
Assessment of neuroinflammation in patients with idiopathic rapid-eye-movement sleep behaviour disorder: A case-control study.

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NEUROINFLAMMATION IN IDIOPATHIC RAPID-EYE-MOVEMENT SLEEP BEHAVIOUR DISORDER: A CASE-CONTROL STUDY

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Abstract

Background

Longitudinal follow-up studies in patients with idiopathic rapid-eye-movement sleep behaviour disorder (iRBD) have shown that the majority eventually develop the synucleinopathies Parkinson’s disease, dementia with Lewy bodies or multiple system atrophy. Neuroinflammation in the form of microglial activation is present in synucleinopathies and provides a possible therapeutic target to halt or delay the neurodegenerative process. This prospective case-control positron emission tomography (PET) study investigates whether neuroinflammation is present in iRBD and its possible relation to nigrostriatal dopamine function.

Methods

Twenty consecutive patients with polysomnography-confirmed iRBD and no clinical evidence of parkinsonism and cognitive impairment were recruited between March 2015 and October 2016 in our tertiary sleep centres in Spain (Barcelona) and Denmark (Aarhus). We assessed levels of microglial activation in the substantia nigra, putamen, and caudate using $^{11}$C-PK11195 PET and dopamine terminal function in putamen and caudate with $^{18}$F-DOPA PET. Nineteen healthy controls underwent either $^{11}$C-PK11195 PET (n=10) or $^{18}$F-DOPA PET (n=9) for comparison.
Findings

$^{11}$C-PK11195 binding was increased in the substantia nigra of iRBD patients compared to controls on the left side (Student’s t test, mean difference = 0.153, 95% CI [0.055 to 0.250], p=0.003) but not on the right side (0.121, [-0.007 to 0.250], p=0.064). $^{11}$C-PK11195 binding was not significantly increased in the putamen and caudate of iRBD patients. $^{18}$F-DOPA uptake was reduced in iRBD in the left (-0.0032, [-0.0044 to -0.0021], p<0.0001) and right putamen (-0.0032, [-0.0044 to -0.0020], p<0.0001) but not in the caudate nuclei.

Interpretation

In patients with iRBD, PET imaging detects increased microglial activation in the substantia nigra along with reduced putamen dopaminergic function. Further studies including more subjects and longitudinal follow-up of the present patients are needed to support our current findings and evaluate if the presence of activated microglia in iRBD represents a marker of short-term conversion to a clinically defined synucleinopathy.

Funding

The study was funded by the Danish Council for Independent Research and the Spanish Instituto de Salud Carlos III.
Background

Parkinson disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) are three progressive neurodegenerative disorders characterised by abnormal aggregation and accumulation of the protein \(\alpha\)-synuclein in neurons, neurites, or oligodendroglial cells and dopamine deficiency in the nigrostriatal system. Accumulative data indicate that the aggregation of \(\alpha\)-synuclein in affected individuals occurs years before the occurrence of the cardinal symptoms of these disorders, namely parkinsonism and dementia.\(^1\)\(^2\) Therefore, the identification of subjects in the prodromal phase of these diseases, along with detection of biomarkers showing early pathological changes indicating manifest disease, is a high priority. This may contribute to the rational development of therapeutic strategies to halt or delay disease progression in its earliest stages.\(^3\)

Rapid-eye-movement (REM) sleep behaviour disorder (RBD) is a parasomnia characterized by dream-enacting behaviours, nightmares and lack of muscle atonia during REM sleep. Available data indicate that the idiopathic form of RBD (iRBD) precedes the development of PD, DLB, and much more rarely MSA.\(^4\)\(^6\) Prospective studies of iRBD patients show a risk of 41% for progression to PD or DLB within 5 years from iRBD diagnosis (cohort size \(n=279\)), and up to 91% by 14 years
(cohort size n=174).\textsuperscript{4} Imaging studies in iRBD patients have detected findings similar to those observed in patients with established PD and DLB such as impaired integrity of the dopaminergic nigrostriatal pathway.\textsuperscript{7-9} Additionally, Lewy type pathology, a neuropathological hallmark of PD and DLB, has been observed in the brains of iRBD cases.\textsuperscript{10,11} Neuroinflammation, a possible disease-causing mechanism that is present in patients manifesting synucleinopathies has not been investigated in iRBD with \textit{in vivo} molecular imaging. Using \textsuperscript{11}C-PK11195 positron emission tomography (PET), an \textit{in vivo} marker of microglial activation, several studies have shown increased brain signal in manifest PD, DLB, and MSA.\textsuperscript{12-15} Microglia are the resident immune cells of the central nervous system, and account for the intrinsic inflammatory response to neuronal damage. It is thought that protracted activation of microglia cells may contribute to the development and progression of the neurodegenerative process.\textsuperscript{16} Modulation of microglial activation could provide a potential therapeutic strategy for slowing disease progression, particularly in its earliest stages.\textsuperscript{17-20} The objective of this \textit{in vivo} PET imaging study was to examine the hypothesis that patients with iRBD show increased nigrostriatal levels of activated microglia as part of the neuropathological process. Additionally, we aimed to investigate the relationship between neuroinflammation and
Methods

Study population

This prospective case-control study was conducted between March 23rd, 2015, and October 19th, 2016. Twenty consecutive patients with polysomnography-confirmed iRBD according to established criteria\textsuperscript{17} were recruited from tertiary Sleep Clinics at Aarhus University Hospital, Denmark (n=10) and the Multidisciplinary Sleep Unit of the Hospital Clinic de Barcelona, Spain (n=10). Prior to inclusion, patients underwent a full clinical history and examination to exclude a neurological condition.\textsuperscript{18-20} None of the patients had motor or cognitive complaints. A healthy control group consisting of nineteen subjects who had no motor or cognitive complaints, a normal neurological examination, and a mean group age similar to the iRBD group were recruited to participate in the current study through newspaper advertisements (44 subjects responded, the first eligible subjects were included). They were randomly selected to receive either $^{11}$C-PK11195 PET (n=10) or $^{18}$F-DOPA PET (n=9). Absence of RBD symptoms in the controls was screened by a questionnaire\textsuperscript{21}, and a comprehensive clinical
history of the individuals and their bed-partner. None of the included subjects were regular users of anti-inflammatory drugs or were taking antidepressants at the time of this study.

The study received approval from the local Ethics Committee at both centers. All subjects gave informed written consent according to the Declaration of Helsinki before enrolment into the study.

**PET and MRI imaging**

All subjects had PET imaging at the Department of Nuclear Medicine & PET Centre, Aarhus University Hospital, Denmark. Dynamic PET scans were performed with a Siemens High-Resolution Research Tomograph (ECAT HRRT; CTI/Siemens). Patients from Spain flew to Denmark to undergo PET studies. $^{11}$C-PK11195 PET was used to assess levels and extent of brain microglial activation and $^{18}$F-DOPA PET to assess nigrostriatal dopamine function.

Patients had the PET studies over two separate days while control subjects had either $^{11}$C-PK11195 PET or $^{18}$F-DOPA PET to reduce their radiation exposure. Detailed information on PET scanning procedure is provided in online appendix.
Participants had a structural MRI 3D T\textsubscript{1} MP2RAGE sequence for co-registration of the PET images (3 T MAGNETOM Skyra, Siemens Healthcare).

**Image analysis**

PET images were analysed using regions of interest (ROI) with the PNEURO module of PMOD software v 3.6 (PMOD technologies Ltd. Switzerland). The following predefined regions of interest were investigated: Substantia nigra, dorsal putamen (posterior to the anterior commissure), and the caudate nucleus. However, since \textsuperscript{18}F-DOPA PET is a marker of dopamine nigrostriatal terminal function rather than perikarya integrity the substantia nigra ROI was only used to sample \textsuperscript{11}C-PK11195 binding.

Parametric images of regional \textsuperscript{11}C-PK11195 binding potentials (BP\textsubscript{ND}) (Figure 1) were generated using individual reference tissue non-specific input functions and the simplified reference tissue model.\textsuperscript{22} Microglia activation can potentially be present throughout the brain in subjects with neurodegenerative disorders. Given this, one cannot be confident that a reference tissue region devoid of specific ligand binding is present. We, therefore, used a supervised cluster-analysis approach to extract brain voxels behaving kinetically similar to normal grey matter as a reference
tissue input function for each individual subject. Since this method can be sensitive to error, similarity between extracted reference tissue input functions from patients and controls were assessed with a repeated measurement analysis ($\chi^2 > 0.05$), as previously described.$^{12,14,15,22}$ The supervised cluster analysis model estimates tracer binding in ROIs relative to a cluster of reference voxels behaving kinetically similar to normal grey matter tissue. In this model, target voxels with binding lower than the reference cluster will provide negative binding potential values.

Parametric images of $^{18}$F-DOPA influx constants (Ki) (Figure 1) were generated with the Patlak graphical approach$^{23}$ using occipital lobe grey matter as a non-specific reference tissue input function.$^{24}$ See online appendix for details on MRI and PET images co-registration and normalisation.

A threshold for abnormality in regional $^{11}$C-PK11195 binding and $^{18}$F-DOPA uptake was defined as a statistical deviation from the norm. Briefly, regional $^{11}$C-PK11195 binding were classified as abnormal when the $\text{BP}_{\text{ND}}$ was two or more standard deviations above the control mean ($z$-score $\geq 2$) - the control values from both left and right side were averaged. Patients were classified as having abnormal regional $^{18}$F-DOPA uptake when their Ki values fell below two or more standard deviations from the controls mean value ($z$-score $\leq -2$).$^{25}$
Statistical analysis

Statistical calculations and graphical presentations were performed using Stata IC 14 (StataCorp LP, Texas, USA) and PRISM 6 (GraphPad, Software, La Jolla, CA). Normal distribution of outcome measures (\(^{18}\text{F-DOPA Ki-values and }^{11}\text{C-PK11195 BP}_{\text{ND}}\)) was assessed with normal probability plots and checked with the D’Agostino-Pearson normality test. Grubbs test was used to exclude any possible outliers. Between-group comparisons for continuous data of \(^{18}\text{F-DOPA Ki-values and }^{11}\text{C-PK11195 BP}_{\text{ND}}\) in predefined ROIs were interrogated with an unpaired two-tailed Student’s \(t\) test (alpha=5.0%). Welch’s correction was used for data with unequal variances. Two-tailed Pearson product-moment correlations (alpha=5.0%) were used to assess relationship between ROIs and tracers. In details, we first assessed the relationship between levels of \(^{11}\text{C-PK11195 binding in the substantia nigra and striatal structures due to their anatomical connection. We then assessed the relationship between levels of }^{11}\text{C-PK11195 binding in the substantia nigra and }^{18}\text{F-dopa uptake in the putamen and caudate to investigate whether higher neuroinflammation around the nigral dopamine cell bodies correlated with more severe dopaminergic dysfunction in striatal areas due to terminal dysfunction. Finally, we investigate possible correlations between neuroinflammation}}\)
and dopaminergic terminal dysfunction within the striatal areas.

Role of the funding source

The funding source had no influence on the study design, collection, analysis and interpretation of data, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Demographics and clinical characteristics of iRBD patients and controls are reported in Table 1. There were no differences in age between patients and controls. No adverse events occurred during the study.

$^{11}$C-PK11195 binding was significantly increased in the left substantia nigra of the iRBD patients compared to controls (mean difference = 0.153, 95% CI [0.055 to 0.250], p=0.003) and not in the right nigra (0.121, [-0.007 to 0.250], p=0.064). The
iRBD group showed no differences in mean $^{11}$C-PK11195 binding in the putamen and caudate nucleus bilaterally (Putamen left: 0.047, [-0.068 to 0.161], p=0.41 and right: 0.044, [-0.074 to 0.163], p=0.45. Caudate left: 0.076, [-0.053 to 0.205], p=0.24 and right: 0.024, [-0.098 to 0.147], p=0.69) (Table 2).

Individual analysis showed that 7/20 (35%) iRBD patients had abnormally raised $^{11}$C-PK11195 binding in the substantia nigra, and 2/20 (10%) in the putamen and caudate nucleus (Table 3). One subject had bilateral abnormal binding in all three regions investigated; substantia nigra, putamen, and caudate nucleus.

$^{18}$F-DOPA uptake was reduced in the putamen bilaterally in the iRBD patients compared to controls (left: -0.0032, [-0.0044] to -0.0021, p<0.0001 and right: -0.0032, [-0.0044 to -0.0020], p<0.0001). The average putaminal decrease in the iRBD group was 24.6% (95% CI 18.6 to 30.6). No changes in $^{18}$F-DOPA uptake where observed in the caudate nucleus at the group-level (left: -0.0012, [-0.0025 to 0.00006], p=0.06 and right: -0.0009, [-0.0022 to 0.0003], p=0.1) (Table 2).

At an individual level, 18/20 (90%) iRBD patients had abnormally reduced $^{18}$F-DOPA uptake in the putamen in one or both sides and 7/20 (35%) patients had reduced $^{18}$F-DOPA uptake in the caudate nucleus (Table 3). All seven patients with reduced $^{18}$F-DOPA uptake in the caudate nucleus also had reduced
putaminal uptake ipsilaterally. Six of these seven patients had bilateral abnormally reduced uptake throughout the striatum.

In the iRBD cohort, increased $^{11}$C-PK11195 binding in the substantia nigra correlated positively with increased binding of $^{11}$C-PK11195 in the ipsilateral putamen (left; Pearson coefficient $r = 0.59$, 95% CI [0.20 to 0.82], $p=0.006$ and right; $0.62$, [0.24 to 0.83], $p=0.004$), and in the caudate nucleus on the right side (left; $= 0.34$, 95% CI [-0.12 to 0.68], $p=0.1$ and right; $0.55$, [0.15 to 0.80], $p=0.01$) (Figure 2). Increased $^{11}$C-PK11195 binding in the left substantia nigra correlated significantly with decreased $^{18}$F-DOPA uptake in the ipsilateral caudate nucleus on the left side (Left $r = -0.53$, [-0.79 to -0.11], $p=0.02$) (Figure 2). No significant correlations were found between right substantia nigra $^{11}$C-PK11195 binding and $^{18}$F-DOPA uptake in the right caudate ($r = -0.13$, [-0.54 to 0.33], $p=0.6$) or between substantia nigra $^{11}$C-PK11195 binding and $^{18}$F-DOPA uptake in the ipsilateral putamen (left; $= -0.26$, 95% CI [-0.63 to 0.21], $p=0.3$ and right; $-0.03$, [-0.46 to 0.42], $p=0.9$) (Figure 2).

Within the striatum, no significant correlations were found between $^{11}$C-PK11195 binding and $^{18}$F-DOPA uptake in the putamen (left; $= -0.23$, 95% CI [-0.61 to 0.23], $p=0.3$ and right; $-0.23$, [-0.61 to 0.23], $p=0.3$) (Figure 2). In the caudate nucleus, a significant correlation was seen only on the left
side (left $r = -0.47$, 95% CI [-0.75 to -0.03], $p=0.04$; right $r = -0.28$, [-0.64 to 0.19], $p=0.2$) (Figure 2).

The analysis of individuals revealed that all seven patients with abnormally raised $^{11}$C-PK11195 binding in the substantia nigra also had abnormally reduced $^{18}$F-DOPA uptake in the ipsilateral putamen. One patient had bilaterally abnormal increased $^{11}$C-PK11195 binding in the substantia nigra, putamen, and caudate nucleus and decreased $^{18}$F-DOPA uptake in the putamen and caudate nucleus. Six patients had reduced $^{18}$F-DOPA uptake in the putamen but their $^{11}$C-PK11195 binding in the ipsilateral substantia nigra was below the upper limit of the 95% CI of control subjects.

No significant correlation was observed between $^{11}$C-PK11195 binding within the substantia nigra and patient reported duration of RBD ($r = 0.2$, [-0.28 to 0.58], $p=0.4$) or from time of iRBD diagnosis to PET examination ($r = 0.3$, [-0.18 to 0.65], $p=0.2$).

**Discussion**

This study represents the first in vivo observations indicating that raised microglial activation in iRBD is an early component in the neurodegenerative process along with reduced nigrostriatal dopaminergic function.
The current study showed a larger degree of neuroinflammation in the substantia nigra than the striatum in iRBD patients. This was the case for both the group level analysis and on an individual level where 7/20 (35%) subjects had raised $^{11}$C-PK11195 binding in the substantia nigra compared to 2/20 (10%) subjects in the putamen and caudate nucleus (Table 3). Previous $^{11}$C-PK11195 PET studies in patients with PD, DLB, or MSA,\textsuperscript{12,14,15,26} have reported raised levels of microglia activation in the striatum with less consistent involvement of the substantia nigra.\textsuperscript{12,14} Patients with higher levels of microglia activation in the substantia nigra also displayed higher $^{11}$C-PK11195 binding in the ipsilateral putamen. This could reflect the dysfunction of dopaminergic terminals and synapses associated with pathological changes in the dopaminergic neurons of the substantia nigra.

Significantly decreased levels of $^{18}$F-DOPA uptake were found in the putamen of our iRBD patients. This supports findings of previous studies investigating iRBD patients, using ligands for the dopamine transporter (DaT) to measure the integrity of nigrostriatal dopaminergic function.\textsuperscript{8,27-30} In our analysis of individual subjects 18/20 (90%) had reduced putaminal $^{18}$F-DOPA uptake on at least one side and 14/20 (70%) bilaterally (Table 3). This represents a higher frequency of patients with a dopaminergic decrement than that reported across the majority
of previous series where 30-40% of the iRBD patients had an abnormal DaT-single photon computed emission tomography (SPECT). This may be explained by the use of high-resolution PET imaging with delineation of regions of interest for each individual from their MRI. The mean decrease of 24.6% in putaminal $^{18}$F-DOPA uptake in iRBD patients compared to controls is in line with studies on Hoehn and Yahr stage 1 unilateral early PD cases which have reported a 20-35% putaminal reduction contralateral to the non-affected limbs and a 43-52% decrease contralateral to the affected limbs. At the group-level, we observed no reduction of mean $^{18}$F-DOPA uptake in the caudate nucleus. This is in line with the fact that dopaminergic function remains relatively preserved in the caudate nucleus when compared with the putamen in patients with early PD. However, some individual subjects were noted to have a decrement in the caudate, which also has been observed in DaT-SPECT studies by others. Currently, it is unknown if a greater reduction in $^{18}$F-DOPA uptake in the caudate nucleus and putamen in individual subjects is a short-term marker for conversion towards a specific type of synucleinopathy.

We found a significant correlation between raised $^{11}$C-PK11195 binding in the substantia nigra and reduced $^{18}$F-DOPA uptake in the ipsilateral caudate nucleus. This finding might relate to potential progression in some of our cases to DLB, where a
more pronounced involvement of the caudate nucleus is observed.\textsuperscript{35}

We did not find any significant correlations between raised \textsuperscript{11}C-PK11195 binding in the substantia nigra or the putamen and reduced \textsuperscript{18}F-DOPA uptake in the ipsilateral putamen (Figure 2C+D). This accord with similar observations between \textsuperscript{11}C-PK11195 and \textsuperscript{18}F-DOPA within nigrostriatal structures in patients with PD.\textsuperscript{12}

Interestingly, while abnormally raised levels of microglial activation in the substantia nigra were only seen in a subgroup 7/20 (35\%) of our iRBD patients, we found all these patients to have reduced \textsuperscript{18}F-DOPA uptake in the ipsilateral putamen. Recent reports have suggested that impaired intracellular trafficking, including dopamine function in the terminals may be an early abnormal phenomenon occurring in PD development.\textsuperscript{36,37} This assumption might explain our observation of reduced putaminal \textsuperscript{18}F-DOPA uptake in the absence of detectable nigral microglial activation in some iRBD patients.

The pathogenesis of PD is complex involving a variety of genetic, environmental, cellular, and molecular factors. There is mounting evidence that the immune system plays an important role since microglial and also astroglial activation are present and have a deleterious effect on neuronal function.\textsuperscript{38} Moreover, epidemiologic studies have shown reduced risk of developing PD in users of non-aspirin non-steroidal
anti-inflammatory drugs\textsuperscript{39} and pharmacological immunosuppression can reduce neurodegeneration in rodent models of PD.\textsuperscript{40} Consequently, research efforts have focused on neuroinflammation as a therapeutic target in early manifested PD. Our current finding of neuroinflammation occurring in the central nervous system of iRBD patients sheds light on the early pathological events occurring in disorders associated to Lewy type pathology. However, the cross-sectional nature of our study does not enable to clarify whether neuroinflammation is a primary event in the neurodegenerative process or whether neuronal dysfunction leads to secondary neuroinflammation.

This study has some limitations. First, the size of our cohort is relatively small. Therefore, our findings need to be confirmed in larger cohorts of patients. Second, this PET study used \textsuperscript{11}C-PK11195 as a marker of translocator protein (TSPO) expressed by activated microglia cells and astrocytes rather than one of the newer TSPO tracers available. This has enabled us to compare our \textsuperscript{11}C-PK11195 PET findings with those of previous studies of microglial activation in synucleinopathies. Additionally, \textsuperscript{11}C-PK11195 binding affinity is not influenced by the TSPO polymorphism carried by individuals. This is an issue with the newer tracers where exclusion of patients with low or mixed-affinity binding can potentially lead to a selection bias. We acknowledge, however, that \textsuperscript{11}C-PK11195 provides a lower specific to background signal
ratio than newer microglial markers and our results might underestimate the true extent of microglia activation, particularly in the case of subtle increases. Another possible limitation of the study is that the healthy controls were self-referred as they responded to newspaper advertisements. However, they had to fulfill all the inclusion/exclusion criteria and they were randomly selected to receive either $^{11}$C-PK11195 PET or $^{18}$F-DOPA PET.

Finally, in this study we have assumed that a raised level of microglial activation is pathogenic. This assumption is at variance with a recent published study in early Alzheimer’s disease where it was reported that raised microglial activation appeared to have a protective effect. Longitudinal follow-up of our iRBD individuals will reveal the clinical significance of the current observations.

In summary, we have shown that raised microglial activation in the substantia nigra and reduced presynaptic nigrostriatal dopaminergic function can be detected in iRBD patients using a combination of $^{11}$C-PK11195 and $^{18}$F-DOPA PET. This result implies that neuroinflammation and dopaminergic deficit occurs in iRBD patients, a condition that represents the prodromal stage of the synucleinopathies. Further studies involving larger series of iRBD subjects are needed to support our current observations and long-term follow-up with clinical and neuroimaging assessment of these patients may elucidate
whether the raised levels of microglial activation in iRBD represents a marker of short-term conversion to clinically defined synucleinopathies.

Contributors

Study design: NP, AI, DJB, ET, KØ, MGS.
Data acquisition: MGS, AI, MS, MO, KBS, AG, DV, JS, CG, NP.
All authors contributed to data analysis and the writing of the manuscript.

Declaration of interests

KØ reports grants from The Danish Parkinson Association, The Danish Council for Independent Research, and Lundbeck Foundation, during the conduct of the study and personal fees from Medtronic Inc., UCB, Fertin Pharma, and AbbVie outside the submitted work. DJB reports grants from The Danish Council for Independent Research, Lundbeck Foundation, The Danish Parkinson Association, European Union FP7 programme, and Alzheimer Research UK and personal fees from GE Healthcare and Plexxikon outside the submitted work. ET reports grants from the MJFox Foundation and the Instituto de Salud Carlos III. NP reports grants from The Danish Council for Independent
Research during the conduct of the study. The other authors declare no competing interests.

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## Tables

### Table 1

Characteristics and demographics of study population

<table>
<thead>
<tr>
<th></th>
<th>iRBD (n=20)</th>
<th>Controls $^{11}$C-PK11195 (n=10)</th>
<th>Controls $^{18}$F-DOPA (n=9)</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.6 (6.3)</td>
<td>65.8 (4.3)</td>
<td>64.6 (3.6)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>17 (85%)</td>
<td>10 (100%)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td>Female</td>
<td>3 (15%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>iRBD follow-up duration (years)</td>
<td>3.8 (3.4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age at iRBD diagnosis (years)</td>
<td>62.8 (6.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Duration of iRBD symptoms at PET examination</td>
<td>9.4 (5.3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$^{11}$C-PK11195 PET</td>
<td>20</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>$^{18}$F-DOPA PET</td>
<td>20</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation) or n (%).

iRBD = idiopathic REM sleep behaviour disorder.
Table 2

$^{11}$C-PK11195 $\text{BP}_{\text{ND}}$ and $^{18}$F-DOPA $\text{Ki}$ values for iRBD patients and controls

<table>
<thead>
<tr>
<th>$^{11}$C-PK11195</th>
<th>$^{18}$F-DOPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IRBD</td>
</tr>
<tr>
<td></td>
<td>n=20</td>
</tr>
<tr>
<td>Left Substantia nigra</td>
<td>0.156 (0.134)</td>
</tr>
<tr>
<td>Right Substantia nigra</td>
<td>0.140 (0.175)</td>
</tr>
<tr>
<td>Left Putamen</td>
<td>0.115 (0.152)</td>
</tr>
<tr>
<td>Right Putamen</td>
<td>0.140 (0.159)</td>
</tr>
<tr>
<td>Left caudate</td>
<td>-0.143 (0.147)</td>
</tr>
<tr>
<td>Right caudate</td>
<td>-0.152 (0.161)</td>
</tr>
</tbody>
</table>

Mean tracer uptake ($\text{BP}_{\text{ND}}$ or Ki values) and standard deviation (SD) and mean difference with 95% confidence interval (95% CI). iRBD = idiopathic REM sleep behaviour disorder.
Table 3

Number of iRBD patients with abnormal $^{11}$C-PK11195 BP$_{ND}$ and $^{18}$F-DOPA Ki values

| Region       | $^{11}$C-PK11195 | | | | $^{18}$F-DOPA | | | |
|--------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|              | Left | Right | Bilateral | Abnormal subjects | Left | Right | Bilateral | Abnormal subjects |
| Substantia nigra | 5    | 4     | 2         | 7                | -    | -     | -         | -                |
|               | (25%) | (20%) | (10%)     | (35%)            | -    | -     | -         | -                |
| Putamen      | 2    | 2     | 2         | 2                | 16   | 16    | 14        | 18               |
|               | (10%) | (10%) | (10%)     | (10%)            | (80%) | (80%) | (70%)     | (90%)            |
| Caudate      | 1    | 2     | 1         | 2                | 6    | 7     | 6         | 7                |
|               | (5%)  | (10%) | (5%)      | (10%)            | (30%) | (35%) | (30%)     | (35%)            |

A threshold for abnormality in regional $^{11}$C-PK11195 binding and $^{18}$F-DOPA uptake was defined as a statistical deviation from the norm. Briefly, regional $^{11}$C-PK11195 binding were classified as abnormal when the BP$_{ND}$ was two or more standard deviations above the control mean (z-score ≥ 2) - the control values from both left and right side were averaged. Patients were classified as having abnormal regional $^{18}$F-DOPA uptake when their Ki values fell below two or more standard deviations from the controls mean value (z-score ≤ -2). iRBD = idiopathic REM sleep behaviour disorder.
Figures

Figure 1

$^{18}$F-DOPA and $^{11}$C-PK11195 images of an iRBD patient and control subjects.
**Figure 2**

Correlations between tracer binding and regions of interests in iRBD patients (n=20)
Legends

Figure 1. $^{18}$F-DOPA and $^{11}$C-PK11195 images of an iRBD patient and control subjects

Images in the upper panel show striatal $^{18}$F-DOPA uptake in a patient with idiopathic rapid-eye-movement sleep behaviour disorder (iRBD) (right) and a healthy control (left). Images in the lower panel show $^{11}$C-PK11195 binding in the substantia nigra of an iRBD patient (right) and a healthy control (left). PET images are overlaid on the individual’s MR image. Scalebars indicates Ki values for $^{18}$F-DOPA and BP$_{ND}$ for $^{11}$C-PK11195.

Figure 2. Correlations between tracer binding and regions of interests in iRBD patients (n=20)

Each symbol represents values from 20 iRBD patients, left and right side is indicated by different colour. Pearson correlation coefficient is indicated for both left and right side.
Research in context

Evidence before this study

Prospective studies of idiopathic rapid-eye-movement sleep behaviour disorder (iRBD) patients have shown that up to 91% of cases develop the synucleinopathies Parkinson’s disease, dementia with Lewy bodies, or multiple system atrophy after 14 years of follow-up. Thus, iRBD is considered a harbinger of these conditions.

We have reviewed the reported evidence of early neuroimaging changes detected in vivo in iRBD patients by searching PubMed in May 2017 for published articles containing the MESH terms “Parkinson disease”, “Lewy Body Disease”, “Multiple System Atrophy”, “REM sleep behaviour disorder”, “Positron-Emission Tomography”, “Tomography Emission-Computed Single-Photon”, “Magnetic Resonance Imaging”, and “Proton Magnetic Resonance Spectroscopy”.

We found that previous neuroimaging studies consistently report the presence of nigrostriatal dopamine decrement in iRBD. Additionally, some studies observed relatively increased glucose metabolism and blood flow in the pons of iRBD patients. MRI changes in the brainstem have also been reported, and a single study observed microstructural changes related to neurodegeneration in the substantia nigra of iRBD patients. These neuroimaging observations combined with post-mortem descriptions of Lewy pathology in the brainstem of iRBD
patients indicate the presence of an underlying neurodegenerative process. Studies in patients affected by neurodegenerative disorders and their animal models have implicated microglial activation as a characteristic feature of the neuropathological process. $^{11}$C-PK11195 PET, an in vivo marker of translocator protein expressed by activated microglia cells, has shown raised nigrostriatal binding in patients with established synucleinopathies. Microglial activation may cause cell death and thus contribute to the development and progression of the neurodegenerative process and in the light of these observations, we hypothesized that microglial activation might also be increased in the nigrostriatal structures of iRBD patients.

**Added value of this study**

This is the first study in iRBD patients using in vivo PET imaging to investigate the occurrence of intrinsic neuroinflammation in the nigrostriatal system, a potential pathological mechanism contributing to the progression towards full-blown synucleinopathies. $^{11}$C-PK11195 PET, a marker of microglial activation, and $^{18}$F-DOPA PET, a marker of dopa decarboxylase activity, were used to investigate levels of nigrostriatal neuroinflammation and dopaminergic nigrostriatal function in iRBD patients, respectively. Significantly
increased levels of microglial activation were found in the substantia nigra of iRBD patients. This was correlated with increased levels of microglia activation in the ipsilateral putamen. Additionally, we found decreased $^{18}$F-DOPA uptake in the putamen bilaterally in the iRBD patients compared to controls.

**Implications of all available evidence**

This study presents the first in vivo observations indicating that increased microglial activation in iRBD is an early component in the neurodegenerative process along with nigrostriatal dopaminergic deficits. This finding marks microglial cells as a potential therapeutic target for future disease-modifying strategies, which could be tested to halt or delay progression of the underlying neurodegenerative process. Future follow-up studies may elucidate whether high levels of microglial activation are associated with a more rapid progression towards full-blown synucleinopathies and whether these imaging modalities are useful to monitor the effect of disease-modifying strategies.
References


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