Chapter 2

ANALYSIS OF EXTRASTRIATAL
$^{123}$I-FP-CIT BINDING
CONTRIBUTES TO THE
DIFFERENTIAL DIAGNOSIS
OF PARKINSONIAN DISEASES

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ABSTRACT

$^{123}$I-FP-CIT SPECT can visualise and quantify striatal dopamine transporter (DAT) binding in vivo. In addition, $^{123}$I-FP-CIT has modest affinity for the serotonin transporter (SERT), predominantly represented in extrastriatal binding. Based on previous imaging studies that have suggested more pronounced degeneration of other monoaminergic systems in multiple system atrophy (MSA) and progressive supranuclear palsy (PSP) than in Parkinson’s disease (PD), we hypothesised that, in addition to striatal DAT binding, there would be differences in extrastriatal $^{123}$I-FP-CIT SPECT binding to SERT between MSA, PSP and PD. **Methods:** We included patients with parkinsonian type MSA (MSA-P, n=9), cerebellar type MSA (MSA-C, n=7), PSP (n=13) and PD (n=30). $^{123}$I-FP-CIT binding was analysed using region of interest (ROI) as well as voxel-based methods in both the DAT-rich striatum (caudate nucleus and putamen) and SERT-rich extrastriatal brain regions (thalamus, hypothalamus and pons). For SERT analysis, patients on selective serotonin reuptake inhibitor were excluded (n=48 remained). **Results:** In the ROI-analyses, extrastriatal $^{123}$I-FP-CIT binding ratios in the hypothalamus were significantly lower in PSP than in MSA-C patients, and we observed significantly lower striatal $^{123}$I-FP-CIT binding ratios in the caudate nucleus of PSP patients compared to both PD and MSA-C patients. In the posterior putamen, binding ratios were significantly lower in MSA-P, PSP and PD compared with MSA-C patients. Striatal ROI outcomes were confirmed by the voxel-based analyses that additionally showed a significantly lower hypothalamic binding in PSP and MSA-P compared with PD. **Conclusion:** Striatal $^{123}$I-FP-CIT binding to DAT and hypothalamic $^{123}$I-FP-CIT binding to SERT is significantly lower in MSA-P and PSP than in PD and MSA-C patients, and might therefore be of interest for differential diagnosis.
INTRODUCTION

Parkinson’s disease (PD) is a common degenerative brain disease that affects approximately 1% of persons over 60 years of age (15). Loss of dopaminergic neurons in the substantia nigra plays a major role in the aetiology of the motor symptoms that characterise PD: i.e. bradykinesia, rigidity, postural instability and resting tremor (4). These motor symptoms are also associated with other degenerative brain diseases in which dopaminergic neurons are affected: progressive supranuclear palsy (PSP; estimated prevalence 6.4/100,000 (65)) and multiple system atrophy (MSA; estimated prevalence 5.4/100,000 (65)). Clinically and neuropathologically, MSA can be divided into MSA with predominantly parkinsonian features (MSA-P) or with cerebellar features (MSA-C), occurring in an estimated ratio of 2:1, respectively (65). While MSA-C commonly shows more distinguishable clinical symptoms, the similarity of the motor symptoms in PD, MSA-P and PSP, especially in the early clinical stages, often makes it difficult to unequivocally establish an accurate clinical diagnosis. Indeed, it is not uncommon that the clinical diagnosis needs to be adjusted during the course of the disease (66), thereby inducing frustrating uncertainty of prognosis and treatment outcomes in patients and health care professionals.

\[ ^{123}\text{I}-\text{N-}\omega\text{-fluoropropyl-2\textbeta-carbomethoxy-3\textbeta-(4-iodophenyl)nortropane (}\ ^{123}\text{I-}\text{FP-CIT,}\] is a well-validated single-photon emission computed tomography (SPECT) tracer with high affinity for the dopamine transporter (DAT). Studies have shown that \(^{123}\text{I-}\text{FP-CIT}\) SPECT scans can visualise loss of nigrostriatal dopaminergic neurons (49). Consequently, this molecular imaging technique can help to distinguish PD, MSA-P or PSP from other movement disorders not characterised by dopaminergic degeneration (e.g., essential tremor) (51). However, to differentiate between PD, MSA-P and PSP using DAT imaging by \(^{123}\text{I-}\text{FP-CIT}\) SPECT remains a challenge. Although some studies have demonstrated lower striatal DAT binding in MSA-P and PSP patients than in PD patients, findings have so far been inconsistent (51, 66-74). Moreover, at the individual level there is a clear overlap in binding ratios between MSA-P, PSP and PD patients, which precludes a role for striatal DAT imaging with \(^{123}\text{I-}\text{FP-CIT}\) SPECT to differentiate between degenerative parkinsonian diseases in daily clinical practice.

In addition to its affinity for DAT, \(^{123}\text{I-}\text{FP-CIT}\) also has modest affinity for the serotonin transporter (SERT) (50), which is located on the presynaptic membrane of serotonergic neurons (75). \(^{123}\text{I-}\text{FP-CIT}\) binding to SERT is predominantly visible in extrastriatal regions: the diencephalon (hypothalamus and thalamus), the midbrain and the pons (52, 53, 56). Lower midbrain SERT binding has been reported for PSP (64) and MSA-P (70) in comparison to PD, using \(^{123}\text{I-}\text{FP-CIT}\) and \(^{123}\text{I-}\text{\beta-CIT}\) respectively, a radiotracer chemically similar to \(^{123}\text{I-}\text{FP-CIT}\). In this cross-sectional study, we therefore analysed both striatal and extrastriatal \(^{123}\text{I-}\text{FP-CIT}\) binding in patients with PD, PSP, MSA-P and MSA-C. We hypothesised that both striatal and extrastriatal \(^{123}\text{I-}\text{FP-CIT}\) binding would be lower in PSP and MSA-P than in PD and MSA-C.
MATERIALS AND METHODS

Participants
We performed a cross-sectional study on consecutive cases presented between May 2008 and December 2015 to the outpatient clinic for movement disorders of the VU University Medical Center (VUmc) in Amsterdam, the Netherlands. Clinical data and—for some patients—\(^{123}\)I-FP-CIT SPECT scans were acquired, and a consensus diagnosis was established by a multi-disciplinary team including movement disorders specialists. A clinical diagnosis of PD was based on the UK PD Society Brain Bank criteria (4, 6); for MSA-P and MSA-C, revised American Autonomic Society/American Academy of Neurology criteria (76) were used; a diagnosis of PSP was established using the National Institute of Neurological Disorders and Stroke and Society for Progressive Supranuclear Palsy criteria (40). After patients provided informed consent all data were registered in a pseudonymised database for research purposes. This procedure was approved by the local Medical Ethics Committee of VUmc.

We made a selection from this database of parkinsonian patients of whom an \(^{123}\)I-FP-CIT SPECT scan was available. The initial clinical diagnosis was confirmed with their medical records. Patients no longer returning to VUmc for clinical follow-up were approached by letter, and their current clinical diagnosis was retrieved from their neurologist. This procedure was approved by the local Medical Ethics Committee of VUmc. Of all patients meeting selection criteria, 16 had a clinical diagnosis of MSA (nine MSA-P and seven MSA-C) and 13 of PSP. MSA and PSP patients were age- and gender-matched with 30 PD patients, selected from the research database, blinded for \(^{123}\)I-FP-CIT SPECT scan outcome.

Clinical characteristics
We assessed severity of motor symptoms using the Unified Parkinson’s disease rating scale, motor section (UPDRS-III) (77). The Scales for Outcomes of Parkinson’s Disease – Autonomic symptoms subscale was used to assess autonomic symptoms (78). Hoehn & Year (79) disease stages were determined for PD patients only, since this scale was specifically designed for PD. Disease duration was defined as the time between the onset of motor symptoms, as subjectively reported by the patients, and the day of \(^{123}\)I-FP-CIT imaging.

\(^{123}\)I-FP-CIT SPECT–image acquisition and pre-processing
\(^{123}\)I-FP-CIT scans were acquired and pre-processed as described earlier by Vriend et al. (80). In short: 3-4 hours before images were acquired, \(^{123}\)I-FP-CIT was intravenously administered in a dose of approximately 185 MBq (specific activity >185 MBq/nmol; radiochemical purity >99%; produced as DaTSCAN according to GMP criteria at GE Healthcare, Eindhoven, The Netherlands). Static images were obtained for 30 minutes using a dual-head gamma camera (E.Cam; Siemens, Munich, Germany) with a fan-beam collimator. Reconstructed images were subsequently reoriented and normalised to Montreal Neurological Institute space in Statistical
Parametric Mapping 8 software (SPM 8; Wellcome Trust Centre for Neuroimaging, London, UK) using a standardised in-house $^{123}$I-FP-CIT SPECT template as described by Vriend et al. (81).

$^{123}$I-FP-CIT SPECT–image analysis

Region of Interest (ROI) analyses.

We defined ROIs for the DAT-rich caudate nucleus and the SERT-rich thalamus from the automated anatomical labelling atlas; the DAT-rich posterior putamen was based on the putamen in this atlas as described elsewhere (80); we based the SERT-rich pons ROI on the Talairach Daemon (TD) Lobes atlas; and the SERT-rich hypothalamus was based on the TD Brodmann area + atlas, and because of its small size, this region was two times dilated. All ROIs were implemented in the WFU Pickatlas (Version 3.0.5; Wake Forest University, Winston-Salem, NC, USA) (Supplemental Figure 1). We used non-specific $^{123}$I-FP-CIT binding in the cerebellum as a reference (REF; WFU Pickatlas, automated anatomical labelling atlas; bilateral Crus 2). The ratios of specific to non-specific binding (binding ratios), were calculated in SPM8 by: $\frac{(\text{ROI} - \text{REF})}{\text{REF}}$, and used as outcome measures.

Analysis with DaTQUANT.

In routine clinical practice, software is commonly used to automatically analyse striatal DAT binding, which offers the practical advantage that time-consuming (pre)processing steps are not needed. Therefore, we also used the DaTQUANT image quantification software, developed by GE Healthcare (Amersham, United Kingdom) to analyse the striatal DAT binding. This software package is written with posterior putamen and caudate nucleus as standard ROIs, and binding activity in the occipital lobe as a reference. Specific to non-specific binding ratios in the striatum were calculated as described by Siepel et al., using $\frac{[\text{mean counts ROI} - \text{mean counts reference}]}{\text{mean counts occipital lobe}}$ (82).

Voxel-based analyses.

In addition to the ROI analyses, we performed voxel-based analyses on the ROIs that showed significant between-group differences using the ROI approach. All voxels in the $^{123}$I-FP-CIT SPECT scan were adjusted by the mean binding in the cerebellar reference region according to: $\frac{[\text{voxel} - \text{REF}]}{\text{REF}}$. Voxel-based between-group analyses were performed in SPM8 and explicitly masked for the relevant ROI. Statistical threshold was set to $P<0.05$, Family-Wise Error corrected for multiple comparisons. Age was included as nuisance covariate in all analyses.

Statistics

Since selective serotonin reuptake inhibitors (SSRIs) can influence striatal and extrastriatal $^{123}$I-FP-CIT SERT binding (53), we performed the extrastriatal group analyses after excluding the patients using SSRIs ($n=48$: without SSRIs); striatal analyses were performed with and without patients using SSRIs to assert its influence. For the ROI and DaTQUANT analyses, normality of
data was assessed by plotting histograms, examining Q-Q plots, and using Kolmogorov-Smirnov test for normality. We used one-way analysis of variance (ANOVA) tests where appropriate. We used Hochberg GT2 correction for the post-hoc tests, and set the alpha level to \( P<0.05 \). Although all groups were matched for age, we performed additional analysis of covariance to test for the influence of inter-individual age differences on \(^{123}I\)-FP-CIT binding. Assumptions for analysis of covariance were met; including homogeneity of the variances and regression slopes. We analysed data that did not approximate a normal distribution by non-parametric Kruskal-Wallis tests. We performed Chi-square tests to test for equal distribution of gender among the groups. For ROI analyses and patients characteristics we used SPSS 22 (IBM Corp, Armonk, NY).

**RESULTS**

**Group characteristics**

Group characteristics are summarised in Table 1. There were no significant group differences in gender \( \chi^2 (3)=2.986, P=0.394 \) and disease duration \( K-W=4.98; P=0.173 \). There was a slight difference in age \( F(3,55)=2.322, P=0.085 \), where the MSA-P group was younger. PD patients had a median Hoehn & Yahr score of 2.5 (IQR 1.5). SSRIs were used by five PD (16.7%), three MSA-P (33.3%) and three PSP (23.1%) patients on the day of scanning, comprising citalopram \( n=5 \), paroxetine \( n=2 \), sertraline \( n=2 \), fluvoxamine \( n=1 \) and fluoxetine \( n=1 \). Excluding SSRI users had an effect on disease duration only, explained by a longer duration in PSP patients compared with the other parkinsonisms \( K-W=8.785; P=0.032 \).

**Table 1. Clinical characteristics.**

<table>
<thead>
<tr>
<th>Test statistic/df/P-value</th>
<th>PD</th>
<th>MSA-P</th>
<th>MSA-C</th>
<th>PSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>30</td>
<td>9</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Gender (f/m)</td>
<td>14/16</td>
<td>7/2</td>
<td>4/3</td>
<td>6/7</td>
</tr>
<tr>
<td>Age at DAT-SPECT</td>
<td>66.39 ± 7.55</td>
<td>61.37 ± 9.61</td>
<td>67.72 ± 10.63</td>
<td>70.46 ± 6.29</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>3.59 ± 2.95</td>
<td>3.15 ± 2.593</td>
<td>3.57 ± 1.43</td>
<td>5.69 ± 4.71</td>
</tr>
<tr>
<td>UPDRS III</td>
<td>26.8 ± 12.4</td>
<td>41.38 ± 22.83</td>
<td>36.50 ± 7.78</td>
<td>33.17 ± 12.13</td>
</tr>
<tr>
<td>SCOPA-AUT</td>
<td>35.57 ± 8.45</td>
<td>44.88 ± 12.05</td>
<td>53.00 ± 11.31</td>
<td>36.62 ± 6.62</td>
</tr>
<tr>
<td>SSRI, n(%)</td>
<td>5 (16.7)</td>
<td>3 (33.3)</td>
<td>0 (0)</td>
<td>3 (23.1)</td>
</tr>
</tbody>
</table>

(values given are mean ± standard deviation, unless otherwise specified; UPDRS III, Unified Parkinson’s disease rating scale: motor evaluation; SCOPA-AUT, Scales for Outcomes of Parkinson’s Disease—AUTonomic symptoms; K-W, Kruskal-Wallis test; \( \chi^2 \), Chi squared test; PD, Parkinson’s disease; MSA-P, Multiple system atrophy with predominantly parkinsonism; MSA-C, Multiple system atrophy with cerebellar features; PSP, progressive supranuclear palsy. *For this analysis MSA-P and MSA-C were pooled into one group.)
**ROI-based $^{123}I$-FP-CIT SPECT analyses**

**Striatal DAT binding.**

We found a significant between-group difference in $^{123}I$-FP-CIT binding ratios for all four striatal ROIs, corrected for age [caudate right: $F(3,55)=6.621, P=0.001$; caudate left: $F(3,55)=7.619, P<0.001$; posterior putamen right: $F(3,55)=6.588, P=0.001$; posterior putamen left: $F(3,55)=7.559, P<0.001$] (Table 2). Post-hoc analyses showed lower $^{123}I$-FP-CIT binding ratios in PSP in the bilateral caudate nuclei [$P=0.010$ right, $P=0.001$ left], and a trend towards lower binding ratios in the caudate of MSA-P patients [$P=0.081$ right, $P=0.078$ left] than in PD patients. MSA-C patients showed higher striatal binding ratios as compared to PSP [$P=0.005$ right; $P=0.004$ left, and posterior putamen: $P=0.003$ right; $P=0.001$ left], MSA-P [posterior putamen: $P=0.002$ right; $P=0.001$ left], and PD (left posterior putamen: $P=0.020$) (Figure 1).

Excluding patients on SSRIs had no effect on these reported results (data not shown).

**Table 2. Between-group differences in $^{123}I$-FP-CIT binding.**

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>PD</th>
<th>MSA-P</th>
<th>MSA-C</th>
<th>PSP</th>
<th>$F/df/P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate left</td>
<td>1.82 ± 0.37</td>
<td>1.39 ± 0.47</td>
<td>1.99 ± 0.54</td>
<td>1.22 ± 0.53</td>
<td>7.619/3,55/&lt;0.001</td>
</tr>
<tr>
<td>Caudate right</td>
<td>1.89 ± 0.41</td>
<td>1.43 ± 0.54</td>
<td>2.16 ± 0.55</td>
<td>1.36 ± 0.56</td>
<td>6.621/3,55/0.001</td>
</tr>
<tr>
<td>Putamen left</td>
<td>1.57 ± 0.33</td>
<td>1.22 ± 0.51</td>
<td>2.12 ± 0.51</td>
<td>1.28 ± 0.52</td>
<td>7.559/3,55/&lt;0.001</td>
</tr>
<tr>
<td>Putamen right</td>
<td>1.49 ± 0.39</td>
<td>1.08 ± 0.50</td>
<td>1.95 ± 0.46</td>
<td>1.18 ± 0.54</td>
<td>6.588/3,55/0.001</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.67 ± 0.16</td>
<td>0.45 ± 0.28</td>
<td>0.78 ± 0.30</td>
<td>0.47 ± 0.28</td>
<td>4.307/3,43/0.012</td>
</tr>
<tr>
<td>Thalamus left</td>
<td>0.79 ± 0.18</td>
<td>0.58 ± 0.27</td>
<td>0.80 ± 0.29</td>
<td>0.70 ± 0.29</td>
<td>1.576/3,43/0.236</td>
</tr>
<tr>
<td>Thalamus right</td>
<td>0.81 ± 0.18</td>
<td>0.68 ± 0.24</td>
<td>0.90 ± 0.20</td>
<td>0.68 ± 0.26</td>
<td>2.332/3,43/0.087</td>
</tr>
<tr>
<td>Pons</td>
<td>0.55 ± 0.03</td>
<td>0.41 ± 0.09</td>
<td>0.50 ± 0.05</td>
<td>0.48 ± 0.07</td>
<td>0.803/3,43/0.375</td>
</tr>
</tbody>
</table>

Extrastriatal groups (below line) depicted for patients without SSRI; $F$, df and $P$-values given are between-group analysis of covariance results, corrected for age; values given are mean ± standard deviation, unless otherwise specified; df, degrees of freedom; PD, Parkinson’s disease; MSA-P, Multiple system atrophy with predominantly parkinsonism; MSA-C, Multiple system atrophy with cerebellar features; PSP, progressive supranuclear palsy

**Figure 1.** Binding ratios per region of interest. *, statistically significant difference; +, trend. Striatal regions, including patients using SSRIs.

Extrastriatal $^{123}I$-FP-CIT in parkinsonisms 21
Consistent with our ROI-analysis, the DaTQUANT analysis showed between-group differences in the right posterior putamen, however, not on the left side \([F(3,55)=6.046, P=0.001 \text{ right}; F(3,55)=0.877, P=0.459 \text{ left}]\). There were higher binding ratios in PD than in PSP patients in the bilateral caudate nuclei \([P=0.035 \text{ right}, P=0.044 \text{ left}]\), and at trend-significant level in the left caudate nucleus \([P=0.067]\) compared with MSA-P patients. Patients with MSA-C had higher binding ratios than PSP patients in the bilateral caudate nuclei \([\text{MSA-C versus PSP: } P=0.004 \text{ right}; P=0.008 \text{ left}; \text{MSA-C versus MSA-P: } P=0.022 \text{ right}; P=0.011 \text{ left}]\) and the right posterior putamen \([\text{MSA-C versus PSP: } P=0.006; \text{MSA-C versus MSA-P: } P=0.001]\). MSA-C also showed higher binding ratios than PD in the right posterior putamen \([P=0.012]\).

**Extrastriatal SERT binding.**

\(^{123}\text{I}-\text{FP-CIT}\) binding differed significantly between diagnostic groups in the hypothalamus \([F(3,43)=4.307, P=0.010]\), but in none of the other extrastriatal ROIs (Table 2). The group difference in hypothalamic binding was due to higher binding ratios in MSA-C patients than in PSP patients \([P=0.044]\), and MSA-P patients at trend-significant level \([P=0.065]\). (Figure 2 for hypothalamus; for other regions see Supplemental Figure 2).

![Figure 2](image)

*Figure 2.* Binding ratios in the hypothalamus, without patients using SSRIs. Data on thalamus and pons are provided in the supplementary results.

**Voxel-wise \(^{123}\text{I}-\text{FP-CIT}\) SPECT imaging analysis**

**Striatal DAT binding.**

We observed significant between-group differences in striatal \(^{123}\text{I}-\text{FP-CIT}\) binding ratios. In PD patients, binding ratios were higher in the caudate nucleus than in patients with PSP (both caudate nuclei) and MSA-P (right caudate nucleus). MSA-C patients had higher binding ratios than PSP patients (both caudate nuclei), and MSA-P patients (right caudate nucleus). In the posterior putamen, MSA-C patients showed bilaterally higher \(^{123}\text{I}-\text{FP-CIT}\) binding ratios compared with all
other groups; PD patients showed higher $^{123}$I-FP-CIT binding ratios compared with PSP patients in the right posterior putamen (Table 3; Supplemental Figures 3-6).

**Table 3. Voxel-by-voxel analyses.**

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Group1 &gt; Group2</th>
<th>$K_e$</th>
<th>$P_{fwe \text{ peak-voxel}}$</th>
<th>$T$</th>
<th>$x/y/z \text{ (mm)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate nucleus left (df\ (1,54))</td>
<td>PD</td>
<td>13</td>
<td>0.005</td>
<td>4.80</td>
<td>-14/4/20</td>
</tr>
<tr>
<td></td>
<td>MSA-C</td>
<td>4</td>
<td>0.008</td>
<td>4.67</td>
<td>-12/18/12</td>
</tr>
<tr>
<td>Caudate nucleus right (df\ (1,54))</td>
<td>PD</td>
<td>22</td>
<td>0.005</td>
<td>4.85</td>
<td>12/4/20</td>
</tr>
<tr>
<td></td>
<td>MSA-C</td>
<td>2</td>
<td>0.024</td>
<td>4.30</td>
<td>16/0/24</td>
</tr>
<tr>
<td></td>
<td>MSA-P</td>
<td>2</td>
<td>0.018</td>
<td>4.41</td>
<td>18/0/18</td>
</tr>
<tr>
<td>Posterior putamen left (df\ (1,54))</td>
<td>MSA-C</td>
<td>41</td>
<td>0.004</td>
<td>4.69</td>
<td>-26/2/4</td>
</tr>
<tr>
<td></td>
<td>MSA-P</td>
<td>16</td>
<td>0.007</td>
<td>4.51</td>
<td>-26/2/4</td>
</tr>
<tr>
<td></td>
<td>PD</td>
<td>33</td>
<td>0.003</td>
<td>4.83</td>
<td>-26/14/6</td>
</tr>
<tr>
<td>Posterior putamen right (df\ (1,54))</td>
<td>PD</td>
<td>2</td>
<td>0.018</td>
<td>4.17</td>
<td>36/16/8</td>
</tr>
<tr>
<td></td>
<td>MSA-C</td>
<td>30</td>
<td>0.004</td>
<td>4.73</td>
<td>28/10/2</td>
</tr>
<tr>
<td></td>
<td>MSA-P</td>
<td>28</td>
<td>0.001</td>
<td>5.22</td>
<td>28/10/2</td>
</tr>
<tr>
<td></td>
<td>PD</td>
<td>13</td>
<td>0.002</td>
<td>4.88</td>
<td>32/12/8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>&lt;0.001</td>
<td>5.73</td>
<td>28/10/2</td>
</tr>
<tr>
<td>Hypothalamus* (df\ (1,43))</td>
<td>PD</td>
<td>17</td>
<td>&lt;0.001</td>
<td>5.93</td>
<td>-4/0/10</td>
</tr>
<tr>
<td></td>
<td>MSA-P</td>
<td>6</td>
<td>0.002</td>
<td>4.79</td>
<td>-6/0/10</td>
</tr>
<tr>
<td></td>
<td>MSA-C</td>
<td>8</td>
<td>&lt;0.001</td>
<td>5.47</td>
<td>-2/0/8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>0.002</td>
<td>4.79</td>
<td>-6/0/10</td>
</tr>
</tbody>
</table>

Difference between two diagnoses for specific cluster; \(df\), degrees of freedom; $P_{fwe}$, family-wise error corrected \(P\)-values; $K_e$, cluster extend in no of voxels; \(T\), \(T\)-statistic; \(x/y/z\), location of significantly most different between groups cluster from midpoint in millimetre; PD, Parkinson’s disease; MSA-P, Multiple system atrophy with predominantly parkinsonism; MSA-C, Multiple system atrophy with cerebellar features; PSP, progressive supranuclear palsy. *For this analysis, patients not on SSRI’s were included.

**Extrastriatal SERT binding.**

Figure 3 shows the results of the between-group analysis for the hypothalamus. Post-hoc tests revealed that PD and MSA-C had higher binding ratios than PSP and MSA-P patients (Table 3).

**MRI scans**

MRI scans of the brain were available for 10 out of 13 PSP patients (77%): six scans showed mesencephalic atrophy, typical for PSP, and four were rated as normal. MRI scans were also available for six out of the nine MSA-P patients (67%): two scans showed atrophy in the basal ganglia, one cerebellar atrophy with a hot cross bun sign, typical for MSA, and three showed global cortical atrophy. A brain MRI was available for five out of the seven (71%) examined MSA-C patients, which showed atrophy of the cerebellum, except for one which showed global cortical atrophy. MRI scans were available in 24 out of the 30 examined PD patients (80%): In 18
PD patients, the MRI scan showed no atrophy, whereas four scans showed slight global cortical atrophy and two had a more advanced global cortical atrophy.

**Figure 3.** Voxel-by-voxel analysis of hypothalamus at (x,y,z) -2,0,-8. Lower panel shows quantification per diagnosis.

**DISCUSSION**

The current study explored the use of both striatal and extrastriatal binding ratios derived from a single $^{123}$I-FP-CIT SPECT to help distinguish between patients with clinical diagnoses of PD, MSA-P, MSA-C and PSP. $^{123}$I-FP-CIT binding ratios in the caudate, putamen and in the hypothalamus were significantly lower in PSP and MSA-P patients compared with PD or MSA-C patients.

In the striatal analysis, we found significantly lower $^{123}$I-FP-CIT binding ratios in the caudate nucleus of PSP patients in comparison to PD patients, and a trend-significant difference in caudate binding between MSA-P and PD patients. Several previous studies have demonstrated lower overall striatal DAT binding in PSP and MSA-P compared to PD, with relative sparing of the caudate nucleus in PD (66, 68, 69, 74). Already in early disease stages, patients initially diagnosed with PD that later convert to MSA or PSP, have lower DAT binding in the caudate
nucleus than patients in whom a diagnosis of PD is maintained (66). In addition, our findings in the posterior putamen in the ROI analysis corroborate the results of earlier studies by Oh and Messa (68, 69). The automated DAT binding analyses resulted in comparable data, suggesting this type of approach is useful when assessing striatal binding in routine clinical practice.

In the present study, ROI-based analysis revealed lower extrastriatal $^{123}$I-FP-CIT binding in the hypothalamus in PSP patients and a trend towards lower binding in MSA-P patients, both compared with MSA-C patients. This finding was confirmed by our voxel-based analysis, although this analysis additionally showed lower $^{123}$I-FP-CIT binding in PSP and MSA-P patients compared with PD patients. This is likely due to the difference in the method of the binding ratio calculation (i.e. mean binding vs. voxel-wise). Combining the results of both analyses, hypothalamic SERT availability appears to be reduced in MSA-P and PSP compared with PD and MSA-C patients.

Literature on the role of extrastriatal SERT in the differential diagnosis of PD, MSA and PSP is scarce. Nevertheless, higher SERT binding has been observed in the midbrain and pons of PD patients relative to MSA-P and PSP patients (64, 70), and whole brain analysis has been reported to be informative before (74). In the hypothalamus, SERT has been shown to be ubiquitously present in controls in a post-mortem human brain study (83). In line with this, PET studies using selective SERT tracers as well as an $^{123}$I-FP-CIT SPECT study have shown specific hypothalamic binding in healthy controls (84, 85). Moreover, $^{123}$I-FP-CIT binding in the hypothalamus of rats could be blocked with an SSRI, but not with a DAT blocker (49). Therefore, it is likely that $^{123}$I-FP-CIT binding in the hypothalamus represents predominantly binding to the SERT.

Regarding observations on the role of serotonin in the hypothalamus in MSA or PSP patients, loss of serotonergic neurons in the hypothalamus has been shown in an autopsy study in MSA (86). Chinaglia and co-workers (87) found a decrease of SERT binding in the cortex and caudate nucleus in PSP, although findings were not consistent (88). Nevertheless, although no autopsy study looked specifically into SERT expression in the hypothalamus in MSA or PSP, our present data may motivate examination of SERT in the hypothalamus in future autopsy studies.

Data on hypothalamic tracer binding in the differential diagnosis is also not abundant, yet lower in vivo SERT availability in the hypothalamus of MSA patients compared with healthy controls; lower hypothalamic $^{18}$F-FP-CIT binding (reflecting predominantly SERT binding) in PSP than in PD patients; and lower SERT-specific $^{11}$C-DASB binding (which reflects SERT binding, since $^{11}$C-DASB is a selective SERT tracer) in PD patients than healthy controls have been reported previously (89-91). To our knowledge, a $^{11}$C-DASB PET study that examined the availability of SERT in the hypothalamus in MSA and/or PSP as compared to PD has not been performed before.

The hypothalamus is an important brain area for autonomic functions, such as the stress responses: these functions are partly regulated by serotonin (92). Since patients with MSA-P have prominent autonomic symptoms and neuronal inclusion bodies have been demonstrated in the hypothalamus in both MSA-P and MSA-C (93), a logical assumption would be that loss
of SERT-expressing neurons, represented by lower $^{123}$I-FP-CIT binding, accounts for dysregulation of these autonomic functions in MSA-P. While some studies looked into the relationship between loss of serotonin and autonomic symptoms in MSA, to our knowledge, no studies examined the role of serotonin in the hypothalamus in PSP. However, our present data may underscore the need to look into this in future studies.

We observed marked differences in both striatal DAT and extrastriatal SERT binding ratios when comparing MSA-P with MSA-C patients, with overall higher binding ratios in MSA-C patients. In fact, two MSA-C patients had scans that in the clinic were diagnostically rated as normal, something that has also been observed previously (94, 95) (see Supplemental Fig. 7 for an example). Our results are consistent with another study that observed less severe loss of DAT binding in striatal regions in MSA-C compared with MSA-P patients, as measured by $^{18}$F-FP-CIT positron emission tomography (PET) (96). Jakobson Mo et al. found lower striatal $^{123}$I-FP-CIT binding ratios in PD and PSP compared with MSA patients; however, they made no distinction between MSA-P and MSA-C, which possibly explains why they found lower binding in PD than in MSA in contrast with our results (51).

Some patients in this study were using SSRIs, which may interfere with striatal $^{123}$I-FP-CIT binding in healthy subjects (53). However, we did not find binding differences when we compared patients using SSRIs to those without SSRIs (data not shown). This has previously been described for PD patients chronically using SSRIs in a comparison with patients not using SSRIs, when corrected for disease duration (97). Apparently, the striatal effects of SSRI use on striatal $^{123}$I-FP-CIT binding are small in chronically treated parkinsonian patients. Considering the use of SSRIs is a reality in daily clinical practice: in our sample 18.6% of patients were using an SSRI on the day they were scanned. It is important to know that SSRI use in parkinsonian patients is not a confounder in analysis of striatal $^{123}$I-FP-CIT binding ratios. Noticeably, use of an SSRI probably does confound the analysis of extrastriatal binding; therefore, we excluded SSRI users from our extrastriatal analysis.

A limitation of this study is the fact that the limited resolution of SPECT cameras might impede an accurate measurement of small areas such as the hypothalamus. To cope with this, we dilated the mask of the hypothalamus. Nevertheless, these results are in need of replication, for example with the SERT-selective PET tracer $^{11}$C-DASB. Furthermore, we had a small sample size for patients with MSA and PSP, a consequence of the low incidence of MSA and PSP in the population (65). The sample was, however, well documented and most of the patients had a substantial follow-up, which is imperative to deal with the problem of clinical diagnostics where decision-adjusting symptoms might arise during the course of the disease. Although our study had a retrospective design, which in itself also harbours some limitations, we were able to achieve a relatively high degree of diagnostic certainty. Particularly in the MSA-P patients, the MRI scans showed signs of atrophy in the basal ganglia, which supported the clinical diagnosis. We did not correct for atrophy in this study, since MRI scans were not available for each
subject. Consequently, we cannot exclude that (part) of the loss of DAT binding in the putamen, particularly in the MSA-P patients, is caused by local atrophy.

CONCLUSION

This hypothesis-generating study suggests the presence of information relevant for the difference in pathophysiology, and possibly in the differential diagnosis of PD, PSP, MSA-P and MSA-C by examining $^{123}$I-FP-CIT binding in extrastriatal brain areas.
**SUPPLEMENT**

*Supplemental Figure 1:* Regions of interest (ROIs) used in the analysis. Top panel: striatal ROIs. Lower panel: extrastriatal ROIs.

*Supplemental Figure 2:* Extrastriatal binding ratios per region of interest, without patients using SSRIs.
Supplemental Figure 3: Voxel-by-voxel analysis of caudate nucleus left at \((x,y,z)\) -14,4,20. Lower panel shows quantification per diagnosis.

Supplemental Figure 4: Voxel-by-voxel analysis of caudate nucleus right at \((x,y,z)\) 18,0,18. Lower panel shows quantification per diagnosis.
**Supplemental Figure 5:** Voxel-by-voxel analysis of posterior putamen left at (x,y,z) -26,-2,4. Lower panel shows quantification per diagnosis.

**Supplemental Figure 6:** Voxel-by-voxel analysis of posterior putamen right at (x,y,z) 28,-10,2. Lower panel shows quantification per diagnosis.
Supplemental Figure 7: Array of $^{123}I$-FP-CIT SPECT scans of different patients.