the 1st and 2nd scan of the day (Fig. 4; 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 BPND 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 (Fig. 4; multivariate time x region interaction F=4.173, df=5.4, p=0.096).

There was a trend for the change in BPND during the infusion to be smaller in tic subjects (Fig. 5; time x diagnosis interaction F=4.211, df=1.8, p=0.074). In individual regions, 0.05 < p < 0.10 for the NA, Pu, and Cd VOIs.

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 BPND in the post-LD and post-placebo scans.

In the VOI analysis, there was no 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; Fig. 6). In these regions BPND decreased in control subjects, consistent with increased dopamine release during the levodopa infusion, whereas the mean effect in the tic subjects was in the opposite direction.

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 negra (peak t(12) = 9.0, FDR corrected p=0.002; Fig. 7A). 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; Fig. 7B). The mean regional change in BPND with levodopa is shown in Fig. 7C. Note that in both these clusters, the BPND 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 × 10−6; Bonferroni threshold 1.17 × 10−6), but the cluster volume was only 0.1 ml, not significant by FDR correction (Fig. 7D).
third statistically significant cluster was centered at the posterior edge of the occipital lobe; both the location and the observation that in this cluster the BP<sub>ND</sub> on placebo was negative in half the subjects suggests that this cluster likely does not reflect D2R binding.

**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 in baseline binding. However, a recently published study by Denys and colleagues reported decreased RAC* binding at baseline in the putamen and right caudate nucleus (Denys et al. 2013). Outside the striatum, two PET studies using higher affinity D2R radioligands indicated decreased binding at baseline in the 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 necessarily limited in sample size.

**Change in striatal BP<sub>ND</sub> on the placebo day**

**Implications for other RAC* challenge studies**

BP<sub>ND</sub> increased from before to during the placebo infusion in the striatum, thalamus and frontal lobe VOIs, especially in control subjects (Figs. 4, 5). 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<sub>ND</sub> 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.

The observation in the present study that BP<sub>ND</sub> increased from the first to second scan of the day is consistent with this background, and has implications for RAC* challenge PET studies in general, because essentially all such studies use a before- vs. after-intervention design. Our results and those of Alakurtti et al. (2011) suggest that the before-after design is flawed in that BP<sub>ND</sub> 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<sub>ND</sub> 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<sub>ND</sub> 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 and Stoessl...
The presumed decrease in dopamine release during the placebo infusion could indicate that control subjects accommodate to the scanner environment over the course of the study day. 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; Panagopoulou et al. 2013).

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 and Jokela 2012). Boredom is also a typical clinical manifestation of ADHD, which can be diagnosed in about half of TS subjects, and is 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). But there is also evidence in subjects without dopamine deficiency: intravenous levodopa is rapidly taken up from the bloodstream into the brain and converted into dopamine, and several studies show that it then boosts synaptic dopamine release (reviewed in Gordon et al. 2007). For instance, exogenous levodopa produces 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 substantial phasic release of dopamine.
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 (Flöel et al. 2008). That 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 Flöel 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 (Brodman’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. D₂ and D₃ 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 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 ([¹⁸F]fallypride) in SN, consistent with greater dopamine release (Zald et al. 2009). Functional MRI studies have also demonstrated substantia nigra signal related to stimulus novelty or to the Novelty Seeking trait (Bunzeck and 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 [¹¹C]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 [¹⁸F]fallypride or [¹¹C]FLB-457, 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 BP₅₀Ds suspect. For
instance, some regional RAC* BP NDs 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 (Fig. 3B), allowing statistically reliable estimates of BP ND that were uniformly positive. Similarly RAC* displacement in thalamus has previously shown adequate counting statistics and reliability (Alakurtti et al. 2011; Hirvonen et al. 2003).

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). The validity concern is less worrisome in substantia nigra, where D2 and D3 receptors are well characterized, and in human thalamus, which contains predominantly D3 rather than D2 receptors (Sun et al. 2012). There are precedents for interpreting substantia nigra RAC* displacement in terms of synaptic dopamine release (Egerton et al. 2009).

Finally, the limited sample size for the comparison of the TS and control groups likely prevented identifying some true differences (type II 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.

Acknowledgments

The authors gratefully acknowledge funding and recruitment assistance from the Tourette Syndrome Association, and technical assistance from Johanna M. Hartlein, R.N., M.S.N.; Meghan C. Campbell, Ph.D.; Kathryn Vehe, Pharm.D.; Michael P. McEvilly; Susan Loftin; and Stephen Moerlein, Ph.D., BCNP. Manuscript preparation was supported in part by NIH grant K24 MH087913. These data were presented in part at the 14th International Congress of Parkinson’s Disease and Movement Disorders, Buenos Aires, 16 June 2010 (Black et al. 2010). The experiments presented here complied with the current laws of the United States of America.
References


Black KJ (2010a) An evidence-based review of treatment efficacy in tic disorders: A report of the ANPA Committee on Research 21st annual meeting, American Neuropsychiatric Association, Tampa, FL


Black KJ, Snyder AZ, Mink JW, Revilla FJ, Tolia VN, Moerlein SM, Perlmutter JS ([submitted]) Spatial reorganization of putaminal dopamine D2-like receptors in cranial and hand dystonia. PLoS ONE


Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleishmann RL, Hill CL, Heninger GR,


patients. European Neuropsychopharmacology 11: 69-74

doi: 10.1002/ana.21809


Zald DH, Cowan RL, Riccardi P, Baldwin RM, Ansari MS, Li R, Shelby ES, Smith CE,
**Tables**

**Table 1** Subject characteristics and adequacy of matching

<table>
<thead>
<tr>
<th>Measure</th>
<th>Tic Subjects (N=5)</th>
<th>Controls (N=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean ± S.D.)</td>
<td>33.8 ± 12.9</td>
<td>32.8 ± 11.1</td>
</tr>
<tr>
<td>Sex, male (N)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Race, Caucasian (N)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Handedness, right (N)</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>OCD dx (N)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ADHD dx (N)</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2** Clinical characteristics of the Tourette syndrome group

<table>
<thead>
<tr>
<th>Scale</th>
<th>Scores (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCI score</td>
<td>36.8 ± 22.0</td>
</tr>
<tr>
<td>YGTSS</td>
<td></td>
</tr>
<tr>
<td>Motor tic score</td>
<td>10.6 ± 3.4</td>
</tr>
<tr>
<td>Vocal tic score</td>
<td>7.8 ± 4.0</td>
</tr>
<tr>
<td>Impairment score</td>
<td>9.4 ± 9.8</td>
</tr>
<tr>
<td>TSSR score</td>
<td></td>
</tr>
<tr>
<td>Motor</td>
<td>9.3 ± 5.9</td>
</tr>
<tr>
<td>Vocal</td>
<td>3.2 ± 2.3</td>
</tr>
<tr>
<td>Total</td>
<td>12.5 ± 7.9</td>
</tr>
<tr>
<td>ADHD Rating Scale</td>
<td>11.6 ± 10.7</td>
</tr>
</tbody>
</table>

**Legend to Table 2:**

The Y-BOCS was completed for only 1 tic subject; the score was 9 on day 1 and 14 on day 2.

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

**Table 3** Levodopa plasma concentrations in ng/ml, mean ± SD

<table>
<thead>
<tr>
<th>Time</th>
<th>Controls</th>
<th>Tic subjects</th>
<th>p (t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak (10’ into infusion)</td>
<td>1591.5 ± 232.5</td>
<td>1938.8 ± 726.3</td>
<td>0.36</td>
</tr>
<tr>
<td>Just before RAC* scan</td>
<td>788.0 ± 152.4</td>
<td>992.4 ± 322.9</td>
<td>0.26</td>
</tr>
<tr>
<td>Just after RAC* scan</td>
<td>529.5 ± 149.2</td>
<td>662.8 ± 136.1</td>
<td>0.21</td>
</tr>
</tbody>
</table>
Figure Captions

Fig. 1 Study overview

Fig. 2 Automated striatal VOIs

Fig. 3 Decay-corrected time-activity curves for the right putamen VOI (filled circles), the frontal lobe VOI (+’s), and the cerebellar reference region (empty circles) from one subject’s pre-levodopa PET scan

Fig. 4 Mean difference in $B_{ND}$ across all 10 subjects from before to during the infusion on the placebo day

Fig. 5 Change in $B_{ND}$ with placebo infusion, tic vs. control groups; the $p$ values shown are for difference between groups, from $t$ tests for each region

Fig. 6 Levodopa-induced change in $B_{ND}$, tic vs. control groups. The mean difference in $B_{ND}$ during levodopa vs. placebo infusion is shown for each group. FL, Frontal lobe; Th, Thalamus; Pl, Pallidum; NA, Nucleus Accumbens; Cd, Caudate; Pu, Putamen

Fig. 7 Differences in the RAC* binding response to levodopa between TS and control subjects, thresholded at uncorrected $p = 0.001$, in color, laid over the MRI template image (grayscale). a, b: Significant clusters, with blue lines crossing at substantia nigra in (a), 3 views, and parahippocampal gyrus in (b). (c) Levodopa-induced change in $B_{ND}$, TS vs. control, in the clusters shown in A and B. R., Right; PHG, parahippocampal gyrus. (d) Blue lines cross at the peak voxel from the same comparison.
Figure 1.

Day 1: raclo\textsubscript{1} → PET

Day 2: raclo\textsubscript{3} → PET

Infusion rate:

Infusion rate:

Day 1 raclo\textsubscript{1} → PET

Day 2 raclo\textsubscript{3} → PET

Infusion rate:

Infusion rate:

Saline or levodopa

Saline or levodopa

Time

Time
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.
Figure 7.