ORIGINAL ARTICLE

Cholinergic denervation in patients with idiopathic rapid eye movement sleep behaviour disorder

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Background and purpose: Cholinergic dysfunction appears to play a role in the cognitive impairment observed in Parkinson's disease and dementia with Lewy bodies. The occurrence of cholinergic dysfunction in the early stages of these conditions, however, has not been investigated. The objective of this study was to investigate cholinergic function in patients with idiopathic rapid eye movement sleep behaviour disorder (iRBD), a disorder recognized to be an early stage of both Parkinson's disease and dementia with Lewy bodies.

Methods: A total of 21 patients with polysomnography-confirmed iRBD with no evidence of parkinsonism and cognitive impairment and 10 controls underwent positron emission tomography (PET) to assess brain acetylcholinesterase levels (^{11}C-donepezil PET) and nigrostriatal dopaminergic function (^{18}F-DOPA PET). Clinical examination included the Movement Disorder Society–Unified Parkinson's Disease Rating Scale part III, Mini Mental State Examination and Montreal Cognitive Assessment.

Results: The ^{11}C-donepezil PET was successfully performed in 17 patients with iRBD and nine controls. Compared with controls, patients with iRBD showed a mean 7.65% reduction in neocortical ^{11}C-donepezil levels (P = 0.005). Bilateral superior temporal cortex, occipital cortex, cingulate cortex and dorsolateral prefrontal cortex showed the most significant reductions at voxel level.

Conclusion: Reduced neocortical ^{11}C-donepezil binding in our patients indicates cholinergic denervation and suggests that the projections from the nucleus basalis of Meynert, which supplies cholinergic innervation to the neocortex, are dysfunctional in iRBD. Longitudinal studies will clarify if these changes are predictive of future cognitive impairment in these patients.

Introduction

Idiopathic rapid eye movement (REM) sleep behaviour disorder (iRBD) is a parasomnia characterized by loss of normal muscle atonia during REM sleep with accompanying dream enactment behaviour [1]. The disorder is associated with an increased risk of developing Parkinson's disease (PD) or dementia with Lewy bodies (DLB) [1,2]. Clinically, both PD and DLB are characterized by progressive parkinsonism and cognitive decline [3,4]. Parkinsonian patients with dementia near the onset of motor symptoms may be classified as

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patients with DLB. They show rapidly progressive cognitive impairment at the beginning of disease, whereas patients with idiopathic PD show only subtle signs of cognitive impairment at onset and may develop dementia later in the course of their disease [3–5].

There is strong evidence that cholinergic denervation of cortical areas [6–10] due to degeneration of the nucleus basalis of Meynert [6,11,12] plays a role in the cognitive impairment of PD and DLB. However, the integrity of the cholinergic neurotransmitter system in the prodromal phase of PD and DLB is unknown. Although patients with iRBD report no cognitive complaints in clinical settings, neuropsychological tests in these patients indicate abnormalities, particularly on tests of visuospatial abilities [13,14]. In this study, we have used ^{11}C-donepezil positron emission tomography (PET), an *in-vivo* marker of acetylcholinesterase (AChE) levels, to investigate the functional integrity of the cholinergic neurotransmitter system in patients with iRBD, who presumably are in a prodromal phase of PD and DLB.

Methods

Study population

A total of 21 patients with iRBD with a polysomnography-confirmed diagnosis according to established criteria [15] were recruited from tertiary sleep clinics at Aarhus University Hospital (n = 11) and the Multidisciplinary Sleep Unit at Hospital Clínic de Barcelona (n = 10). Prior to inclusion, patients gave a full clinical history and had a complete neurological examination to exclude parkinsonism, dementia and other neurological conditions [16,17]. No patients reported symptoms of hallucinations. Ten control subjects with no sleep, motor or cognitive complaints or neurological diseases were recruited through advertisements. Controls were screened for absence of REM sleep behaviour disorder symptoms by a comprehensive clinical history and questionnaire [18]. No patients or control subjects were receiving medication affecting the cholinergic or dopaminergic neurotransmitter system.

All subjects gave informed written consent according to the Declaration of Helsinki before enrolment into the study. The study protocol was approved by the Central Denmark Region Committee on Health Research and the ethics committee of the Hospital Clínic de Barcelona.

Clinical examination

Motor symptoms and signs of PD were assessed with the Movement Disorder Society–Unified Parkinson's Disease Rating Scale part III. The cognitive status of patients and controls was rated with the Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA).

Positron emission tomography and magnetic resonance imaging

All PET scans were performed at the Department of Nuclear Medicine & PET Centre, Aarhus University Hospital. Patients from Barcelona flew to Aarhus to undergo the examination.

Characteristics of the PET scanner and PET procedure are described in the Supporting Information.

To enable co-registration of their PET images, all subjects underwent a high-resolution T1-weighted magnetic resonance imaging (MRI) scan (3T MAGNETOM Skyra, Siemens Healthcare, Erlangen, Germany).

Image analysis

Kinetic modelling of ^{11}C-donepezil time–activity curves sampled from brain volumes of interest (VOIs) was performed with PMOD software (v 3.6, PMOD Technologies Ltd, Zürich, Switzerland). Distribution volume ratio (DVR) of the PET ligand ^{11}C-donepezil was quantified using the Logan reference tissue graphical model [20]. In our study, the reference region chosen was the centrum semiovale and a population average k_2 value of 0.08901 was assigned, computed from a validation study analysing previously performed ^{11}C-donepezil PET scans with an arterial plasma input function in healthy controls and patients with PD dementia (see Supporting Information for full details of the validation analysis).

We assessed similarity between extracted reference tissue input functions from patients and controls with a repeated measurement analysis (χ^2 test, $P > 0.05$).

The VOI analysis was performed as follows. MRI scans were spatially transformed into stereotaxic Montreal Neurological Institute (MNI) space and segmented into grey matter, white matter and cerebrospinal fluid. The segmented images were normalized to the Hammers 1-mm probabilistic atlas in MNI space (implemented in PMOD software). Individual object maps were produced by convolving the individual segmented images with the atlas mask. Summed ^{11}C-donepezil PET images were rigidly matched to the MRI image and afterward normalized into atlas space using the transformation matrix generated from the MRI normalization.

The VOIs where ^{11}C-donepezil was sampled included two regions and were bilateral grey matter tissue in whole neocortex and thalamus. VOI analysis

was performed to estimate the magnitude of a possible reduction in AChE levels. These specific regions were selected *a priori* because cortex receives extensive cholinergic projections from the nucleus basalis of Meynert and thalamus from the pedunculopontine and laterodorsal tegmental nuclei, and denervation may be observed *in vivo* in both PD and DLB [21–24]. Cholinergic denervation in cortical regions has been reported to be associated with impaired attention and hyposmia, and in thalamus with impaired gait and balance [7,23,25,26].

The ^{18}F-DOPA PET influx constants (Ki) were computed with the multiple time graphical Patlak/Gjedde model as previously described in detail [19]. Briefly, we used occipital lobe grey matter as a non-specific reference tissue input function and bilateral caudate nucleus and putamen as VOI.

Statistical parametric mapping

Following VOI analysis, Statistical Parametric Mapping (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK) was used exploratively to topographically localize significant reductions in ^{11}C-donepezil binding potentials (BPs) in patients with iRBD. Parametric ^{11}C-donepezil BP maps in MNI space of patients with iRBD and controls were compared with a two-tailed Student's *t*-test. Analysis was performed at a whole-brain level without any *a-priori* hypothesis. Images were smoothed to 8-mm full width at half maximum Gaussian kernel to minimize the inter-subject variability in the anatomic position of gyri. As normal ageing has an impact on the cholinergic neurotransmitter system, the age of the individual subject was added as a covariate in the voxel-based analysis [7,21,22]. The initial peak threshold was set to $P < 0.01$ (uncorrected) with a cluster extent threshold of 50 voxels. Clusters of voxels from this analysis that also survived family-wise error correction ($P < 0.05$, family-wise error-corrected at the cluster level) are shown in Fig. 3.

None of our patients with iRBD had cognitive complaints or displayed overt cognitive impairment. However, we used explorative SPM to assess whether patterns of cortical ^{11}C-donepezil BPs were different in iRBD with high (>26) or low (≤26) MoCA score compared with controls.

Statistical analysis

Statistical analysis and graphical presentations were performed in Stata IC 14.2 (StataCorp LP, College Station, TX, USA) and Prism 6 (GraphPad Software, La Jolla, CA, USA). Variables were examined with qnorm plots and D'Agostino Shapiro–Wilks test for normality of distribution.

Groups were matched on age. However, normal ageing has an impact on the cholinergic neurotransmitter system and therefore the impact of age on ^{11}C-donepezil VOI analysis was interrogated and found to be insignificant. Group differences were interrogated with a parametric two-tailed Student's *t*-test ($\alpha = 5.0\%$) and categorical data with Fisher's exact test. Correlations between outcome measures were assessed with two-tailed Pearson product-moment correlations ($\alpha = 5.0\%$). Associations between significant results in the ^{11}C-donepezil VOI analysis and clinical test scores (MMSE, MoCA and subitem tests in MoCA) and averaged ^{18}F-DOPA uptake from left and right side in caudate and putamen were assessed.

Results

Three patients with iRBD and one control were not included in the analysis (see Supporting Information). Demographic and clinical characteristics of the 17 patients with iRBD who completed the study are reported in Table 1.

The patients with IRBD had a significant mean reduction in neocortical ^{11}C-donepezil DVR of 7.65% (range −16.3% to 0.0%) compared with controls ($P = 0.005$) (Fig. 1), whereas mean thalamic ^{11}C-donepezil DVR was similar in patients with iRBD and controls ($P = 0.13$) (Fig. 1).

Across the iRBD group, neocortical ^{11}C-donepezil DVRs correlated positively with ^{18}F-DOPA influx constant (Ki) values of nigrostriatal dopaminergic dysfunction [averaged left and right ^{18}F-DOPA uptake in caudate ($r = 0.59$, $P = 0.013$) and putamen ($r = 0.52$, $P = 0.034$), Fig. 2] but not with clinical measures (MMSE score, $r = 0.13$, $P = 0.62$; MoCA

Table 1 Demographic and clinical characteristics

	iRBD	Controls	*P*-value
^{11}C-donepezil PET (*n*)	17	9	
Age (years)	65.3 ± 6.3	64.3 ± 6.9	$P > 0.05$
Sex (female/male)	2/15	0/9	$P > 0.05$
iRBD disease duration (years)	3.5 ± 3.3	–	
UPDRS-III score	3.1 ± 2.4	1.2 ± 2	$P > 0.05$
MMSE score	28.2 ± 1.7	29.6 ± 0.7	$P = 0.03$
MMSE score < 24	0	0	
MoCA score	25.8 ± 2.6	26.8 ± 2.7	$P > 0.05$
MoCA score < 26	8	2	
MoCA score < 21	1	0	

Data are given as mean ± SD. The patients with idiopathic rapid eye movement sleep behaviour disorder (iRBD) with a Montreal Cognitive Assessment (MoCA) score of 20 had a Mini Mental State Examination (MMSE) score of 24. PET, positron emission tomography; UPDRS-III, Unified Parkinson's Disease Rating Scale part III.

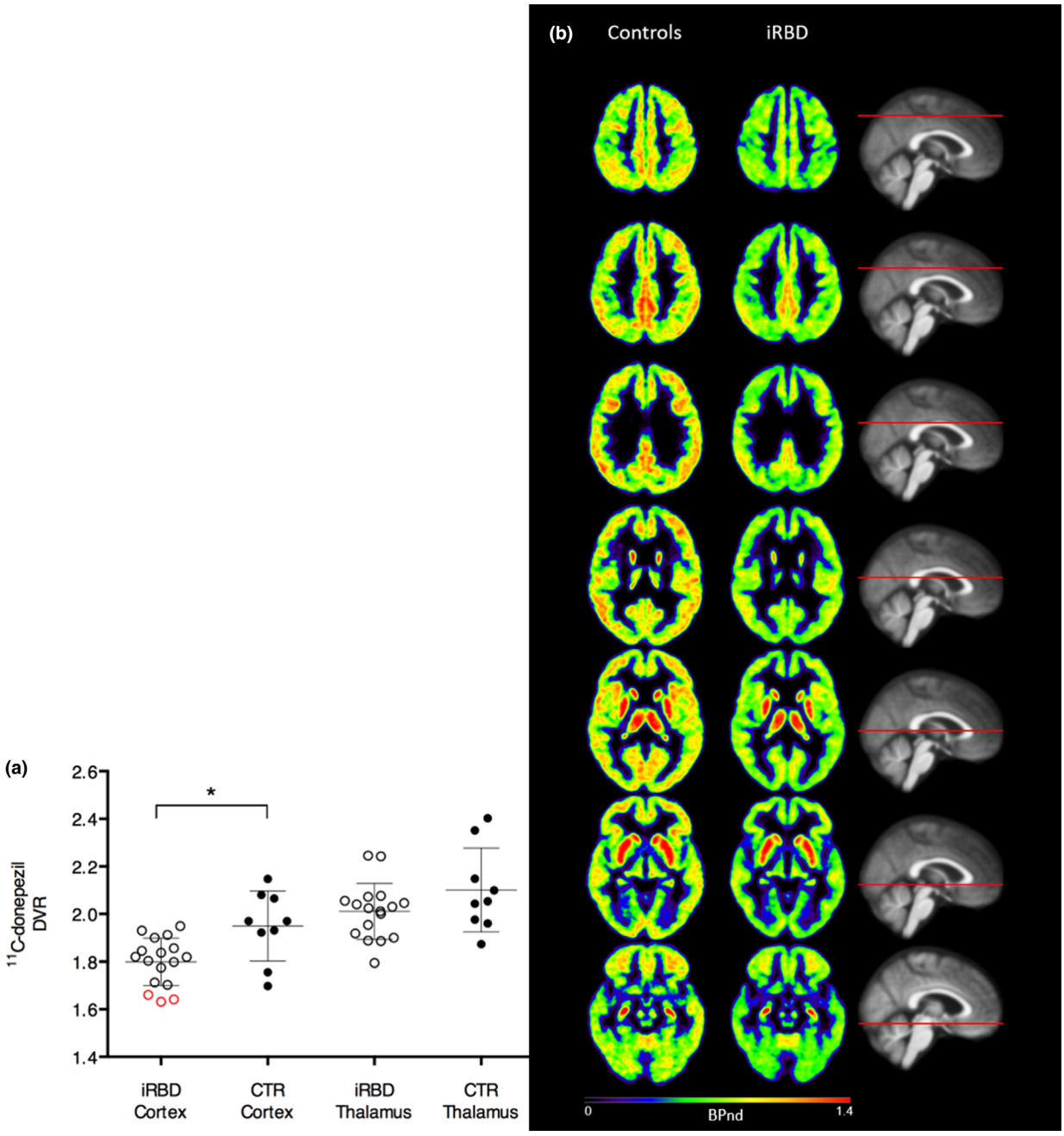

Figure 1 ^{11}C-donepezil distribution volume ratio (DVR) in volumes of interest and averaged ^{11}C-donepezil binding potential (BP) maps in study groups. (a) ^{11}C-donepezil DVRs in the idiopathic rapid eye movement sleep behaviour disorder (iRBD) group were 7.65% lower in cortex compared with control subjects (CTRs). Red circles indicate three subjects with iRBD with ^{11}C-donepezil DVRs ≥2 SDs below the average value of CTRs. Mean neocortical ^{11}C-donepezil DVR: iRBD, 1.800; CTR, 1.949; difference −0.149 [95% confidence interval (CI), −0.249 to −0.049] (P = 0.005). Mean thalamic ^{11}C-donepezil DVR: iRBD, 2.011; CTR, 2.101; difference −0.090 (95% CI, −0.209 to 0.030) (P = 0.134). (b) Parametric ^{11}C-donepezil BP maps generated with Logan reference plot with fixed k_2 value. Images are averaged in the CTR (n = 9) and iRBD (n = 17) group. Colour scale indicates ^{11}C-donepezil BPs for patients with iRBD and CTRs. * = $P < 0.05$. [Colour figure can be viewed at wileyonlinelibrary.com]

score, r = −0.25, P = 0.34) or subitems of the MoCA visuospatial tests (cube and clock). No significant correlations were observed between ^{11}C-donepezil DVRs and iRBD disease duration or patient's age.

Voxel-based analysis with SPM revealed that the most significant reductions in neocortical ^{11}C-donepezil BPs in patients with iRBD compared with controls were located in the bilateral superior temporal cortex,

occipital cortex, posterior and anterior cingulate cortex and dorsolateral prefrontal cortex (Fig. 3).

Interestingly, SPM also showed that, compared with controls, the subgroup of patients with iRBD with MoCA score ≤ 26 (n = 10) had more extensive cortical reduction in ^{11}C-donepezil (frontal, occipital and temporal areas) than patients with MoCA score > 26 (n = 7; occipital and temporal areas only) (Fig. 4).

Discussion

We observed a significant reduction in neocortical ^{11}C-donepezil DVR in patients with iRBD compared with controls. This novel finding suggests that cholinergic denervation in neocortical regions may occur in the earliest stages of developing synucleinopathies and seems to be more severe in the subgroup of patients with lower MoCA score. Previous *in-vivo* PET studies assessing the cholinergic system in clinically defined synucleinopathies have reported that PD patients with dementia show a 23% reduction in neocortical ^{11}C-donepezil distribution volumes [21]. Studies measuring reductions in N-[11C]-methylpiperidin-4-yl acetate (^{11}C-MP4A) k_3 values, a measure of AChE activity, observed 23%–27% reductions in PD dementia [22,27]. In non-demented patients with PD, a mean reduction in neocortical ^{11}C-MP4A k_3 values of around 11% was observed [22,23]. Taken together, the results indicate that function of the cholinergic system as reflected by *in-vivo* PET is affected throughout the course of the disorder even prior to the occurrence of clinical cognitive impairment and parkinsonian motor symptoms.

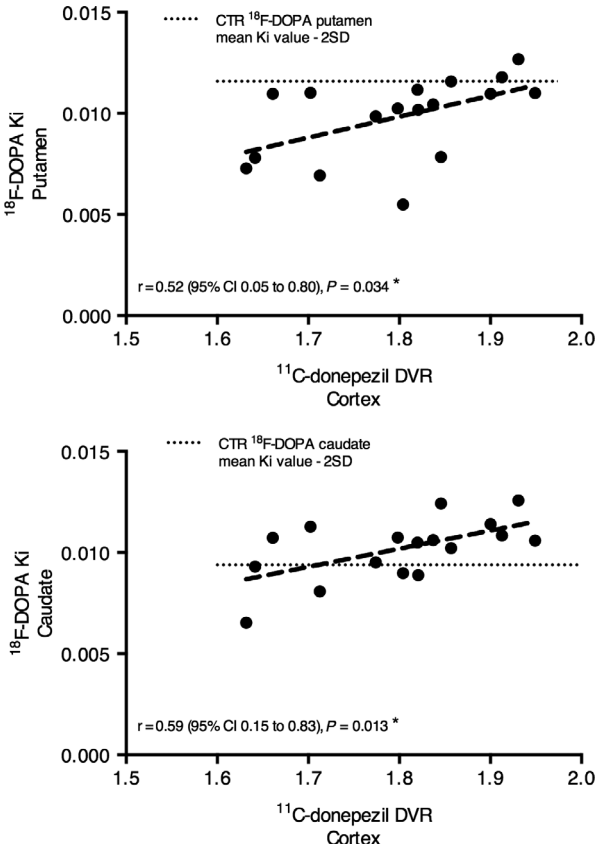

Figure 2 Correlations between ^{11}C-donepezil distribution volume ratio (DVR) and ^{18}F-DOPA influx constant (Ki) values. ^{11}C-donepezil DVRs in cortex in patients with idiopathic rapid eye movement sleep behaviour disorder correlate positively with ^{18}F-DOPA uptake in averaged caudate (P = 0.013) and putamen (P = 0.034). The horizontal line indicates the ^{18}F-DOPA mean level minus 2 SDs in control subjects (CTRs) [19]. CI, confidence interval of r. * = P < 0.05.

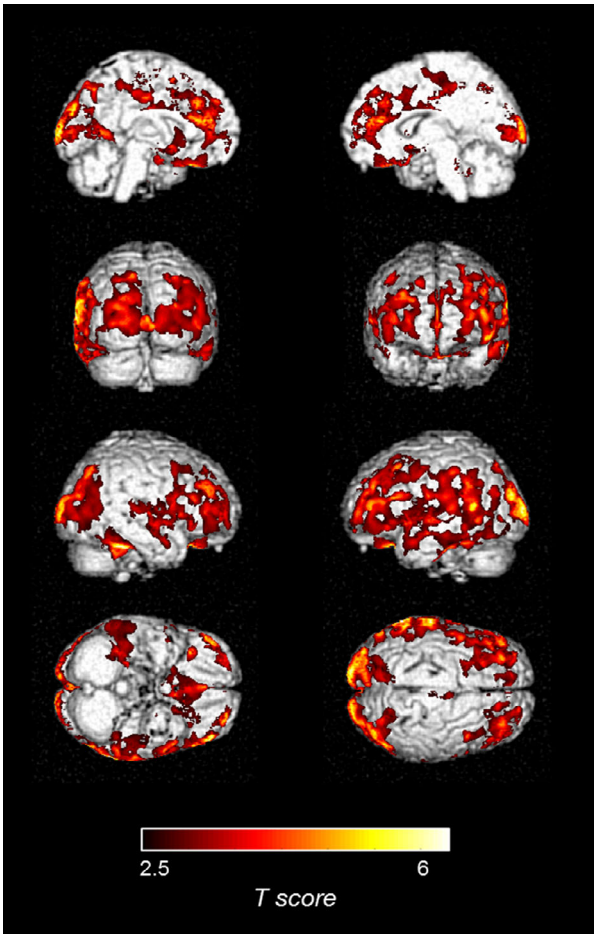

Figure 3 Reductions in cortical ^{11}C-donepezil binding potentials (BPs) in idiopathic rapid eye movement sleep behaviour disorder compared with controls. Neocortical areas where ^{11}C-donepezil BPs are reduced compared with healthy controls. Cluster defining threshold (P = 0.01) with a cluster extent threshold of 50 voxels. All clusters displayed survived family-wise error correction (P < 0.05) at the cluster level. [Colour figure can be viewed at wileyonlinelibrary.com]

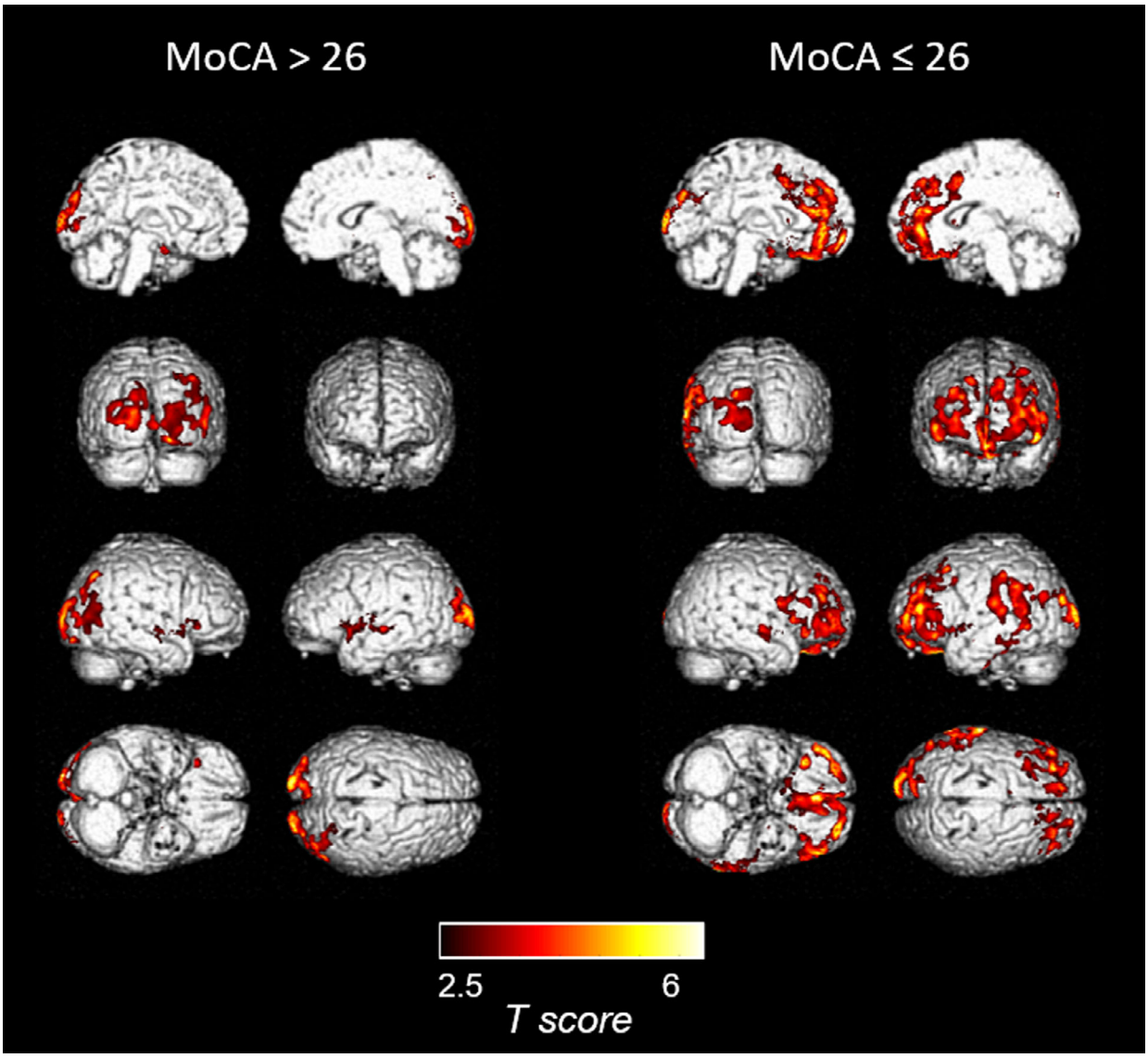

Figure 4 Reductions in cortical ^{11}C-donepezil binding potentials in idiopathic rapid eye movement sleep behaviour disorder (iRBD) with high and low Montreal Cognitive Assessment (MoCA) score compared with controls. Neocortical areas where ^{11}C-donepezil BPs are reduced compared with healthy controls when the iRBD cohort is separated into two subgroups, i.e. MoCA score > 26 ($n = 7$) and MoCA score ≤ 26 ($n = 10$). Reductions are more confined to occipital and temporal areas in subjects with higher MoCA scores, whereas those with a lower MoCA score also have the involvement of more frontal areas. Cluster defining threshold ($P = 0.01$) with a cluster extent threshold of 50 voxels. All clusters displayed survived family-wise error correction ($P < 0.05$) at the cluster level. [Colour figure can be viewed at wileyonlinelibrary.com]

The iRBD study population includes a predominance of male subjects as seen previously in large cohorts of patients with iRBD [28], which might bias results as a previous study in patients with PD showed a greater cholinergic deficit in males compared with females [29]. However, the control group also contained only male subjects and the two female patients with iRBD did not have a more preserved cholinergic neurotransmitter system compared with male patients.

The topographical analysis of cholinergic denervation in our patients with iRBD showed a greater denervation in the superior lateral temporal and occipital cortex. This is in accordance with previous investigations in patients with clinically manifest synucleinopathies [21,22,24,30] and one observation in patients with iRBD [31], suggesting that the observed neocortical changes in our iRBD population mark early pathophysiological events in the development of these synucleinopathies.

Our previous investigation of this iRBD population detected that the majority had an impaired nigrostriatal dopaminergic function with reduced ^{18}F-DOPA uptake in the putamen together with increased microglial activation in the substantia nigra [19]. In addition, the cholinergic findings reported here show that *in-vivo* PET examinations can expose the current state of pathophysiological involvement in an iRBD population.

Three of our subjects with iRBD had reductions in neocortical ^{11}C-donepezil DVRs that were ≥2 SDs below the average value of controls (Fig. 1, red circles), suggesting that these subjects could have a higher risk of future cognitive impairment.

The finding of a significant association between neocortical ^{11}C-donepezil DVR and caudate ^{18}F-DOPA uptake in this study shows that these patients with iRBD have a parallel involvement of different neurotransmitter systems. One previous study in patients with PD observed that involvement of both systems may contribute to the development of cognitive impairment, possibly with a more pronounced contribution from the cholinergic denervation [32]. A significant correlation was also observed between neocortical ^{11}C-donepezil DVR and ^{18}F-DOPA uptake in the putamen, suggesting that some individual patients with iRBD have a more advanced degree of dopaminergic involvement in their disease development. Longitudinal clinical evaluations have reported that patients with iRBD can develop parkinsonism and cognitive impairment concomitantly [1].

Our finding that only the subgroup of patients with iRBD with lower MoCA score has frontal reduction in ^{11}C-donepezil would suggest a close relation between cognitive function and cortical ^{11}C-donepezil binding. The absence of a direct association between neocortical ^{11}C-donepezil binding in the VOI and behavioural measures of cognition (MMSE and MoCA scores) is most likely to be due to the fact that this study only included subjects with no cognitive complaints and a single large VOI was used for the neocortex. Previous PET studies in patients with PD with normal cognition [22] or dementia [21] have not found an association between neocortical cholinergic denervation and MMSE scores, although Bohnen and colleagues did note a correlation ($R = 0.36$, $P = 0.02$) with MMSE scores in patients with PD without dementia, possibly reflecting the greater power in their study (PD, $n = 44$) [25]. The lack of correlation could also be influenced by the proposed non-linear relationship between cholinergic denervation and cognitive impairment as previously suggested [6].

In contrast to the significant reduction in neocortical ^{11}C-donepezil DVR, only a smaller (4.3%) non-significant reduction was observed in thalamic ^{11}C-donepezil DVR of patients with iRBD compared with controls. The thalamus receives cholinergic projecting terminals from the pedunculopontine and laterodorsal tegmental nuclei in the brainstem. Degeneration of cholinergic neurons in the pedunculopontine tegmental nucleus is observed at post-mortem in patients with PD and previous examinations of the cholinergic system in patients with PD with alternative PET tracers have observed a more pronounced cholinergic denervation in neocortex compared with thalamus [23,24,33]. Cholinergic denervation of the thalamus in patients with PD has been associated with walking and balance difficulties [25]. The thalamic ^{11}C-donepezil levels in our patients with iRBD were similar to controls, probably reflecting that they are in a prodromal disease stage without balance problems and changes at this stage are minimal.

The number of subjects participating in our study, although small, was fairly typical for a PET study. However, this limits the power of the study. ^{11}C-donepezil is a reversible AChE inhibitor that provides a reliable marker of cholinergic innervation of the brain. However, it should be noted that AChE is also present in cholinoceptive neurons present in cortical layers 3 and 5 [34]. Despite this, the majority of the cortical AChE is believed to stem from cholinergic fibres projecting from the basal nucleus of Meynert [35]. ^{11}C-donepezil also binds to the sigma-1 receptor present in cortex [36]and activity of this receptor is believed to have neuroprotective effects [37]. Therefore, the reduction of ^{11}C-donepezil binding observed in our patients could, in part, also be related to loss and/or downregulation of sigma-1 receptor activity. To our knowledge, the brain density of sigma-1 receptors in the prodromal phase of PD and DLB is unknown. Therefore, we cannot conclude which effect binding of ^{11}C-donepezil to the sigma-1 receptor has on the current results. We did not use an arterial line for blood sampling during the ^{11}C-donepezil PET and were therefore not able to employ an arterial plasma input function to generate volumes of tracer distribution, which could be seen as a limitation of our study. Nonetheless, Logan kinetic modelling applied using a population k_2 value and a white matter reference region computes DVRs and BPs that are strongly correlated to those derived by compartmental kinetic modelling with arterial line input functions (see Supporting Information for validation studies). Moreover, extracted time–activity curves from the iRBD population and controls were not significantly different (χ^2 test, $P > 0.05$). In addition, the findings of the current study are in strong agreement with previous observations of cholinergic denervation in patients with manifest PD.

Finally, the cross-sectional design of the study does not allow us to evaluate whether subjects with a greater neocortical reduction in ^{11}C-donepezil DVR are at a higher risk of developing cognitive impairment in the future. This needs to be established through clinical follow-up studies of these patients.

In conclusion, this *in-vivo* PET study detected significant neocortical cholinergic denervation in patients with iRBD, most of whom are likely to represent a prodromal stage of PD or DLB. These findings suggest that cholinergic neurons in the basal nucleus of Meynert are dysfunctional and possibly degenerating in the earliest stages of this condition. Degeneration of cholinergic neurons in the basal nucleus of Meynert has also been observed at post-mortem in patients with iRBD [38]. This emphasizes that synucleinopathies should be considered as multisystem disorders in the prodromal stages of the disease.

The findings of this study shed light on the early stages of synucleinopathy disorders and may help to understand mechanisms underlying the development of dementia in these conditions and to rationalize therapeutic approaches such as acetylcholine esterase blockers. The full clinical impact of these findings needs to be thoroughly established in future follow-up studies of these patients.

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Disclosure of conflicts of interest

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow-chart of ^{11}C-donepezil brain positron emission tomography scan inclusion.

Figure S2. Column bar-graph of k_2 values in the reference region.

Figure S3. Time–activity curves from the reference region.

Figure S4. Correlations between Logan plot and Logan reference model.

Figure S5. Statistical parametric mapping analysis between controls and patients with Parkinson's disease with dementia.

Figure S6. Correlations between Logan plot and Logan reference model.

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