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Decreased noradrenaline transporter density in the motor cortex of Parkinson’s disease patients

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Abstract

Background: Reduced noradrenaline levels have been reported to occur in the motor cortex of PD patients at post-mortem. Imaging techniques have recently become available to specifically study noradrenergic terminal function in vivo using PET.

Methods: 30 PD patients and 12 healthy control subjects, comparable across age, sex, and cognitive performance underwent PET imaging with $^{11}$C-MeNER, a specific ligand of the noradrenaline transporter (NAT). Cortical NAT binding was compared at a voxel level using Statistical Parametric Mapping while cortical thickness was assessed using FreeSurfer software with MRI.

Results: PD patients showed reduced $^{11}$C-MeNER binding in the primary motor cortex unrelated to cortical thickness; other cortical regions did not differ between groups. In a subgroup analysis, patients with higher Hoehn & Yahr stage exhibited more pronounced $^{11}$C-MeNER binding reductions.

Conclusion: Loss of cortical noradrenergic projections to the primary motor cortex occurs in PD associated with disease stage.
Introduction

Parkinson’s disease (PD) is a neurodegenerative disorder affecting several major neurotransmitter systems. In particular, post-mortem studies have reported considerable degeneration of the locus coeruleus (LC), which supplies the cortex with noradrenaline. At post-mortem, multiple brain regions were reported to exhibit reduced levels of noradrenaline, including cerebellar cortex, thalamus, and cerebral cortex including primary motor cortex. In vivo imaging using positron emission tomography (PET) with $^{18}$F-DOPA is a marker of aromatic aminoacid decarboxylase activity in monoaminergic terminals, including those of dopaminergic, serotonergic and noradrenergic neurons. Moore and colleagues reported a decreased signal specifically in PD motor cortex. Newer PET ligands based on a reboxetine structure, such as $^{11}$C-MeNER, provide a specific marker of noradrenaline transporter function, and a recently proposed MeNER PET analytical approach allowed in vivo estimation of cortical noradrenaline transporter availability in healthy subjects.

The objective of this $^{11}$C-MeNER PET study was to examine the hypothesis that noradrenaline transporter function is decreased in cortical regions of PD patients compared to matched healthy control (HC) subjects.
Methods

Study design and participants
We recruited 30 PD patients for imaging with $^{11}$C-MeNER PET. Inclusion criteria were: diagnosis of PD according to the Movement Disorder Society (MDS) consensus,\textsuperscript{10} age 50 - 85 years, a geriatric depression scale (GDS-15) score <6, and Montreal cognitive assessment (MoCA) score >22. Exclusion criteria were: a diagnosis of dementia, receiving medication acting on the noradrenergic transporter, and significant white matter lesions. Total levodopa-equivalent daily doses (LEDD) were calculated as previously recommended.\textsuperscript{11} Olfaction was tested with the 16-item Sniffin’ Sticks battery. Motor symptoms were scored using the MDS Unified PD Rating Scale part III (MDS-UPDRS III) after 12 hours of medication abstinence. Clinical disease duration and modified Hoehn & Yahr stage were recorded. Presence of REM sleep behavior disorder (RBD) was assessed with overnight polysomnography and a clinical interview for features of dream enactment.\textsuperscript{12} Demography of PD patients was compared to 12 age and sex matched, cognitively normal (MoCA >26) and non-depressed (GDS-15 <6) HC subjects without a history of neurological disorders and with a normal brain MRI (Table 1). A volume of interest analysis of NAT density in subcortical structures of the 42 subjects was previously reported.\textsuperscript{12}

The study was approved by the local ethical committee. All subjects gave informed written consent according to the Declaration of Helsinki.

$^{11}$C-MeNER PET
Details of $^{11}$C-MeNER radiosynthesis and image reconstruction and normalization are provided as supplementary material. A 4 mm Gaussian filter was applied to the normalized images before calculation of parametric maps of binding potentials non displaceable (£BP_{ND}$) using the simplified reference tissue model 2 (SRTM2). Voxel-wise calculations in PXMOD were initiated with time activity curves of the thalamus as a high binding region and the caudate as a low binding region; the efflux constant of the reference region was fixed at $k2^* = 0.021 \text{ min}^{-1}$ as previously published.\textsuperscript{7,9} $BP_{ND}$ values of the left + right precentral and postcentral gyrus were extracted for group comparisons.

MRI
An MPRAGE sequence was acquired on a Siemens Magnetom Tim Trio scanner using a 32 channel headcoil and the following parameters: TR/TI/TE, 960/2420/3.7ms; flipangle, 9 degrees; number of slices, 176; FOV 256x256mm; 1mm isotropic resolution, and an iPAT factor of 2 using grappa. Cortical grey
matter thickness was estimated using the standard presets in FreeSurfer 5.3 (http://surfer.nmr.mgh.harvard.edu/).

**Statistical parametric mapping (SPM)**

All individual parametric maps were non-rigidly transformed to an $^{11}$C-MeNER spatial template derived from an averaged image of the 12 HC subjects. Statistical Parametric Mapping 12 (SPM12) in Matlab2015b was used for between group comparisons of $^{11}$C-MeNER binding potentials at a voxel level (two-sample t-test between PD patients and HC subjects). Parametric maps were smoothed with a 4 mm Gaussian filter, and an average cortical grey matter mask was used for explicit masking within the SPM software. Voxel-level analysis threshold was $p < 0.005$ (uncorrected) and subsequently a family-wise error (FWE) cluster-level correction at $p < 0.05$ was applied. Surface projections of resulting voxels were overlaid on a rendered brain of one HC subject.

**Statistical analysis**

We analyzed the data with the Statistical Package for the Social Sciences (SPSS) version 24. Group data were presented as mean ± standard deviation. Group comparisons were interrogated with the Student’s t, Mann-Whitney, and Chi-square statistics as appropriate; a normal distribution of data was assessed with the Shapiro-Wilk test. Volume of interest (VOI) analysis of $B_{PD}$ values was tested with a repeated measures analysis of variance (ANOVA). Significance was accepted at $p < 0.05$. 
Results

Demographic and clinical characteristics of PD patients and HC subjects are summarized in table 1. Groups did not differ with regards to age, sex and MoCA scores. In the voxel-wise comparison, SPM localised lower cortical $^{11}$C-MeNER binding in PD patients tracking the primary motor cortex and, to a lesser extent, the primary sensory cortex (Figure 1A). Similarly, VOI analysis of the precentral and postcentral gyrus showed significant reduced $^{11}$C-MeNER BP$_{ND}$ values in PD patients (ANOVA, p = 0.006; precentral left $0.166 \pm 0.081$ vs. $0.044 \pm 0.123$, p = 0.003, precentral right $0.124 \pm 0.087$ vs. $0.022 \pm 0.122$, p = 0.012, postcentral left $0.131 \pm 0.074$ vs. $0.022 \pm 0.127$, p = 0.008, postcentral right $0.125 \pm 0.96$ vs. $0.016 \pm 0.115$, p = 0.006). To correct for potential partial volume effects on binding signals, we compared cortical thickness of the primary motor cortex between groups, which did not differ between PD patients and HC subjects ($2.44 \pm 0.20$ versus $2.43 \pm 0.24$ mm; p = 0.80, and for primary sensory cortex $1.94 \pm 0.13$ versus $1.95 \pm 0.18$ mm; p = 0.76, respectively). Subgroup analysis of PD patients divided by a cut-off at Hoehn & Yahr stage 2.5 (resulting in n = 15 PD subjects in each group), revealed more widespread reductions of $^{11}$C-MeNER binding in PD patients with a higher disease stage when contrasted against healthy controls (Figure 1B). However, a direct comparison of mild and more severely affected PD groups revealed no significant NAT cortical binding differences.
Discussion

Previous $^{11}$C-MeNER studies on PD patients from our group targeted subcortical high binding areas of MeNER and revealed a reduced $^{11}$C-MeNER signal in the thalamus and hypothalamus as well as brainstem nuclei. Using the recently proposed imaging analysis approach for cortical MeNER uptake from Moriguchi and colleagues, we here report first in vivo evidence of impaired noradrenergic function in the primary motor cortex in PD patients using a specific PET ligand for noradrenergic transporter binding. Additionally, we show that patients with more advanced clinical disease have a greater decline of $^{11}$C-MeNER uptake in the primary motor cortex.

Growing evidence suggest an involvement of multiple neurotransmitters in PD and, in line with the results from the present study, point to significant dysfunction of the noradrenergic system associated with more severe motor disability. The LC is affected by alpha synuclein aggregates in Braak stage 2 of PD during its trajectory which precedes and may even surpass later involvement of the substantia nigra. Evidence from animal studies has implicated noradrenergic dysfunction in the appearance and aggravation of motor symptoms. Several studies reported that deficient noradrenergic innervation accompanied dopaminergic cell loss in toxin-derived PD models. In mice with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced dopaminergic lesions, the PD-like motor phenotype is only manifested when there is also noradrenergic co-deficiency. Noradrenaline deficits also attenuated beneficial levodopa replacement effects on motor symptoms in rats lesioned with 6-hydroxydopamine. Additionally, amphetamines could restore locomotor activity in a dopamine-independent way in a novel PD mouse model. Data on manipulation of the noradrenergic system to improve motor function in human PD is scarce. One small scale, open-label trialled duloxetine, a combined serotonin and noradrenaline reuptake inhibitor (SNRI), and reported beneficial effects on motor symptoms in PD. Of note, paroxetine, which is a selective serotonin reuptake inhibitor (SSRI), did not improve motor symptoms. Methylphenidate, which is a dual dopamine and noradrenaline reuptake inhibitor, showed beneficial effects on motor symptoms in PD; however, its effects became insignificant with co-administration of high doses of levodopa. The noradrenergic prodrug droxidopa is approved for orthostatic hypotension in PD and was reported to improve UPDRS III scores in one study.

PD is pathologically characterized not only by perturbed function of the substantia nigra and basal ganglia circuitry, but also of cortical networks, including dysfunction of primary motor cortex connections. Neural plasticity of the primary motor cortex is altered in PD, and facilitation of its excitability by transcranial direct current stimulation has shown to improve motor performance in PD patients. Concordantly, abnormal suppression of electroencephalographic activity during a motor task was observed in PD patients. Using $^{18}$F-DOPA PET, Moore and colleagues reported that the primary motor cortex exhibited a monoaminergic deficit in early PD which then spread anteriorly and laterally with disease.
A cortical deficit of the noradrenergic system has also been reported in a post-mortem study.² Our in vivo results confirm a noradrenergic influence on motor symptoms and altered functioning of the primary motor cortex in PD.

Several limitations of our study have to be mentioned. Cortical binding potentials of ¹¹C-MeNER tend to be low, lowering statistical power.⁹ However, we recruited a relatively robust number of 42 subjects for PET and employed corrected SPM analyses as well as VOI based statistics. As specific binding was very low in the cortex of PD patients, we also minimized subgroup analysis to avoid low statistical power. Larger sample sizes and future noradrenergic PET ligands with higher signal to noise properties may be able to detect more subtle deficits in PD and might facilitate analysis of specific PD subgroups and their association to cortical noradrenergic innervation. These studies should also address laterality of PD symptoms and its correlation to cortical noradrenergic innervation. We also did not assess potential reductions of other monoamines. Future studies using multi-tracer approaches are needed for quantifying and comparing deficits of specific monoamines in the motor cortex of PD.⁶

In conclusion, this study provides in vivo evidence for altered noradrenergic transmission in the motor cortex in PD patients, which is associated with greater disease severity. Thus, our results suggest that noradrenergic dysfunction make a contribution to the motor deterioration in PD.
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Contributors

Research project: A. Conception: MS, PB, DJB; B. Organization: MS, PB, DJB, YF; C. Execution: MS, PB, AKH, TDF, KK, YF, AN
Statistical Analysis: A. Design: MS, PB, DJB; B. Execution: MS, PB; C. Review and Critique: all authors
Manuscript: A. Writing of the first draft: MS, PB; B. Review and Critique: all authors.

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References


Figure legend

**Figure 1: Reduction of $^{11}$C-MeNER binding in the motor cortex of PD patients**

Voxels with significantly lower $^{11}$C-MeNER binding potentials in PD patients compared to HC subjects are given in purple. Maps were derived with statistical parametric mapping 12 (SPM 12) using a voxel threshold of $p < 0.005$ (uncorrected) and a cluster threshold of $p < 0.05$ with family-wise error correction. **A:** Comparison of all 30 PD patients contrasted against 12 HC subjects. **B:** Subgroup analysis of PD patients with Hoehn & Yahr stage $< 2.5$ (left; $n = 15$) and patients with Hoehn & Yahr stage $\geq 2.5$ (right; $n = 15$) contrasted against 12 HC subjects. In PD patients with Hoehn & Yahr stage $< 2.5$, voxels with threshold at $p < 0.005$ (uncorrected) are presented without cluster correction as no cluster survived the family-wise error cluster correction. Abbreviations: HC = healthy control, PD = Parkinson’s disease.
### Tables

#### Table 1: Demographics and clinical characteristics

<table>
<thead>
<tr>
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<th>HC (n=12)</th>
<th>PD (n=30)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Age</td>
<td>67.3 ± 6.3</td>
<td>66.6 ± 9.1</td>
<td>ns&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex (male / female)</td>
<td>9 / 3</td>
<td>22 / 8</td>
<td>ns&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sniffing sticks, correct</td>
<td>11.1 ± 2.8</td>
<td>7.1 ± 2.7</td>
<td>&lt;0.001&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>GDS-15, total</td>
<td>0.2 ± 0.4</td>
<td>1.0 ± 1.4</td>
<td>ns&lt;sup&gt;¤&lt;/sup&gt;</td>
</tr>
<tr>
<td>MoCA, total</td>
<td>28.3 ± 0.9</td>
<td>26.8 ± 2.2</td>
<td>ns&lt;sup&gt;¤&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**PD characteristics**

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<table>
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<tr>
<td>Disease duration [years]</td>
<td>6.4 ± 4.3</td>
</tr>
<tr>
<td>Hoehn &amp; Yahr</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>MDS-UPDRS III, total</td>
<td>36.1 ± 11.5</td>
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<tr>
<td>LEDD [mg]</td>
<td>620 ± 404</td>
</tr>
<tr>
<td>RBD (yes / no)</td>
<td>16 / 14</td>
</tr>
</tbody>
</table>

<sup>†</sup> = parametric test (Student’s t-test),<sup>¤</sup> = non-parametric test (Mann-Whitney test),<sup>§</sup> = χ² test. Abbreviations: GDS-15 = Geriatric Depression Scale, HC = healthy control subject, LEDD = levodopa daily equivalent dose, MDS-UPDRS III = Movement Disorder Society Unified Parkinson’s disease Rating Scale part III, MoCA = Montreal Cognitive Assessment, ns = not significant, PD = Parkinson’s disease, RBD = REM sleep behavior disorder.
Supplementary material

11C-MeNER PET image acquisition and reconstruction

The synthesis of 11C-MeNER was performed accordingly to a previously published protocol.1 A six minute transmission scan was acquired for attenuation correction prior to each 11C-MeNER emission scan. Radiotracer was intravenously injected and 11C-MeNER doses did not differ between groups (HC, 646.3 ± 119.6, PD, 707.6 ± 106.5 MBq; p = 0.146). Dynamic PET was acquired over 90 minutes in list-mode on a Siemens high-resolution research tomograph (HRRT) and then rebinned into 27 time frames (6 x 30 s, 3 x 60 s, 2 x 120 s, and 16 x 300 s). Image series were reconstructed using 3D OSEM (ordered subsets expectation maximization) and resolution recovery modelling (PSF) with 10 iterations and 16 subsets. Reconstructed images consisted of 207 axial image slices with an isotropic 1.22 mm voxel size. Reconstructed images were processed with PMOD 3.8 and its relevant toolboxes, including time frame realignment for motion correction. All PET images were then normalized into Montreal Neurological Institute (MNI) space including rigid matching of subject’s PET to the anatomical magnetic resonance image (MRI), and MRI segmentation using the PNEURO tool. Volumes of interest (VOIs) of the caudate, thalamus and precentral as well postcentral gyrus were extracted from the PMOD built-in atlas.

References: