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Evaluation of the noradrenergic system in Parkinson’s disease
An $^{11}$C-MeNER positron emission tomography & neuromelanin magnetic resonance imaging study

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Abstract
Pathological involvement of the noradrenergic locus coeruleus occurs early in Parkinson’s disease, and widespread noradrenaline reductions are found at post-mortem. Rapid eye movement sleep behavior disorder (RBD) accompanies Parkinson’s disease and its presence predicts an unfavorable disease course with a higher propensity to cognitive impairment and orthostatic hypotension. MRI can detect neuromelanin in the locus coeruleus while $^{11}$C-MeNER PET is a marker of noradrenaline transporter availability. Here, we use both imaging modalities to study the association of RBD, cognition and autonomic dysfunction in Parkinson’s disease with loss of noradrenergic function. Thirty non-demented Parkinson’s disease patients (16 patients with RBD and 14 without RBD, comparable across age (66.6 ± 6.7 years), sex (22 males), and disease stage (Hoehn & Yahr, 2.3 ± 0.5)), had imaging of the locus coeruleus with neuromelanin sensitive MRI and brain noradrenaline transporter availability with $^{11}$C-MeNER PET. RBD was confirmed with polysomnography; cognitive function was assessed with a neuropsychological test-battery, and blood pressure changes on tilting were documented; results were compared to 12 matched control subjects. We found that Parkinson’s disease patients with RBD showed decreased locus coeruleus neuromelanin signal on MRI (p < 0.001) and widespread reduced binding of $^{11}$C-MeNER (p < 0.001) which correlated with amount of REM sleep without atonia. Parkinson’s disease with RBD was also associated with a higher incidence of cognitive impairment, slowed electroencephalographic activity, and orthostatic hypotension. Reduced $^{11}$C-MeNER binding correlated with electroencephalographic slowing, cognitive performance, and orthostatic hypotension. In conclusion, reduced noradrenergic function in Parkinson’s disease was linked to the presence of RBD and associated with cognitive deterioration and orthostatic hypotension. Noradrenergic impairment may contribute to the high prevalence of these non-motor symptoms in Parkinson’s disease, and may be of relevance when treating these conditions in Parkinson’s disease.

Keywords: Parkinson’s disease, REM sleep behavior disorder, positron emission tomography, noradrenaline
Introduction
Noradrenergic innervation of the brain arises almost exclusively from the neuromelanin containing cells of the locus coeruleus. Depositions of aggregated alpha-synuclein in the locus coeruleus occur early in Braak stage 2 of Parkinson’s disease (Braak et al., 2001). Post-mortem studies have detected a substantial noradrenergic deficit in Parkinson’s disease patients (Kish et al., 1984). However, in vivo assessment of the noradrenergic system has been hampered by the lack of suitable imaging tools. Recently, neuromelanin-sensitive magnetic resonance imaging (MRI) sequences have been introduced, capable of delineating the pigment in the cell bodies in the locus coeruleus (Keren et al., 2015). Additionally, $^{11}$C-MeNER, a positron emission tomography (PET) reboxetine analogue with a high specificity for noradrenaline transporters has been developed (Schou et al., 2003). These imaging modalities have been successfully used to study locus coeruleus changes and noradrenergic transporter function in Parkinson’s disease (Garcia-Lorenzo et al., 2013; Nahimi et al., 2017).

Rapid eye movement (REM) sleep behavior disorder (RBD) is a sleep disorder defined by deficient muscle atonia and dream enactment during REM sleep. Parkinson’s disease patients with RBD (PD$^{RBD+}$) are at high risk of developing cognitive impairment (Jozwiak et al., 2017). Additionally, this subpopulation also displays a high prevalence of autonomic disturbances (Postuma et al., 2008). Interestingly, these non-motor symptoms often coincide suggesting a common neurobiological factor (Udow et al., 2016). The noradrenergic system mediates cognitive attention, regulates the sleep cycle and influences cardiac function via widespread projections from the locus coeruleus to the cortex, brainstem nuclei and the hypothalamus (Rodovalho et al., 2006; Vazey and Aston-Jones, 2012); thus, noradrenergic degeneration might be the candidate. The pathophysiology of RBD is not fully understood, but rodent models indicate a substantial role of the sublaterodorsal nucleus in establishing REM atonia, which corresponds to the locus subcoeruleus in humans (Valencia Garcia et al., 2017). These cells contain neuromelanin in humans and are located dorsoventrally to the locus coeruleus; both parts are often assimilated into the subcoeruleus/coeruleus complex (Braak et al., 2001; Garcia-Lorenzo et al., 2013). Recent neuromelanin sensitive MR sequences have revealed a loss of neuromelanin signal from this complex in PD$^{RBD+}$ (Garcia-Lorenzo et al., 2013). We hypothesize that damage to the subcoeruleus/coeruleus complex might represent a common pathway for RBD genesis, cognitive decline, and orthostatic dysregulation.
The objective of this combined $^{11}$C-MeNER PET and neuromelanin-sensitive MRI study was to examine the hypothesis that the noradrenergic system is more severely affected in PD$^{RBD^+}$ compared to Parkinson’s disease patients without RBD (PD$^{RBD^-}$). Additionally, we aimed to investigate the relationship between levels of noradrenergic function, cognition and autonomic regulation in Parkinson’s disease.
Material and methods

Study design and participants
We recruited 30 Parkinson’s disease patients distributed as 14 PD<sup>RBD</sup>- and 16 PD<sup>RBD+</sup> patients, comparable in age, sex, disease stage, and performance on the Montreal Cognitive Assessment (MoCA) (Table 1). Inclusion criteria were: Diagnosis of Parkinson’s disease according to the Movement Disorder Society (MDS) consensus criteria (Postuma <em>et al.</em>, 2015), age 50 - 85 years, a geriatric depression scale (GDS-15) score <6 and MoCA >22. Exclusion criteria were: A diagnosis of dementia, medication interacting with the noradrenaline transporter, and significant white matter lesions. Patients were recruited through advertisement in the Danish Parkinson’s disease magazine and from collaborating neurological clinics. Total levodopa-equivalent daily doses were calculated as previously recommended (Tomlinson <em>et al.</em>, 2010). Motor symptoms were quantified using the MDS Unified Parkinson’s disease Rating Scale part III (MDS-UPDRS III) after 12 hours of medication abstinence. Olfaction was tested with the 16-item Sniffin’ Sticks, RBD symptoms were assessed using the RBD symptom questionnaire (RBDSQ). Parkinson’s disease patients were compared to 12 age and sex matched, cognitive normal (MoCA >26) and non-depressed (GDS-15 <6) healthy control subjects without history of neurological disorders and with normal brain MRI (Table 1). Six additional healthy control subjects - part of an in-house neuromelanin MRI control sample - fulfilling aforementioned criteria were included for neuromelanin MRI analysis. All healthy control subjects were recruited through newspaper advertisements.

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

MRI
Subjects were scanned with a 3 Tesla SIEMENS MAGNETOM TRIO scanner; the protocol included T1 and fluid-attenuated inversion recovery images. Neuromelanin sensitive images were acquired with 2D axial turbo spin echo T1-weighted sequences (repetition time / echo time: 600 ms / 9.9 ms, 16 averages, voxel size: 0.4 × 0.4 × 1.8 mm). Planes were acquired perpendicular to the dorsal brainstem, and a locus coeruleus-to-pons ratio was calculated using PMOD 3.8: A template volume of interest (VOI) for each side of the locus coeruleus, derived from previously published Montreal Neurological Institute atlas coordinates (Keren <em>et al.</em>, 2009), was transformed
to the individual MRI, and adjusted manually for precise localization if needed. The 10 voxels with highest intensity were extracted from each VOI and divided by the background level derived from a rectangular VOI centered in the pons, and both values were averaged. Blinded inter-rater assessment (MS and TF) revealed high agreement ($R^2 = 0.913$), and the average of both raters of MRI neuromelanin locus coeruleus-to-pons ratios was taken for group comparisons. To assess the association of MRI and $^{11}$C-MeNER PET signals, we interrogated correlations between locus coeruleus-to-pons ratios and $^{11}$C-MeNER DVRs.

**$^{11}$C-MeNER PET**

$^{11}$C-MeNER radiosynthesis was performed as described previously (Nahimi et al., 2017). After a transmission scan and at the start of a bolus administration of $^{11}$C-MeNER, a 90-minute dynamic PET scan was acquired with an ECAT HRRT in list mode. We analyzed PET images with relevant toolboxes of PMOD 3.8, including time frame realignment for motion correction. All PET images were then normalized into Montreal Neurological Institute space including rigid matching of subject’s PET to the anatomical MRI, and MRI segmentation. We defined six noradrenaline transporter rich regions (thalamus, hypothalamus, red nucleus, locus coeruleus, median raphe, dorsal raphe) for VOI analysis and used the occipital cortex (OC) as a reference region; thalamus and OC were defined from the built-in Hammers N30R83 atlas. VOIs of the locus coeruleus and dorsal and median raphe were drawn accordingly to published details (Keren et al., 2009; Kranz et al., 2012). Red nucleus VOIs were defined on individual FLAIR images and transformed to Montreal Neurological Institute space; hypothalamus VOIs were defined by their surrounding structures. To optimize kinetic modelling, we smoothed PET images with a 4 mm Gaussian filter and extracted time activity curves from VOIs for calculation of distribution volume ratios (DVRs) with the simplified reference tissue model 2 (SRTM2) for group comparisons. For illustration purpose (Figure 1), parametric maps of SRTM2 DVRs were generated. More details of PET analysis are given in the supplementary material.

**Polysomnography and electroencephalogram**

We used a SOMNOscreen™ plus Tele+Video device for overnight video-polysomnography including ten electroencephalogram electrodes, right and left electrooculogram, nasal pressure
monitoring, thoracic and abdominal effort belts, electrocardiogram, pulse oximetry, electromyogram of the submental and tibialis anterior muscles, and video monitoring. Presence of RBD was judged according to the current consensus criteria from the American Academy of Sleep Medicine. Quantitative electromyogram analysis of ‘phasic’ (representing brief activity less than 15 s), ‘tonic’ (representing sustained activity longer than 15 s), and ‘any’ (representing any activity) submental muscle activity during REM sleep was performed with a validated computer algorithm, which was recently developed by our group and shown to have high congruency with human visual ratings (R² > 0.9). The semi-automated computer algorithm was based on previously proposed visual scoring criteria by McCarter and colleagues (McCarter et al., 2014). As REM sleep atonia is supposed to be mediated by brainstem nuclei interplay (Boeve et al., 2007), we correlated the ¹¹C-MeNER PET signal in the corresponding areas (locus coeruleus and raphe nuclei) with measures of quantitative electromyogram analysis.

Electroencephalography was performed under ambient conditions with the subject awake and resting with closed eyes. We extracted 30 s artifact-free epochs to calculate alpha (8 - 13 Hz) and theta (4 - 8 Hz) power, and background frequency (correspondent to the frequency with highest power in the spectrum) using the Natus Viewer software and the built-in band power report. Signals of the O1 and O2 electrodes were pre-filtered with 3 Hz high-pass and 15 Hz low-pass filters, and results of both electroencephalography electrodes were averaged and used for group comparisons. Slowing of electroencephalographic background frequency was reported to herald cognitive decline in Parkinson’s disease patients (Klassen et al., 2011; Latreille et al., 2016), and as locus coeruleus firing pattern was reported to have high congruency to the cortical electroencephalography signal (Bari and Aston-Jones, 2013), we selected the ¹¹C-MeNER binding in the locus coeruleus for correlation analysis with electroencephalography.

Neuropsychological assessment
Cognition was assessed adhering to the 2012 MDS diagnostic criteria for mild cognitive impairment in Parkinson’s disease (Litvan et al., 2011). Mild cognitive impairment was diagnosed when two cognitive tests revealed z-scores below -1.5 and there was a history of cognitive complaints reported by the subject or spouse on our questionnaire (Koster et al., 2015). Tests included Rey auditory verbal learning test, and the location learning test for memory domain; digit span backwards, letter number sequencing task, and Stroop color and word test for working
memory; Wisconsin card sorting test, and phonemic verbal fluency (-s version) for executive functioning; similarities test, semantic verbal fluency (animals), and the 30 item Boston naming test for language abilities; Benton judgement of line orientation, clock drawing and visual puzzles for visuospatial function. Z-scores for each cognitive domain were calculated based on published age corrected norms, if available, and from an in-house Danish cohort of healthy subjects matched for age. A composite score of the average of the five domain z-scores was calculated to estimate global cognitive performance. This global score was used for correlation analysis with the $^{11}$C-MeNER PET signal of the locus coeruleus. Premorbid cognitive function was estimated with the WAIS-IV vocabulary test and scale scores were comparable across groups (healthy control, 10.6 ± 2.4, PD$^{RBD-}$, 10.4 ± 2.0, PD$^{RBD+}$, 9.7 ± 1.7; p = 0.669).

**Autonomic assessment**

Symptoms of autonomic disturbances were assessed with rating scales for outcomes in Parkinson’s disease - autonomic (SCOPA-AUT); due to incomplete response rates on items 22 - 25 (questions about sexual ability), these sections were excluded. After >15 min rest in the supine position, blood pressure was measured for three consecutive minutes after tilting. Orthostatic hypotension was defined as systolic pressure drop of 20 mmHg or diastolic pressure drop of 10 mmHg within the first three minutes of standing (The Consensus Committee of the American Autonomic Society and the American Academy of Neurology, 1996). Blood pressure measurements were not undertaken at a fixed time of day, but were usually performed between 10 am and 2 pm. We calculated the mean changes of systolic pressure and diastolic pressure during the three minutes of upright position and subtracted the mean from the supine blood pressure for group comparisons. We selected the $^{11}$C-MeNER PET signals of locus coeruleus and hypothalamus, which are noradrenergic centers of blood pressure regulation (Rodovalho et al., 2006), for correlation with blood pressure changes. Blood pressure measurements were not undertaken in 2 PD$^{RBD+}$ subjects because of atrial fibrillation and beta-blocker therapy, and in 2 PD$^{RBD+}$ and 1 healthy control subjects because of technical failure of the blood pressure monitor.
**Statistical analysis**

We interrogated the data with the Statistical Package for the Social Sciences (SPSS) version 24. Group data is presented as mean ± standard deviation if not otherwise stated. Group comparisons were performed with one-way analyses of variances (ANOVA), Student’s t-tests, Mann-Whitney tests, Kruskal-Wallis tests, and chi-square tests as appropriate; a normal distribution of data was assessed with Shapiro-Wilk tests, Q-Q plots, and box plots. Univariate correlation analyses were calculated with Pearson’s r and Spearman’s rho as appropriate. For VOI-based PET analysis, a linear mixed model for repeated measures with the random effect ‘subject’, the between-subjects factor ‘group’, and the within-subjects factor ‘brain region’ was calculated; the interaction term of ‘brain region’ * ‘group’ was included in model calculation. Significance was accepted at p < 0.05.
Results

Decreased noradrenergic imaging-markers in PD\textsuperscript{RBD+} patients

In accordance with their RBD diagnosis, PD\textsuperscript{RBD+} patients scored higher on the RBDSQ and had extensive epochs of REM sleep without atonia for all three muscle activity categories. The Parkinson’s disease subgroups and healthy control subjects were otherwise comparable with regard to demographic and clinical characteristics (Table 1). \textsuperscript{11}C-MeNER DVRs differed significantly between the groups and the PD\textsuperscript{RBD+} patients exhibited the lowest mean values in all examined brain regions (group effect $p < 0.001$, healthy control vs PD\textsuperscript{RBD-}, $p = 0.027$, healthy control vs PD\textsuperscript{RBD+}, $p < 0.001$, and PD\textsuperscript{RBD-} vs PD\textsuperscript{RBD+}, $p = 0.001$). The interaction term of ‘brain region’ * ‘group’ was highly significant ($p = 0.004$), and the decrease of regional \textsuperscript{11}C-MeNER DVRs was most pronounced in brainstem nuclei in PD\textsuperscript{RBD+} patients; see Figure 1. PD\textsuperscript{RBD+} patients also displayed the lowest neuromelanin locus coeruleus-to-pons ratio on MRI (group effect $p < 0.001$, healthy control vs PD\textsuperscript{RBD-}, $p = 0.014$, healthy control vs PD\textsuperscript{RBD+}, $p < 0.001$, and PD\textsuperscript{RBD-} vs PD\textsuperscript{RBD+}, $p = 0.026$; Figure 2 A+B). Locus coeruleus-to-pons ratios from neuromelanin MRI correlated with thalamic \textsuperscript{11}C-MeNER DVR levels in pooled analysis but did not correlate with other regional \textsuperscript{11}C-MeNER DVR levels (rho = 0.339, $p = 0.030$ for thalamus; Figure 2 C).

The amount of muscle activity in REM sleep correlated negatively with \textsuperscript{11}C-MeNER DVR of the locus coeruleus in PD\textsuperscript{RBD+} patients ($r = -0.623$, $p = 0.013$ for ‘any’, $r = -0.678$, $p = 0.006$ for ‘tonic’, and $r = -0.551$, $p = 0.033$ for ‘phasic’ activity; Figure 3); no correlations were significant between amount of electromyogram activity and raphe \textsuperscript{11}C-MeNER DVR or MRI locus coeruleus-to-pons ratio.

Correlation of noradrenergic imaging-markers with cognition and autonomic regulation

PD\textsuperscript{RBD+} patients showed a lower performance in all cognitive domains which reached significance in the visuospatial domain, and they had an increased prevalence of mild cognitive impairment (Table 2). PD\textsuperscript{RBD+} patients also showed significantly slower electroencephalographic background frequency compared to PD\textsuperscript{RBD-} patients and healthy control, and by extension, higher theta power and a lower alpha/theta power ratio (Table 2). \textsuperscript{11}C-MeNER DVRs of the locus coeruleus correlated with global cognitive performance in Parkinson’s disease patients as well as in pooled analysis.
with healthy control subjects ($r = 0.399, p = 0.029$, and $r = 0.394, p = 0.010$, respectively); similar correlations were obtained with electroencephalographic background frequency ($r = 0.404, p = 0.033$, and $r = 0.490, p = 0.001$ for pooled analysis, respectively), see Figure 4 A-B. MRI locus coeruleus-to-pons ratios did not correlate with background frequency or cognitive performance.

Subjective complaints of autonomic symptoms were more prevalent in Parkinson’s disease patients compared to healthy control subjects and orthostatic hypotension was most prevalent in $\text{PD}^{\text{RBD}+}$ patients ($50\%, p = 0.026$). Upon tilting, $\text{PD}^{\text{RBD}+}$ patients exhibited a blunted blood pressure response with a significant fall of systolic pressure ($-12.2 \pm 17.9$ mmHg, $p = 0.019$) (Table 2). Blood pressure falls correlated significantly with $^{11}\text{C-MeNER DVR}$s of the locus coeruleus and hypothalamus in the pooled analysis (systolic pressure: $r = 0.375, p = 0.022$ & $r = 0.286, p = 0.086$; diastolic pressure: $r = 0.339, p = 0.040$ & $r = 0.367, p = 0.025$), but significance was not achieved in the analysis of Parkinson’s disease patients alone (Figure 4 C-D). No correlation was observed between MRI locus coeruleus-to-pons ratios and blood pressure changes.
Discussion

In this study, we examined structure and function of the noradrenergic system in Parkinson’s disease patients with multimodal imaging. We targeted the pigmented cell bodies of the locus coeruleus with neuromelanin sensitive MRI, and noradrenaline transporters in relevant brain regions with $^{11}$C-MeNER PET. Both modalities showed marked reductions of signal in Parkinson’s disease patients, most pronounced in PD$^{RBD+}$ patients, and decline of $^{11}$C-MeNER binding was associated with magnitude of abnormal muscle activity during REM sleep. PD$^{RBD+}$ patients showed slowed electroencephalography activity and a higher incidence of cognitive impairment and orthostatic hypotension. These conditions were associated with reduced $^{11}$C-MeNER binding in regulatory noradrenergic brain areas.

Parkinson’s disease patients exhibited the lowest $^{11}$C-MeNER binding in all examined brain regions as well as the lowest neuromelanin signal on MRI; however, neuromelanin MRI and $^{11}$C-MeNER PET signals were not well correlated. Several studies on Parkinson’s disease patients and animal lesion models have reported a lack of correlation between dopaminergic neuronal density in the substantia nigra and the integrity of striatal dopaminergic terminals. When data from subjects with and without dopaminergic deficit were pooled, however, a correlation was observed (Karimi et al., 2013; Kraemmer et al., 2014). A “dying-back” concept of the dopaminergic system has been postulated in the context of Parkinson’s disease, i.e. axonal and terminal degeneration prior to neuronal cell loss, which may also apply to the noradrenergic system (Cheng et al., 2010). Nevertheless, dying back does not explain an absent correlation between structural and functional imaging modalities in the locus coeruleus itself. Monoaminergic transporters and receptors undergo adaptive changes in Parkinson’s disease and so their availability may not show a proportional relationship with the magnitude of neuronal death of corresponding cell bodies. Additionally, while the noradrenergic locus coeruleus signalling is largely integrative, neurons display significant heterogeneity in their regional projections (Kebschull et al., 2016). Thus, it is perhaps not unexpected that the neuromelanin MRI signal as a marker of the integrity of locus coeruleus cell bodies did not correlate with regional noradrenaline transporter density measured with $^{11}$C-MeNER PET in brain structures exhibiting differential noradrenergic inputs. However, since PD$^{RBD+}$ patients showed decreased signal on both imaging modalities, it suggests that noradrenergic damage is profound and widespread in this Parkinson’s disease subpopulation.
In addition to the difference of PD\textsuperscript{RBD+} patients as a group, we also detected a negative correlation of 11\textsuperscript{C}-MeNER binding in the locus coeruleus with the amount of muscle activity in REM sleep, strengthening the association of noradrenergic alterations to key symptoms of RBD. Of note, noradrenergic enhancing drugs increase the amount of muscle activity during REM sleep, and the locus coeruleus is known to cease firing during REM sleep as part of a sleep-stage switch (Lu \textit{et al.}, 2006). This evidence points to a substantial role of noradrenergic signalling in normal REM sleep regulation and as a mediator of REM sleep atonia. However, converging evidence from animal research suggests that activation of the glutamatergic sublaterodorsal nucleus establishes REM atonia (Valencia Garcia \textit{et al.}, 2017). Nevertheless, it remains unsolved, whether the homologous locus subcoeruleus in humans is also glutamatergic, especially since it contains neuromelanin pigment, which is typically expressed in catecholaminergic neurons. Additionally, cells in the locus subcoeruleus express α\textsubscript{2}-adrenergic receptors, which when activated inhibit the output from these cells that is necessary to establish REM atonia (Yang \textit{et al.}, 2014). Thus, deficient noradrenergic signaling might interact at the level of the sleep stage switch, resulting in inefficient REM sleep stage transition with ‘leaking’ of muscle activity during REM sleep. Alternatively, non-physiological activation of the nucleus subcoeruleus during REM sleep may result from insufficient inhibition from the locus coeruleus. Our findings may also simply represent a mutual affection of the locus subcoeruleus/coeruleus complex in Parkinson’s disease, as these structures are well interconnected and situated closely together (Braak \textit{et al.}, 2001). It would have been interesting to explore correlation of RBD symptom duration with noradrenergic imaging parameters in our PD cases but many cases were unable to accurately date the onset of their sleep disorder.

A heterogeneous clinical presentation and prognosis of Parkinson’s disease patients is increasingly recognized, and in a recent longitudinal study, the presence of RBD, mild cognitive impairment, and orthostatic hypotension were the best predictors of a diffuse/malignant phenotype of Parkinson’s disease (Fereshtehnejad \textit{et al.}, 2015). In our study, PD\textsuperscript{RBD+} patients also showed higher propensity to cognitive impairment and orthostatic hypotension in agreement with previous studies (Postuma \textit{et al.}, 2008; Udow \textit{et al.}, 2016; Jozwiak \textit{et al.}, 2017). Hence, a common neurobiological premise for RBD, cognitive deterioration, and orthostatic disturbances in Parkinson’s disease seems plausible and indeed, we detected associations between the noradrenergic system as measured with 11\textsuperscript{C}-MeNER PET and all three conditions. 11\textsuperscript{C}-MeNER not only correlated with global cognitive performance but also with electroencephalographic activity, which was slowed in
PD^{RBD+} patients. Slowing of electroencephalography is commonly seen in several forms of dementia, and was previously shown to be highly predictive for the development of dementia in Parkinson’s disease (Klassen et al., 2011). However, maintaining healthy cognition is dependent on several neurotransmitter systems and particularly the loss of cholinergic signalling is a well-known cause of cognitive decline in Parkinson’s disease (Bohnen et al., 2015). Nevertheless, locus coeruleus terminals exhibit a brain-wide distribution and are known to modulate various neurotransmitters via adaption of their firing pattern and divergent post-synaptic effects due to different noradrenergic receptors (Schwarz and Luo, 2015). This may be particularly important in early to mid-stage Parkinson’s disease when alpha-synuclein pathology has not yet spread to the majority of cortical regions, but is mostly confined to brainstem areas (Braak et al., 2001). Accordingly, and given the results of the present study, the noradrenergic system should be a considered therapeutic target in Parkinson’s disease. Some studies have already pointed to beneficial effects of noradrenergic enhancement by atomoxetine and droxidopa on cognition and blood pressure control (Weintraub et al., 2010; Kaufmann et al., 2014), and studies with these compounds are currently on-going.

Several limitations of our study should be noted. First, the sample size of 42 subjects is modest, but still represents a high standard for PET studies. Some VOIs used in the study are not clearly defined on anatomical MRI and we had to use previously recommended definitions in Montreal Neurological Institute space coordinates (Keren et al., 2009; Kranz et al., 2012). Blood pressure regulating medication was not withdrawn prior to orthostatic testing; however, prevalence of treatment did not differ between groups. We also did not control for circadian variation of blood pressure regulation as measurements were not performed at a fixed daytime. These factors might have contributed to reduced statistical power of our blood pressure analysis.

In conclusion, we found that noradrenergic impairment in Parkinson’s disease is linked to the presence of RBD and correlated with its key characteristic of increased muscle activity during REM sleep. Noradrenergic impairment might also contribute to the high prevalence of cognitive decline and orthostatic hypotension in Parkinson’s disease patients, especially in patients with RBD, and might point to new options for treatment of these conditions.
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Supplementary material

$^{11}$C-MeNER PET image acquisition and choice of reference region

A six minute transmission scan was acquired for attenuation correction prior to each $^{11}$C-MeNER emission scan. Radiotracer doses of intravenously injected $^{11}$C-MeNER did not differ significantly between groups (healthy control, 646.3 ± 119.6, PD$^{RBD-}$, 707.4 ± 63.6, PD$^{RBD+}$, 707.8 ± 138.5 MBq; p = 0.243). Dynamic PET was acquired over 90 minutes in list-mode 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.

Despite brain-wide noradrenergic innervation, regions with variable noradrenaline re-uptake transporter densities have been identified, with lowest binding of the MeNER ligand to the occipital cortex in humans. In line with these results, binding of $^{11}$C-MeNER in the occipital cortex was not significantly displaceable by pharmacologic interventions on the noradrenaline transporter, providing evidence that the occipital cortex is a suitable reference region to study specific binding in brain regions with higher expression of noradrenaline transporters (i.e. thalamus, hypothalamus, red nucleus, locus coeruleus, and raphe nuclei). To calculate specific binding with the simplified reference tissue model 2 (SRTM2), the efflux rate constant of the reference region (k2’) has to be defined and is typically fixed across subjects. The average k2’ for the occipital cortex was previously reported to be 0.021 min$^{-1}$, and was used in our study, since it was in accordance with estimates derived from coupled fits of k2’ within the SRTM2 model using all studied high binding areas (thalamus, hypothalamus, red nucleus, locus coeruleus, and raphe nuclei): averaged k2’ = 0.022 min$^{-1}$ for all studied 42 subjects without differences between Parkinson’s disease patients and healthy control subjects.


References


Figure Legends

Figure 1: $^{11}$C-MeNER Distribution volume ratios (DVR) of PD patients and control subjects

A. Averaged $^{11}$C-MeNER DVR in noradrenaline transporter rich regions (Thal = thalamus, Hypo = hypothalamus, RN = red nucleus, LC = locus coeruleus, DR = dorsal raphe, MR = median raphe) of 12 healthy controls, 14 PD$^{RBD-}$, and 16 PD$^{RBD+}$ subjects; error bars indicate standard error of the mean (SEM); pair-wise comparison as indicated: * = p < 0.05, ** = p < 0.01, *** = p < 0.001. B. Representative sagittal slices of averaged parametric maps of $^{11}$C-MeNER DVR of the groups with corresponding anatomical MRI on the left; upper row is a paramedian section through thalamus, red nucleus, and hypothalamus, lower row is a median section through brainstem nuclei. $^{11}$C-MeNER images are levelled from 0.8 (dark blue) to 1.8 (red). Abbreviations: HC = healthy control, MRI = magnetic resonance imaging, PD$^{RBD-}$ = Parkinson’s disease without REM sleep behaviour disorder, PD$^{RBD+}$ = Parkinson’s disease with REM sleep behaviour disorder.

Figure 2: MRI neuromelanin contrast of the locus coeruleus and its correlation to $^{11}$C-MeNER DVR

A. Averaged neuromelanin MRI locus coeruleus-to-pons ratios of healthy control, PD$^{RBD-}$, and PD$^{RBD+}$ subjects (healthy control, 1.34 ± 0.05, PD$^{RBD-}$, 1.30 ± 0.05, PD$^{RBD+}$, 1.27 ± 0.04); error bars indicate standard error of the mean (SEM); group effect p < 0.001; pair-wise comparison as indicated: * = p < 0.05, *** = p < 0.001. B. Representative coronal sections of the dorsal brainstem on neuromelanin sensitive MRI of a healthy control subject with locus coeruleus-to-pons ratio of 1.36 (upper row) and of a PD$^{RBD+}$ subject with locus coeruleus-to-pons ratio of 1.25 (lower row); images were normalized to the intensity of the pons. The locus coeruleus is typically visible as a bilateral, hyper-intense, longitudinal structure in the dorsal pons in healthy subjects as seen in the upper row, but disappears in PD$^{RBD+}$ subjects. C. Correlation of individual neuromelanin MRI locus coeruleus-to-pons ratios with $^{11}$C-MeNER DVRs of the thalamus (rho = 0.339, p = 0.030 for all groups combined). Abbreviations: DVR = distribution volume ratio, HC = healthy control, LC = locus coeruleus, MRI = magnetic resonance imaging, PD$^{RBD-}$ = Parkinson’s disease without REM sleep behaviour disorder, PD$^{RBD+}$ = Parkinson’s disease with REM sleep behaviour disorder.
Figure 3: Correlation of $^{11}$C-MeNER DVR of the locus coeruleus with extent of altered REM sleep atonia in PD

Each symbol represents one subject and $^{11}$C-MeNER DVR of the locus coeruleus is plotted against the percentage amount of REM sleep with ‘any’ activity (A), ‘tonic’ activity (B), and ‘phasic’ activity (C). Correlations were calculated in the PD$^{RBD+}$ group only ($r = -0.623$, $p = 0.013$ for ‘any’, $r = -0.678$, $p = 0.006$ for ‘tonic’, and $r = -0.551$, $p = 0.033$ for ‘phasic’ activity). As control subjects elicited only marginal muscle activity during REM sleep, they were not included in data presentation. Abbreviations: DVR = distribution volume ratio, PD$^{RBD-}$ = Parkinson’s disease without REM sleep behaviour disorder, PD$^{RBD+}$ = Parkinson’s disease with REM sleep behaviour disorder.

Figure 4: Correlation of $^{11}$C-MeNER DVR with cognitive and autonomic assessments

A-B. Correlation between $^{11}$C-MeNER DVR of the locus coeruleus with global cognition expressed as averaged z-scores of all five cognitive domains ($r = 0.394$, $p = 0.010$), and between $^{11}$C-MeNER DVR of the locus coeruleus with electroencephalographic background frequency expressed as the averaged peak frequency of O1 and O2 electroencephalography leads ($r = 0.490$, $p = 0.001$). C-D. Correlation of changes in systolic blood pressure and diastolic blood pressure with $^{11}$C-MeNER DVR of the hypothalamus (systolic pressure: $r = 0.339$, $p = 0.040$ and diastolic pressure: $r = 0.367$, $p = 0.025$). Comparable correlations were obtained for $^{11}$C-MeNER DVR of the locus coeruleus and blood pressure parameters (not shown). Abbreviations: EEG = electroencephalography, DVR = distribution volume ratio, HC = healthy control, PD$^{RBD-}$ = Parkinson’s disease without REM sleep behaviour disorder, PD$^{RBD+}$ = Parkinson’s disease with REM sleep behaviour disorder.
Table 1: Demographics and clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>HC (n=12)</th>
<th>PD&lt;sup&gt;RBD&lt;/sup&gt;- (n=14)</th>
<th>PD&lt;sup&gt;RBD+&lt;/sup&gt; (n=16)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Age</td>
<td>67.3 ± 6.3</td>
<td>65.4 ± 9.0</td>
<td>67.7 ± 9.3</td>
<td>ns&lt;sup&gt;ǂ&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex (male / female)</td>
<td>9 / 3</td>
<td>10 / 4</td>
<td>12 / 4</td>
<td>ns&lt;sup&gt;§&lt;/sup&gt;</td>
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<tr>
<td>Sniffing sticks, correct</td>
<td>11.1 ± 2.8</td>
<td>7.6 ± 2.4</td>
<td>6.6 ± 2.9</td>
<td>&lt;0.001&lt;sup&gt;ǂ1,2&lt;/sup&gt;</td>
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<tr>
<td>GDS-15, total</td>
<td>0.3 ± 0.8</td>
<td>0.8 ± 1.4</td>
<td>1.2 ± 1.4</td>
<td>ns&lt;sup&gt;ǂ&lt;/sup&gt;</td>
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<tr>
<td>MoCA, total</td>
<td>28.3 ± 0.9</td>
<td>27.2 ± 2.3</td>
<td>26.4 ± 2.2</td>
<td>ns&lt;sup&gt;ǂ&lt;/sup&gt;</td>
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**PD characteristics**

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<tr>
<td>Disease duration [years]</td>
<td>5.0 ± 3.6</td>
<td>7.6 ± 4.6</td>
<td>ns&lt;sup&gt;ǂ&lt;/sup&gt;</td>
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<tr>
<td>Hoehn &amp; Yahr</td>
<td>2.1 ± 0.6</td>
<td>2.4 ± 0.5</td>
<td>ns&lt;sup&gt;ǂ&lt;/sup&gt;</td>
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<tr>
<td>MDS-UPDRS III, total</td>
<td>32.6 ± 11.9</td>
<td>39.3 ± 10.5</td>
<td>ns&lt;sup&gt;ǂ&lt;/sup&gt;</td>
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<tr>
<td>MDS-UPDRS III, tremor</td>
<td>3.1 ± 3.8</td>
<td>3.9 ± 3.7</td>
<td>ns&lt;sup&gt;ǂ&lt;/sup&gt;</td>
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<tr>
<td>LEDD [mg]</td>
<td>547 ± 467</td>
<td>685 ± 342</td>
<td>ns&lt;sup&gt;ǂ&lt;/sup&gt;</td>
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**RBD characteristics**

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<tbody>
<tr>
<td>RBDSQ, total</td>
<td>2.8 ± 1.7</td>
<td>3.3 ± 2.2</td>
<td>7.3 ± 3.4</td>
<td>0.001&lt;sup&gt;ǂ2,3&lt;/sup&gt;</td>
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<tr>
<td>Chin muscle activity:</td>
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<tr>
<td>Tonic [% of REM sleep]</td>
<td>0.0 ± 0.2</td>
<td>0.1 ± 0.2</td>
<td>20.6 ± 21.7</td>
<td>&lt;0.001&lt;sup&gt;ǂ2,3&lt;/sup&gt;</td>
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<tr>
<td>Phasic [% of REM sleep]</td>
<td>1.8 ± 1.9</td>
<td>4.2 ± 1.8</td>
<td>34.6 ± 12.5</td>
<td>&lt;0.001&lt;sup&gt;ǂ1,2,3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Any [% of REM sleep]</td>
<td>1.8 ± 1.9</td>
<td>4.2 ± 1.8</td>
<td>39.8 ± 18.2</td>
<td>&lt;0.001&lt;sup&gt;ǂ1,2,3&lt;/sup&gt;</td>
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<sup>ǂ</sup> = parametric test (Student’s t-test for two groups, and one-way ANOVA for three groups, respectively), □ = non-parametric test (Mann-Whitney test for two groups, and Kruskal-Wallis test for three groups, respectively), § = χ² test; p-values for pair-wise comparison as indicated: 1 = HC versus PD<sup>RBD</sup>−, p <0.05, 2 = HC versus PD<sup>RBD+</sup>, p <0.05, 3 = PD<sup>RBD+</sup> versus PD<sup>RBD</sup>−, p <0.05. 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, RBDSQ = RBD symptom questionnaire, REM = rapid eye movement.
Table 2: Cognitive and autonomic assessment of healthy controls and PD patients

<table>
<thead>
<tr>
<th>Cognitive testing</th>
<th>HC (n=12)</th>
<th>PD&lt;sup&gt;RBD-&lt;/sup&gt; (n=14)</th>
<th>PD&lt;sup&gt;RBD+&lt;/sup&gt; (n=16)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory (z-score)</td>
<td>-0.09 ± 0.92</td>
<td>-0.28 ± 1.01</td>
<td>-0.65 ± 0.85</td>
<td>ns&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Working memory (z-score)</td>
<td>-0.04 ± 0.65</td>
<td>-0.01 ± 0.78</td>
<td>-0.44 ± 0.64</td>
<td>ns&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Executive function (z-score)</td>
<td>0.09 ± 0.91</td>
<td>0.07 ± 1.01</td>
<td>-0.23 ± 1.05</td>
<td>ns&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Language (z-score)</td>
<td>0.35 ± 0.69</td>
<td>0.14 ± 0.60</td>
<td>-0.27 ± 1.11</td>
<td>ns&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Visuomotorial function (z-score)</td>
<td>0.00 ± 0.62</td>
<td>-0.26 ± 0.72</td>
<td>-0.73 ± 0.82</td>
<td>0.037&lt;sup&gt;3,2&lt;/sup&gt;</td>
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<tr>
<td>Global performance (z-score)</td>
<td>0.08 ± 0.61</td>
<td>-0.07 ± 0.67</td>
<td>-0.46 ± 0.62</td>
<td>ns&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mild cognitive impairment [%]</td>
<td>16.7</td>
<td>21.4</td>
<td>50</td>
<td>ns&lt;sup&gt;8&lt;/sup&gt;</td>
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<tr>
<th>EEG</th>
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<tr>
<td>Background frequency [Hz]</td>
<td>9.79 ± 0.82</td>
<td>9.29 ± 0.83</td>
<td>8.52 ± 1.16</td>
<td>0.007&lt;sup&gt;3,2&lt;/sup&gt;</td>
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<tr>
<td>Alpha power [µV²]</td>
<td>22.55 ± 23.55</td>
<td>30.85 ± 26.94</td>
<td>28.54 ± 26.61</td>
<td>ns&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Theta power [µV²]</td>
<td>3.88 ± 2.72</td>
<td>9.95 ± 10.16</td>
<td>20.85 ± 20.41</td>
<td>0.009&lt;sup&gt;3,2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alpha/theta ratio</td>
<td>5.03 ± 2.32</td>
<td>4.67 ± 2.62</td>
<td>2.39 ± 1.90</td>
<td>0.008&lt;sup&gt;3,2&lt;/sup&gt;</td>
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<th>Autonomic assessment</th>
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<tbody>
<tr>
<td>Antihypertensive medication [%]</td>
<td>16.7</td>
<td>28.6</td>
<td>21.4</td>
<td>ns&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>SCOPA-AUT, total</td>
<td>5.0 ± 2.4</td>
<td>12.6 ± 5.6</td>
<td>12.1 ± 7.8</td>
<td>&lt;0.001&lt;sup&gt;1,2&lt;/sup&gt;</td>
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<td>BP changes upon tilting:</td>
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<tr>
<td>Orthostatic hypotension [%]</td>
<td>0</td>
<td>28.6</td>
<td>50</td>
<td>0.026&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Systolic pressure [mmHg]</td>
<td>7.5 ± 12.9</td>
<td>-4.2 ± 16.7</td>
<td>-12.2 ± 17.9</td>
<td>0.019&lt;sup&gt;1,2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diastolic pressure [mmHg]</td>
<td>7.2 ± 6.7</td>
<td>4.5 ± 10.2</td>
<td>2.8 ± 9.1</td>
<td>ns&lt;sup&gt;1&lt;/sup&gt;</td>
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<sup>1</sup> = one-way ANOVA with post-hoc testing, <sup>2</sup> = Kruskal-Wallis and Mann-Whitney test for pair-wise comparison, <sup>3</sup> = χ² test; p-values for pair-wise comparison as indicated: 1 = HC versus PD<sup>RBD-</sup>, p <0.05, 2 = HC versus PD<sup>RBD+</sup>, p <0.05, 3 = PD<sup>RBD+</sup> versus PD<sup>RBD-</sup>, p <0.05. Abbreviations: BP = blood pressure, EEG = electroencephalography, MCI = mild cognitive impairment, ns = not significant, PD = Parkinson's disease, RBD = REM sleep behavior disorder, SCOPA-AUT = scales for outcomes in PD - autonomic.