Chapter 5

SEROotonin TRANSPORTER BINDING AND ANXIETY SYMPTOMS IN PARKINSON’S DISEASE

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ABSTRACT

Anxiety is a common neuropsychiatric symptom in Parkinson’s disease (PD), yet the neural mechanisms have been scarcely investigated. Disturbances in dopaminergic and serotonergic signalling may play a role in its pathophysiology. \( ^{123}\text{I}-\text{N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane (}^{123}\text{I-FP-CIT)} \) is a single-photon emission computed tomography (SPECT) radiotracer, and its binding in striatal and extrastriatal subcortical brain areas represents predominantly binding to the pre-synaptic dopamine transporter (DAT) and the serotonin transporter (SERT), respectively. Availability of DAT and SERT may thus provide an \textit{in vivo} measure for the integrity of both dopamine and serotonin neurons. \textbf{Methods:} We studied the association between anxiety symptoms, measured with an affective subscale of the Beck Anxiety Inventory, and (extra)striatal \(^{123}\text{I-FP-CIT} \) binding in 127 non-demented PD patients with a median disease duration of 2.55 (Inter Quartile Range 2.90) years. We conducted the analyses on patients currently on or not on dopamine replacement therapy (DRT). \textbf{Results:} Severity of anxiety symptoms showed a significant negative association with \(^{123}\text{I-FP-CIT} \) binding ratios in the right thalamus \( [\beta=-0.203, P=0.019; \Delta R^2=0.040] \) (multiple testing \( P_{\text{corr}}<0.020 \)). In the subgroup of patients not on dopamine replacement therapy \( (n=81) \), we found a significant negative association between anxiety and thalamic \(^{123}\text{I-FP-CIT} \) binding ratios bilaterally [right: \( \beta=-0.349, P=0.001; \Delta R^2=0.119 \); left: \( \beta=-0.269, P=0.017, \Delta R^2=0.071 \) \( (P_{\text{corr}}<0.020) \). \textbf{Conclusion:} This study shows that higher levels of anxiety in PD patients are associated with lower thalamic \(^{123}\text{I-FP-CIT} \) binding, pointing towards a contribution of serotonergic degeneration to anxiety symptoms in PD.
INTRODUCTION

Anxiety is a common neuropsychiatric symptom in Parkinson’s disease (PD). It has a higher prevalence in PD than in the general elderly population (35), increases the psychological burden of disease, and is associated with exacerbation of motor symptoms such as dyskinesia, freezing of gait and on/off fluctuations (131-133). In addition, anxiety appears to occur more frequently during wearing-off (134), and can be alleviated by dopaminergic medication (135-137). This suggests an association of anxiety with the waxing and waning of extracellular dopamine levels that results from the degeneration of dopaminergic neurons and the compensatory treatment with dopamine replacement therapy (DRT).

Other studies have suggested that other factors beyond low dopamine levels during the wearing off-phase are involved in the pathophysiology of anxiety of PD (138). In non-PD samples, anxiety has been associated with serotonergic, noradrenergic, GABA-ergic and cholinergic deficits (see (139) for a review). These neurotransmitter systems are also affected in PD. Lower serotonin transporter (SERT) binding, for example, has previously been described in PD (140, 141), and it has also been suggested that degeneration of the serotonergic system plays a role in anxiety in PD (see (34) for a review).

$^{123}$I-N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane ($^{123}$I-FP-CIT) is a single-photon emission computed tomography (SPECT) radiotracer that binds with high affinity to both the presynaptic dopamine transporter (DAT) and to the presynaptic SERT, albeit with lower affinity (50). Previous studies have shown that striatal $^{123}$I-FP-CIT binding (putamen and caudate nucleus) predominantly represents binding to DAT, whereas extrastriatal binding in subcortical brain areas (e.g., thalamus, hypothalamus, midbrain, pons) predominantly represents binding to SERT (53, 56). Consequently, striatal $^{123}$I-FP-CIT binding to DAT and extrastriatal $^{123}$I-FP-CIT binding to SERT could serve as proxy for the integrity of the dopaminergic and serotonergic system, respectively, and allows us to study their involvement in PD-related anxiety symptoms.

Molecular imaging studies that investigated the association between striatal DAT availability and PD-related anxiety have shown mixed results. Some studies reported a positive association (142, 143), while others reported a negative association (144, 145). These studies included PD patients at different disease stages and medication states, which may be a potential explanation for the inconsistency in the reported findings. To the best of our knowledge, the association between SERT availability and anxiety in PD has not yet been studied.

Brain regions that are highly innervated by serotonergic projections have previously been associated with anxiety in PD in a structural brain imaging study (e.g. the amygdala) (146), and are also implicated in anxiety in non-PD samples (e.g. the thalamus) (147); see (148) for a review. Based on the aforementioned results we hypothesised that extrastriatal SERT in the amygdala, hippocampus and thalamus would show a negative association with the severity of anxiety symptoms in PD. Based on the close relationship between anxiety and depression...
(133), the involvement of DAT in depression (81), and the aforementioned results, we also analysed the association between anxiety and DAT binding in the striatum.

MATERIALS AND METHODS

Participants
For this cross-sectional study, we selected PD patients from a database of consecutive cases that presented between May 2008 and July 2015 to the outpatient clinic for movement disorders of the neurology department of the VU University Medical Center (VUmc) in Amsterdam, The Netherlands. Both an 123I-FP-CIT SPECT scan and a T1-weighted magnetic resonance imaging (MRI) brain scan had to be available for a patient to be eligible for participation. In addition, the availability of a Beck Anxiety Inventory (BAI) score was an inclusion criterion. We excluded patients on selective serotonin reuptake inhibitors (SSRIs), since these drugs can influence 123I-FP-CIT binding to the SERT (53). Based upon the mini mental state examination (MMSE) score we excluded patients scoring below 25 from this study to exclude patients with signs of dementia. See flowchart in Figure 1 for in- and exclusion criteria. Movement disorder specialists clinically established a diagnosis of PD according to the UK PD Society Brain Bank criteria (6), supported by an abnormal 123I-FP-CIT SPECT scan in 124 (97.6%) patients. Three patients (2.4%) had a scan that was visually abnormal, but still normal with quantification. All included patients gave written informed consent to use their clinical and neuroimaging data for scientific purposes, and the study was approved by the local medical ethics committee.

Clinical characteristics
We evaluated the severity of the motor symptoms with the Unified PD Rating Scale, motor section (UPDRS-III) (77); of the 46 patients on DRT, 31 patients (67.4%) were in the “on” state, five (10.9%) were in the “off” state, and of another ten patients (21.7%) state was unknown. Levodopa equivalent daily dose (LEDD) was calculated for patients with DRT as described previously (149). On the same day as the UPDRS-III, anxiety symptoms were assessed with the BAI (150) and depressive symptoms with the Beck depression inventory (BDI) (151). Eight patients were on anxiolytics (four on benzodiazepines, two on a tricyclic antidepressant and one on zopiclone).

Beck Anxiety Inventory
Patients were asked to fill out the BAI (150), a 21-item questionnaire asking patients to report anxiety symptoms over the last week, ranging from 0 (not at all) to 3 (severe). A total score of >12 is considered to represent clinically relevant anxiety in PD (152). For this study, up to three missing items were accepted, and in that case the values were imputed with the mean score of the available items. Patients with >3 missing values were excluded. Since many symptoms of
anxiety overlap with the motor symptoms of PD, we used the BAI affective subscale \(\text{BAI}_{\text{affective}}\) to assess ‘affective symptoms’. \(\text{BAI}_{\text{affective}}\) is a subset of BAI items covering the affective aspects of anxiety that are not directly associated with PD-related motor symptoms (153). This subscale was also previously applied to study the volumetric brain correlates of anxiety symptoms in PD (146).

**Selection of patients**

- 260 patients with \(^{123}\text{I}-\text{FP-CIT}-\text{scan and BAI available}
- Exclusion criteria:
  - 3 Invalid BAI
  - 43 No MRI available
  - 12 Invalid MMSE
  - 23 MMSE ≤ 24
  - 13 Diagnosis other than PD
  - 23 Use of SSRIs

- 143 patients evaluated

- Exclusions:
  - 11 \(^{123}\text{I}-\text{FP-CIT} technical imaging problems
  - 2 No good segmentation due to poor contrast
  - 1 MRI with excessive movement
  - 1 Poor coregistration between MRI and \(^{123}\text{I}-\text{FP-CIT}
  - 1 Age several SD below group mean

- 127 patients with \(^{123}\text{I}-\text{FP-CIT} \text{scan, MRI and BAI available for analyses}

*Figure 1. Patients included in the study*

**\(^{123}\text{I}-\text{FP-CIT SPECT–image acquisition and pre-processing***

\(^{123}\text{I}-\text{FP-CIT} \text{was intravenously administered in a dose of approximately 185 MBq (specific activity >185 MBq/nmol; radiochemical purity >99%; produced as DaTSCAN™ according to good-manufacturing-practices criteria at GE Healthcare, Eindhoven, The Netherlands). Static images were obtained for 30 minutes after 3-4 hours using a dual-head gamma camera (E.Cam; Siemens, Munich, Germany) with a fan-beam collimator. Images were reconstructed as described earlier (81), and reoriented to an anterior-posterior commissure plane in Statistical Parametric Mapping 12 software (SPM 12; Wellcome Trust Centre for Neuroimaging, London, UK).*
**MRI T<sub>1</sub>–image acquisition**

Structural images were acquired using a 3D T<sub>1</sub>-weighted sequence on different MRI systems at the VU University Medical Center (Amsterdam, The Netherlands). See supplementary materials for the scan parameters.

**Regions of interest**

We used the striatal caudate nucleus, putamen and nucleus accumbens, and extrastriatal amygdala, hippocampus and thalamus as regions of interest (ROIs). All ROIs were individually constructed using FreeSurfer 5.3 (Athinoula A. Martinos Center for Biomedical Imaging, Boston, MA, USA) with default settings. The putamen was divided into an anterior and posterior putamen by a line perpendicular to the anterior commissure in the mid-sagittal plane. The caudate nucleus and nucleus accumbens FreeSurfer segmentations were combined (left and right separately) to avoid spill over effects when calculating the DAT binding ratios.

All ROIs were visually inspected for segmentation errors and if necessary excluded from analysis; this resulted in one pairwise exclusion for the bilateral anterior and posterior putamen, and two pairwise exclusions for the bilateral caudate/accumbens.

**123<sup>I</sup>-FP-CIT SPECT and T<sub>1</sub> co-registration**

Because 123<sup>I</sup>-FP-CIT SPECT contains insufficient anatomical details, co-registering the SPECT scan to a T<sub>1</sub>-weighted image is often challenging. We therefore devised a method to optimise the process. Voxel intensity in the 123<sup>I</sup>-FP-CIT SPECT scan is highest in the striatum. Using the tools from the FMRIB Software Library (FSL 5.0.8; http://fsl.fmrib.ox.ac.uk/fsl) we added the FreeSurfer segmentations of the striatal regions to each patient’s T<sub>1</sub>-weighted image. The intensity of the striatal regions in the T<sub>1</sub>-weighted image was increased to obtain an image in which – like in an 123<sup>I</sup>-FP-CIT SPECT scan – the striatal regions were easily distinguishable from the background. Co-registration was subsequently successfully performed in SPM12 using the hyperintense striatal regions as a common landmark in both images. See Supplemental Figure 1 for a graphical representation of the processing pipeline.

**123<sup>I</sup>-FP-CIT SPECT–image analysis**

We calculated binding ratios per subject for the ROIs in the DAT-rich striatum and the SERT-rich extrastriatal areas. The cerebellum was used as the reference region (REF; WFU Pickatlas, Wake Forest University, Winston-Salem, NC, USA; automated anatomical labelling atlas; bilateral Crus 2). We converted the REF mask from Montreal Neurological Institute (MNI)-space to subject space by using the inverse normalization parameters that were obtained when converting the T<sub>1</sub>-weighted MRI scan to MNI-space. Binding ratios were calculated according to the following formula: \([(\text{ROI} – \text{REF}) / \text{REF}].\)
Voxel-based analysis

We performed voxel-based multiple regression analyses with age as covariate in SPM 12 to corroborate the findings of our ROI analyses. Masks were applied conform an earlier study. Statistical threshold was set to \( P<0.050 \), Family-Wise Error (FWE) corrected for multiple comparisons.

Statistics

We assessed normality of data by plotting histograms, examining Q-Q plots, and using Kolmogorov-Smirnov tests. After checking for multicollinearity, homoscedasticity and independence of variables, we performed hierarchical multiple regression analyses with BAI\textsubscript{affective} as the independent factor, and age as nuisance covariate. For the regression analyses, we calculated multiple comparison corrected \( p \)-values with Simple Interactive Statistical Analysis (SISA; http://www.quantitativeskills.com/sisa/calculations/bonhlp.htm), a tool that uses the mean association between variables that are mutually correlated (binding ratios in three different bilateral striatal ROIs and three different bilateral extrastriatal ROIs) for the alpha correction \( (r=0.7 \text{ striatal ROIs}, r=0.5 \text{ extrastriatal ROIs}) \), and allows a less stringent correction than the Bonferroni method for multiple comparisons. For the striatal ROIs this resulted in a statistical threshold \( (P_{\text{corr}}) \) of \( P_{\text{corr}}<0.030 \) and for extrastriatal ROIs a \( P_{\text{corr}}<0.020 \). We considered a \( p \)-value between 0.050 and 0.100 for clinical data, or between \( P_{\text{corr}} \) and \( P=0.050 \) for the binding ratios as a trend. Analyses were performed on the total group and on groups stratified for use of DRT \( (n=46 \text{ with medication}, n=81 \text{ without}) \). Post-hoc analyses were performed to check for influence of volume on thalamic findings. For this, ROI volumes obtained with FreeSurfer were added to a hierarchical multiple regression analysis as dependent variable. To avoid possible scanner effects on volume measures, we used only scans that were acquired on one particular scanner (GE Signa HDxt 3T – General Electric, Milwaukee, WI, USA) \( (n=92) \), see supplement for all scan parameters. All analyses of clinical characteristics and ROIs were performed in SPSS 22 (IBM Corp, Armonk, NY, USA).

RESULTS

Group characteristics

The characteristics of the 127 PD patients are summarised in Table 1. The BAI\textsubscript{affective} subscale correlated positively with the BDI score \( [r=0.703, P<0.001] \). This was true for both patients with and without DRT \( [r=0.758, P<0.001; r=0.654, P<0.001, \text{ respectively}] \). BAI\textsubscript{affective} subscale did not correlate with age, disease duration, UPDRS-III or LEDD.
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total group</th>
<th>With DRT</th>
<th>Without DRT</th>
<th>With DRT vs without DRT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>127</td>
<td>46</td>
<td>81</td>
</tr>
<tr>
<td>Number of patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (f/m)</td>
<td></td>
<td>43/84</td>
<td>17/29</td>
<td>26/55</td>
</tr>
<tr>
<td>Age at $^{123}$I-FP-CIT SPECT</td>
<td></td>
<td>64.91 (10.98)</td>
<td>64.52 (10.57)</td>
<td>65.12 (11.27)</td>
</tr>
<tr>
<td>Disease duration, yrs median</td>
<td></td>
<td>2.55 (2.90)</td>
<td>3.91 (5.68)</td>
<td>2.30 (2.17)</td>
</tr>
<tr>
<td>(IQR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPDRS-III</td>
<td></td>
<td>23.02 (10.68)</td>
<td>23.05 (10.16)</td>
<td>23 (11.02)</td>
</tr>
<tr>
<td>BAI_{affective} scores</td>
<td></td>
<td>11.50 (8.32)</td>
<td>14.02 (9.00)</td>
<td>10.07 (7.59)</td>
</tr>
<tr>
<td>BAI&gt;12 (N(%) of patients)</td>
<td></td>
<td>47 (37.00)</td>
<td>21 (45.70)</td>
<td>26 (32.10)</td>
</tr>
<tr>
<td>BDI-scores, median (IQR)</td>
<td></td>
<td>8.00 (9.00)</td>
<td>9.50 (9.45)</td>
<td>7 (6.50)</td>
</tr>
<tr>
<td>LEDD</td>
<td></td>
<td>161.77 (274.78)</td>
<td>446.64 (285.23)</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are in mean (SD), unless otherwise specified. DRT, dopamine replacement therapy; UPDRS-III, Unified Parkinson’s Disease Rating Scale – motor symptoms; BAI, Beck anxiety Inventory; BAI>12, clinically relevant anxiety; BDI, Beck Depression Inventory; LEDD, Levodopa equivalent daily dose; IQR, inter quartile range; U, Mann-Whitney U-test statistic.

ROI-based $^{123}$I-FP-CIT SPECT analyses

Striatal DAT binding

The multiple regression analysis on 1) the total group and 2) separately for the patients with or without DRT did not show any significant associations between striatal $^{123}$I-FP-CIT binding ratios and BAI_{affective} score. In patients without DRT, however, we observed a trend-significant negative association between $^{123}$I-FP-CIT binding ratios in the right anterior putamen and the BAI_{affective} score ($\beta=-0.236, P=0.038; \Delta R^2=0.054$) ($P_{corr}<0.030$).

Extrastriatal SERT binding

In the total group of PD patients, BAI_{affective} scores showed a statistically significant negative association with $^{123}$I-FP-CIT binding ratios in the right thalamus ($\beta=-0.203, P=0.019; \Delta R^2=0.040$), and a trend-significant negative association in the left thalamus ($\beta=-0.186, P=0.039; \Delta R^2=0.034$) and right amygdala ($\beta=-0.200, P=0.026; \Delta R^2=0.039$) ($P_{corr}<0.020$).

In PD patients without DRT, we observed a significant negative association between BAI_{affective} and $^{123}$I-FP-CIT binding in the right and left thalamus [right: $\beta=-0.349, P=0.001; \Delta R^2=0.119$; left: $\beta=-0.269, P=0.017; \Delta R^2=0.071$] ($P_{corr}<0.020$), see Figure 2. This association was not evident in patients using DRT [right: $\beta=-0.016, P=0.913; \Delta R^2=0$; left: $\beta=-0.017, P=0.912; \Delta R^2=0$]. In addition, we saw a trend-significant negative association between BAI_{affective} and $^{123}$I-FP-CIT binding ratios in the right hippocampus for patients without DRT ($\beta=-0.231, P=0.040; \Delta R^2=0.052$) ($P_{corr}<0.020$).
Figure 2. Scatterplots showing association between serotonin transporter (SERT) binding ratios in the thalamus and the affective subscale of the Beck Anxiety Inventory (BAI<sub>affective</sub>).

Voxel-based $^{123}$I-FP-CIT SPECT analysis

Striatal DAT binding

The voxel-based multiple regression analysis of the striatal areas did not show any significant associations between striatal $^{123}$I-FP-CIT binding ratios and BAI<sub>affective</sub> scores corrected for age.

Extrastriatal SERT binding

In line with the results of the ROI analysis, the BAI<sub>affective</sub> score showed a significant negative association with $^{123}$I-FP-CIT binding ratios in the left posterior thalamus, corrected for age (see Table 2, Supplemental Figure 2). For none of the other ROIs did we find a significant association.

Table 2. Voxel-based analysis

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Medication?</th>
<th>$K_e$</th>
<th>$P_{FWE}$ peak-voxel</th>
<th>$T$</th>
<th>x/y/z (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus left</td>
<td>Total group</td>
<td>2</td>
<td>0.044</td>
<td>4.11</td>
<td>-14/-24/0</td>
</tr>
<tr>
<td></td>
<td>No DRT</td>
<td>7</td>
<td>0.019</td>
<td>4.46</td>
<td>-16/-26/0</td>
</tr>
<tr>
<td>Thalamus right</td>
<td>Total group</td>
<td>No significant results</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>No DRT</td>
<td>No significant results</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Analysis on the ROIs with significant association with BAI<sub>affective</sub>. DRT, dopamine replacement therapy; $K_e$, size of significant cluster of voxels; $P_{FWE}$, Family-wise corrected $P$-value; $T$, T-statistic value; x/y/z, millimetres from origo in Montreal Neurological Institute-space.
**Post-hoc analysis: ROI volume**

Volume of the bilateral thalamus was not associated with BAI affective; neither in patients with \( n=37; \) left: \( \beta=0.209, P=0.151; \Delta R^2=0.042; \) right: \( \beta=0.124, P=0.368; \Delta R^2=0.015 \), nor without DRT \( n= 55; \) left: \( \beta= -0.128, P= 0.175; \Delta R^2=0.016; \) right: \( \beta= -0.031, P= 0.745; \Delta R^2=0.001 \).

**DISCUSSION**

In this study we investigated the relationship between anxiety symptoms and \(^{123}\)I-FP-CIT binding in striatal and extrastriatal brain regions in PD patients. We found that \(^{123}\)I-FP-CIT binding ratios in the thalamus, predominantly representing SERT availability (53), were negatively associated with the severity of anxiety symptoms, supporting our hypothesis. This effect was mainly driven by the group of patients not using DRT.

To the best of our knowledge, this is the first study to show an association between SERT binding in the thalamus and anxiety symptoms in a PD population. Using \(^{11}\)C-DASB, a selective SERT PET tracer, Reimold and co-workers have previously reported an association between SERT binding and anxiety in non-PD samples: in patients with unipolar major depression and in patients with obsessive-compulsive disorder (147, 155). Using both \(^{11}\)C-DASB, and the selective DAT PET tracer \(^{11}\)C-PE2I, Maillet et al. compared apathetic and non-apathetic PD patients, and reported lower SERT availability in the thalamus, pallidum and meso-cortico-limbic and mesostriatal pathways in apathetic patients that were more depressed and more anxious. However, they did not observe a direct relationship between anxiety symptoms and SERT binding in the thalamus (156). Others have reported a relationship between thalamic SERT availability and fatigue in PD (140), and evidence of reduced thalamic SERT availability in PD versus healthy controls (113, 157).

Lower SERT availability can be interpreted as 1) downregulation of SERT and/or 2) degeneration of serotonergic projections. Downregulation would result in a higher serotonin availability in the synaptic cleft, while with neurodegeneration there would be lower serotonin availability. Much evidence points to dysfunction of the serotonergic system in the pathophysiology of anxiety; it has been thought that anxiety originates from a lack of serotonin (see (148) for a review). Moreover, evidence of loss of SERT binding in PD patients has been reported (140, 141), as well as a link to anxiety in PD (See for a review (34)). Consequently, we interpret degeneration of the serotonergic system to be the most likely cause of the lower SERT binding in our PD patients.

Studies on the neural correlates of anxiety mainly involve the sensory and limbic circuits, and their reciprocal interactions (158). These networks comprise anatomical regions including nuclei in the thalamus and amygdala (for a review see (159, 160)). The amygdala is associated with both regulation and production of anxiety (see for reviews (161, 162)), and has been shown to be hyperactive in several anxiety disorders (review (158)). In our study, however, we only observed a trend-significant association of SERT availability in the right amygdala with
anxiety. This may be related to the modest affinity of $^{123}$I-FP-CIT for SERT, and the small size of the amygdala ROI, resulting in low specific to non-specific binding. Research with more selective SERT tracers (e.g., $^{11}$C-DASB) is needed for more detailed data on the role of amygdalar SERT in PD-related anxiety.

A growing body of evidence suggests that the thalamus is able to regulate parts of the amygdala. According to a study performed in mice, the thalamus regulates fear processing in the lateral division of the central amygdala, thus coordinating conditioned fear (163). Also in healthy human subjects, a functional connection between the thalamus and the central amygdala has been demonstrated. Using fMRI, this connection was found to be disturbed in patients with generalised anxiety disorder (164). Moreover, Planetta et al. showed with diffusion tensor imaging that the integrity of thalamic fibres projecting from the dorsomedial nucleus of the thalamus to the amygdala, is reduced in de novo PD patients compared with healthy controls (165). Taken together, these results seem to suggest that the thalamus has modulatory effects on the amygdala.

The SPECT data of this study does not offer information about the functional deficit in anxiety, but as argued above we assume lower SERT availability to imply lower serotonin presence. A functional study in ferrets has shown that serotonin has an inhibitory effect on the activity of nuclei in the dorsal thalamus (166). In addition, an fMRI study performed in patients with social anxiety disorder showed that the SSRI paroxetine reduced the activation in the thalamus, compared to placebo (167). Although this currently remains speculation, this may imply that reduced serotonin levels, e.g. due to PD-related neurodegeneration, lead to dysregulation of the thalamus and dysfunctional coupling between the thalamus and amygdala, possibly resulting in increased vulnerability to anxiety.

Noticeably, the presence of a bilaterally significant association of lower thalamic SERT availability with anxiety was restricted to PD patients that were not using DRT. Several studies have reported a relief of anxiety symptoms after administration of DRT (135-137). In our study, however, we observed higher average anxiety scores in patients on DRT. It has been suggested that the mechanisms underlying neuropsychiatric symptoms like anxiety may differ between disease stages; with early PD stages exhibiting stronger serotonergic involvement, and later stages of PD exhibiting predominant dopaminergic involvement (156). Indeed, in our population, the average disease duration in patients on DRT was longer. An alternative explanation is a phenomenon that has been shown in a rodent model of PD, where L-dopa is converted to dopamine in serotonergic neurons. This vesicle-stored dopamine displaces vesicles containing serotonin, resulting in dysregulated serotonin secretion, possibly increasing SERT levels (113, 168).

$^{123}$I-FP-CIT binding ratios in DAT-rich striatal regions did not show any association with severity of anxiety symptoms that survived correction for multi-comparisons. Nevertheless, in patients without DRT, we observed a trend-significant negative association in the right anterior putamen. Previous analyses of the relationship between anxiety and striatal DAT availability in
PD patients have provided mixed results. In one SPECT study, using $^{123}$I-FP-CIT as a radiotracer, anxiety was positively associated with DAT binding (142), whereas in another study DAT and anxiety were negatively correlated (144). Similarly, in SPECT studies using $^{99m}$Tc-TRODAT-1—another DAT tracer—both positive (143) and negative associations (145) between striatal DAT binding and anxiety have been found.

The main strengths of this study are the large number of participants that were scanned using the same SPECT camera and the relatively large number of PD patients that were drug-naïve. Another strength is the use of individual MRI brain scans to more accurately determine $^{123}$I-FP-CIT binding, which was particularly helpful in the extrastriatal brain areas. The present study is however not without limitations. In this study we used a single radiotracer, $^{123}$I-FP-CIT SPECT, to simultaneously study the integrity of the striatal dopaminergic and the extrastriatal serotonergic system. Furthermore, the resolution of SPECT scans compared with PET precludes a thorough determination of the exact thalamic sub-areas involved. Another limitation is that the questionnaire we used assesses anxiety-associated symptoms, and does not allow a formal clinical diagnosis of an anxiety disorder. Furthermore, the amount of variance in anxiety symptoms that could be explained by thalamic SERT was relatively small, suggesting that other neurobiological factors likely contribute to the pathophysiology of PD-related anxiety.

In conclusion, this study shows a significant negative association between severity of anxiety symptoms and serotonergic integrity in the thalamus in PD patients, particularly in patients not using DRT. This observation may help unravelling of the pathogenesis of anxiety symptoms in PD, opening up the possibility of future improvements in the management of PD-related anxiety.

ACKNOWLEDGMENTS

We would like to thank Dr. Anouk Schrantee from the Academic Medical Center, Amsterdam, The Netherlands, for her valuable insights and advice in developing the $^{123}$I-FP-CIT SPECT – MRI coregistration method.
SUPPLEMENT

MRI-scanner specifics
In this study, images were acquired on 6 scanners:

- **GE Signa HDxT 3.0T** (General Electric medical Systems, Milwaukee, WI, USA) with an eight-channel head coil (8HRBRAIN). (TR=7.82 ms, TE=3 ms, TI=450 ms; 256x256 mm; voxel size 1.0 mm x 0.977 mm x 0.977 mm; 172 slices)

- **GE Discovery* MR750 3.0T** (GE medical Systems) with an eight-channel head coil (8HRBRAIN). (TR=8.21 ms, TE=3.22 ms, TI=450 ms; 265x256 mm; voxel size 1.0 mm x 0.977 mm x 0.977 mm; 176 slices).

- **GE Signa HDxt 1.5T** (GE medical Systems) with a Head Neck Spine head coil. (TR=12.3 ms, TE=5.2 ms, TI=450 ms; 256x256 mm; voxel size 1.5 mm x 1.0 mm x 1.0 mm; 172 slices)

- **Toshiba Vantage Titan 3.0T** (Toshiba America Medical Systems Inc., Tustin, CA, USA) with a 32 channel head SPDR coil. (TR=9.5 ms, TE=3.2 ms, TI=800 ms; 256x256 mm; voxel size 1.0 mm x 1.0 mm x 1.0 mm; 176 slices)

- **Siemens Avanto 1.5T** (Siemens Sonata, Erlangen, Germany) with an eight-channel phased-array head coil. (TR=2700 ms, TE=5.2 ms, TI=950 ms; 256x192 mm; voxel size 1.5 mm x 1.0 mm x 1.0 mm; 160 slices)
Supplemental Figure 1. Processing pipeline of the $^{123}$I-FP-CIT SPECT and T1 co-registration. MRI, magnetic resonance imaging; SPECT, single photon emission computed tomography; ROI, region of interest; REF, reference region; BR, binding ratio; MNI, Montreal Neurological Institute.

Supplemental Figure 2. Voxel-based analysis showing significant negative association between $^{123}$I-FP-CIT binding and anxiety in the left thalamus at x=-16, y=-26, z=0.