Microsleep disturbances are associated with noradrenergic dysfunction in Parkinson's disease

Christopher E. J. Doppler[1,2], Julia A. M. Smit[1], Maximilian Hommelsen[2], Aline Seger[1,2], Jacob Horsager[3], Martin B. Kinnerup[3], Allan K. Hansen[3], Tatyana D. Fedorova[3], Karoline Knudsen[3], Marit Otto[4,5], Adjmal Nahimi[3], Per Borghammer[3,*], and Michael Sommerauer[1,2,3,*]

* both authors contributed equally (shared last authorship)

[1]Department of Neurology, University Hospital Cologne, Faculty of Medicine, University of Cologne, Köln, Germany

[2]Institute of Neuroscience and Medicine (INM-3), Forschungszentrum Jülich, Jülich, Germany

[3]Department of Nuclear Medicine and PET Centre, Aarhus University Hospital, Aarhus, Denmark

[4]Department of Neurology, Aarhus University Hospital, Aarhus, Denmark

[5]Department of Clinical Neurophysiology, Aarhus University Hospital, Aarhus, Denmark

Corresponding author: Michael Sommerauer, MD, Department of Neurology, University Hospital Cologne, Faculty of Medicine, University of Cologne, Kerpener Straße 62, 50937 Köln, Germany, michael.sommerauer@uk-koeln.de

Abstract

Study Objectives: Parkinson's disease (PD) commonly involves degeneration of sleep-wake regulating brainstem nuclei; likewise, sleep-wake disturbances are highly prevalent in PD patients. As polysomnography macroparameters typically show only minor changes in PD, we investigated sleep microstructure, particularly cyclic alternating pattern (CAP), and its relation to alterations of the noradrenergic system in these patients.

Methods: We analysed 27 PD patients and 13 healthy control (HC) subjects who underwent over-night polysomnography and ^{11}C-MeNER positron emission tomography for evaluation of noradrenaline transporter density. Sleep macroparameters as well as CAP metrics were evaluated according to the consensus statement from 2001. Statistical analysis comprised group comparisons and correlation analysis of CAP metrics with clinical characteristics of PD patients as well as noradrenaline transporter density.

Results: PD patients and HC subjects were comparable in demographic characteristics (age, sex, body mass index) and polysomnography macroparameters. CAP rate as well as A index differed significantly between groups, with PD patients having a lower CAP rate (46.7 ± 6.6% versus 38.0 ± 11.6%, p = 0.015) and lower A index (49.0 ± 8.7/hour versus 40.1 ± 15.4/hour, p = 0.042). In PD patients, both CAP metrics correlated significantly with diminished noradrenaline transporter density in arousal prompting brainstem nuclei (locus coeruleus,

raphe nuclei) as well as arousal propagating brain structures like thalamus and bitemporal cortex.

Conclusions: Sleep microstructure is more severely altered than sleep macrostructure in PD patients and is associated with widespread dysfunction of the noradrenergic arousal system.

Keywords: microsleep, cyclic alternating pattern, Parkinson's disease, positron emission tomography, noradrenaline

Statement of significance: Parkinson's disease is the second most common neurodegenerative disorder and characterized by a widespread degeneration of brainstem nuclei. We found that noradrenergic system demise in Parkinson's disease is associated with disruption of microsleep structure assessed as metrics of cyclic alternating pattern. Our study provides evidence for involvement of a specific neurotransmitter system in modulation of cyclic alternating pattern occurrence in humans.

Introduction

More than 200 years ago intriguing sleep phenomena were described as a remarkable feature of Parkinson's disease (PD) in James Parkinson's essay on the "Shaking Palsy" [1]. In a more recent survey from 1988, up to 98% of PD patients reported sleep-wake disturbances [2]. Even though PD is still primarily defined by slowing of movements resulting from dopaminergic cell death in the substantia nigra [3], the abundance of patients suffering from disordered sleep is unsurprising: neuropathological changes are present in virtually all key neurotransmitter systems of sleep-wake regulation early in the course of the disease [4]. Surprisingly, despite these dramatic neuropathological changes, assessment of classic sleep macrostructure parameters with polysomnography, i.e. sleep stages, sleep efficiency, and sleep related breathing events, has typically yielded heterogenous results, showing no explicit differences between PD patients and control populations [5,6]. In the most recent meta-analysis of 63 case-control studies using polysomnography, differences in polysomnography variables were relatively modest, i.e. less than a 2% difference in the amount of slow-wave sleep, which cannot explain patients' high burden of disordered sleep [5]. Thus, a closer look on sleep microstructure might elucidate alterations of sleep in PD patients.

The cyclic alternating pattern (CAP) is defined as a periodic electroencephalographic (EEG) activity during non - rapid eye movement (NREM) sleep and represents an essential component of physiological sleep microstructure [7]. CAP is a physiological oscillatory EEG phenomenon involving sequences of salient transient events (A phases) in alternation with background EEG activity (B phases). Depending on the EEG rhythms during the A phase, 3 subtypes can be defined: A1, with EEG synchrony as predominant activity and EEG desynchrony occupying less than 20%; A2, with EEG activity as a mixture of slow and fast

rhythms with 20 - 50% of the A phase occupied by EEG desynchrony; and A3, with EEG activity of predominantly rapid low-voltage rhythms occupying more than 50% of the A phase [7]. CAP can be provoked by internal and external stimuli of arousal, i.e. auditory perturbations [8] or apnea events [9]; thus, CAP appearance is thought to be associated with arousal instability during sleep [7,10].

Little is known about changes of CAP metrics in PD patients, but a recent report on subjects with idiopathic REM sleep behavior disorder (iRBD) - representing a prodromal stage of PD [11] - demonstrated a reduction of CAP rate, which was associated with earlier phenoconversion to motor PD [12]. Interestingly, iRBD subjects already show degeneration of lower brainstem nuclei including the arousal-prompting noradrenergic locus coeruleus and its thalamic and cortical terminals comparable to PD [13–15].

In this study, we aimed to analyse CAP metrics in PD patients compared to matched healthy control (HC) subjects and correlate findings with measures of noradrenergic integrity, i.e. noradrenergic terminal density as measured with ^{11}C-MeNER positron emission tomography (PET). We postulated that PD patients would show a reduced CAP rate similar to iRBD subjects, and this would be associated with altered noradrenergic terminal density.

Methods

Participants

We evaluated 30 PD patients and 12 HC subjects from a previous study [15], and additional 5 subjects (3 PD patients and 2 HC subjects) from an ongoing study at our institution who underwent overnight polysomnography and ^{11}C-MeNER PET. To reduce a potential bias on CAP metrics due to extraordinary high rates of apnea/hypopnea or periodic limb movement (PLM) events [9], we only included subjects with apnea-hypopnea index (AHI) in the range of 0 – 25/hour and PLM index in the range of 0 – 80/hour, resulting in 27 PD patients and 13

HC subjects for final analysis. All subjects were between 50 - 85 years old, non-demented (Montreal Cognitive Assessment > 22), non-depressed (Geriatric Depression Scale 15 < 6), and without known sleep related breathing disorders. Diagnosis of PD was undertaken according to the current consensus criteria [3], differential dopaminergic therapy was ascertained, levodopa equivalent daily doses (LEDD) were estimated as previously recommended [16], disease severity was judged according to Hoehn and Yahr stage, and motor symptoms were quantified according to the Movement Disorder Society Unified Parkinson's disease rating scale part III (MDS-UPDRS III) after 12 hours of medication withdrawal. Patients were recruited through advertisement in the Danish Parkinson's disease magazine and from collaborating neurological clinics. HC subjects were recruited through newspaper advertisements. All subjects gave written informed consent prior to study inclusion.

Polysomnographic data and CAP analysis

We used a mobile SOMNOscreen™ plus device for overnight video-polysomnography including 10 EEG recordings (according to the international 10/20 system: F3, F4, C3, C4, O1, O2, A1, A2, Fpz as grounding, and Cz as reference), electrooculography, surface electromyography of the submental muscle and the tibialis anterior muscles, electrocardiography, nasal pressure monitoring, thoracic and abdominal respiratory effort belts, finger pulse oximetry, and synchronized audio-visual recording. Visual PSG scoring was performed on 30-second epochs including sleep efficiency, sleep cycles, total sleep time, relative amount of stage 1 (N1), stage 2 (N2), stage 3 (N3) sleep, and REM sleep, arousal index (number of arousals per hour of sleep), apnoea-hypopnea index (AHI, number of apnea plus hypopnea events per hour of sleep) and periodic limb movement index (PLMI,

number of periodic leg movements per hour of sleep) according to the American Academy of Sleep (AASM) Manual for the Scoring of Sleep and Associated Events Version 2.5. An arousal was defined as a visible and abrupt shift to higher frequencies in any of the electroencephalogram leads, with a duration of 3 to 15 s [17]. Movement- and breathing-related arousals were included in the further analysis. Patients were allowed to take their regular evening medication during overnight polysomnography, motor assessments were performed in the morning after 12 hours of medication withdrawal.

CAP scoring followed the rules of the consensus criteria published in 2001 [7]. Scoring was performed using DOMINO software by a board-certified sleep expert (MS) who was blinded to clinical features during analysis by anonymizing of the PSG and blocked video recording. EEG channels were presented referenced to the contralateral ear as well as bipolar in a longitudinal montage to optimize A and B phase detection. A phase subtypes (A1, A2, A3) during NREM sleep were tagged directly on the EEG data and exported from DOMINO software into the American Standard Code for Information Interchange (ASCII). Similarly, sleep stage scorings were exported and a custom-made Python script identified NREM episodes including CAP by applying the consensus criteria to the exported data [7]. The following CAP parameters were calculated: CAP rate (CAP time / total NREM time × 100), occurrence rate A1-A3 (amount of A1(-A3) phases / all A phases x 100), mean duration of A and B phases, and average duration of CAP sequences.

^{11}C-MeNER PET imaging

All PET scans were acquired on a Siemens High Resolution Research Tomograph (HRRT) during daytime, less than one week apart of polysomnography recording. ^{11}C-MeNER is a high-affinity and highly selective radioligand to the noradrenergic transporter located presynaptically on brain neurons mostly originating from the locus coeruleus [18]. Ligand

binding is thought to be unaffected by transient fluctuations of endogenous noradrenaline levels. ^{11}C-MeNER radiosynthesis and image acquisition were performed as described previously [19]. In brief, after a transmission scan and intravenous bolus injection of ^{11}C-MeNER, a 90 minutes PET scan was acquired. Images were analysed with PMOD 4.0, including motion correction and normalization into Montreal Neurological Institute (MNI) space via rigid matching of subject's PET to an additionally acquired anatomical T1-weighted MRI. To optimize kinetic modelling, we smoothed PET images with a 4 mm Gaussian filter prior to calculation of parametric maps of distribution volume ratios (DVRs) with the simplified reference tissue model 2 (SRTM2). We used voxel-wise calculations in PXMOD initiated with thalamus and caudate as ^{11}C-MeNER high- and low-binding regions, respectively. DVRs of the thalamus, locus coeruleus, median raphe, and dorsal raphe were extracted for volume of interest analysis for group comparison and correlation analysis as they show high ^{11}C-MeNER binding and represent brain structures tightly engaged in arousal propagation. Thalamus and caudate were defined from the PMOD built-in atlas, and the locus coeruleus as well as dorsal and median raphe were manually drawn accordingly to published MNI coordinates and anatomical landmarks [20,21]. For correlation analysis of cortical ^{11}C-MeNER uptake, all individual parametric maps were non-rigidly transformed to an in-house ^{11}C-MeNER template and additionally smoothed with a 4 mm Gaussian filter prior to voxel-wise analysis [22,23].

Statistical analysis

We analysed the data with Statistical Package for the Social Sciences (SPSS) version 24. Group data are presented as mean ± standard deviation unless otherwise stated. Group comparisons were calculated using Student *t*, Mann-Whitney, and chi-square statistics as

appropriate, univariate correlation analyses were calculated with Pearson's *r*, and multivariate associations were analysed with linear regression analysis. Normal distribution of data was assessed with the Shapiro-Wilk test, Q-Q plots, and box plots. Significance was accepted at $p < 0.05$. For volume of interest-based PET analysis, we calculated a repeated measures analysis of variance (ANOVA) with group as between-subject and brain regions as within-subject factors. Statistical Parametric Mapping 12 (SPM12) software was used for voxel-wise correlation analysis of cortical ^{11}C-MeNER binding including a cortical gray matter mask for explicit masking. The voxel-level analysis threshold was set at $p < 0.005$ uncorrected. Surface projections of the resulting voxels were overlaid on the rendered brain included in the SPM12 package.

Results

Demographic and clinical data

HC subjects and PD patients were comparable in age, sex, and body mass index (table 1). Mean age of all included subjects was 66.1 ± 8.3 years, and 30% were female. PD patients had a mean disease duration of 6.3 ± 4.2 years and an average Hoehn & Yahr stage of 2.2 ± 0.4. Only one patient was treatment-naïve, nine patients were levodopa-naïve. On conventional polysomnographic parameters, PD patients and HC subjects showed largely similar metrics (table 1), but PD patients exhibited a numerical but statistically non-significant decrease of sleep stage N1 (16.2 ± 4.9% versus 12.6 ± 6.2%, $p = 0.079$) and an increase of REM sleep amount (12.3 ± 4.6% versus 18.0 ± 9.3%, $p = 0.045$). Both groups were comparable according to arousal prompting events, i.e. AHI and PLMI, but PD patients

showed a diminished arousal index compared to HC subjects (23.0 ± 4.9/hour versus 15.2 ± 6.1/hour, $p < 0.001$).

CAP sleep microstructure analysis

PD patients exhibited an approximately 19% lower CAP rate than controls (46.7 ± 6.6% versus 38.0 ± 11.6%, $p = 0.015$; table 2). Occurrence of CAP was diminished in PD patients in all NREM sleep stages by a similar amount but reached statistical significance in N2 and N3 only. Duration of averaged CAP sequences was shortened in PD patients (244.0 ± 40.7s versus 193.9 ± 39.5s, $p < 0.001$), despite similar duration for A and B phases between groups. This points to inclusion of less A and B phases in each CAP in PD patients. Accordingly, index of A phases was lower in PD patients compared to healthy controls (49.0 ± 8.7/hour versus 40.1 ± 15.4/hour, $p = 0.042$). Subtype analysis revealed that both, the A1 index and the combined A2+A3 index were numerically lower in PD patients, but only the difference seen in the A2+A3 index reached statistical significance (table 2). The nine levodopa-naïve patients did not differ in any examined CAP measure compared to patients taking levodopa (all $p > 0.05$).

The combined A2+A3 index, but not the A1 index correlated with arousal index independently of group effects ($\beta = 0.525$, $p = 0.002$ for A2+A3 index, and $\beta = -0.236$, $p = 0.169$ for A1 index, respectively; multiple linear regression analysis).

A stepwise multiple linear regression analysis showed an association between worsening motor symptoms (higher MDS-UPDRS III) and lower A index in PD patients ($\beta = -0.402$, $p = 0.038$). No associations were found for age, disease duration, Hoehn & Yahr stage, and LEDD. Similar analysis for CAP rate did not reveal any significant predictor (trend for MDS-

UPDRS III: β = -0.339, p = 0.084). Results remained identical when AHI and PLMI were included in the analysis as potential confounders.

CAP metrics and noradrenaline transporter PET

Consistent with our previous publication [15], PD patients exhibited reduced ^{11}C-MeNER binding in arousal prompting brainstem nuclei and the thalamus (group effect, p = 0.002; pontine raphe, p = 0.115; dorsal raphe, p = 0.003; locus coeruleus, p = 0.004; thalamus p < 0.001; figure 1A). CAP rate as well as A index correlated with ^{11}C-MeNER binding in these regions in PD patients but not in HC subjects (for PD patients: pontine raphe, r = 0.615, p = 0.001; dorsal raphe, r = 0.646, p < 0.001; locus coeruleus, r = 0.371, p = 0.057 (in pooled HC + PD analysis: r = 0.365, p = 0.021); thalamus, r = 0.630, p < 0.001; figure 1B). The findings were robust when MDS-UPDRS III values and levodopa-treatment were included as covariates. CAP rate also correlated with bitemporal cortical ^{11}C-MeNER binding in PD patients (figure 2A). Accordingly, A index correlated with ^{11}C-MeNER binding in similar regions (figure 2B). CAP rate and A index did not correlate with ^{11}C-MeNER binding in the HC group.

Discussion

We demonstrated that despite comparable polysomnographic macroparameters of sleep, PD patients exhibited significant alterations in sleep microstructure assessed as CAP metrics: patients showed reductions in CAP rate and A index and shortened CAP sequence duration. Occurrence of CAP was related to the integrity of the noradrenergic system in arousal-promoting and -propagating brain structures.

Even though arousals are determined as events of sleep interruption and fragmentation, their presence during sleep is a normal physiological phenomenon in all ages - even in the absence of an external or internal trigger [24]. The assessment of CAP expands the view on dynamic organization of sleep by considering physiological rhythms of stable (periods without CAP) and unstable sleep (phases showing CAP) [25]. Additionally, a three-stage hierarchy of arousal strength can be identified within the CAP methodology indicated by the different A phases with gradual decline of EEG synchrony [25]. The A3 phase with predominant EEG desynchrony - and to a lesser extent the A2 phase - are most comparable to the classical 'arousal' definition according to the AASM scoring scheme and both parameters typically correlate, as they did in our sample [9]. However, the arousal index increases linearly with age [24], whereas CAP rate follows a U-shape with a nadir during young adulthood and an increasing propensity in the elderly [26], clearly indicating differences in both assessments - accounting for sleep fragmentation (classical arousal) and sleep instability (CAP) [25]. Accordingly, an increased CAP rate - but also arousal index - can occur upon pathologically elevated sleep disruptive elements such as periodic limb movements, apnea/hypopnea events [9], or auditory pertubations [8]. Nevertheless, CAP also represents physiological oscillations of the sleeping brain showing recurring periods of activation; hence, not only an increase of CAP occurrence but also a decrease might be indicative of defective sleep not capable to grant its full beneficial potential.

Our findings of diminished CAP presence are in line with earlier observations in iRBD subjects which represent a prodromal stage of PD [12]. Therefore, disruption of microsleep structure may occur early in PD genesis due to degeneration of non-dopaminergic pathways. Interestingly, a lower CAP rate was also predictive of more rapid phenoconversion of iRBD subjects to overt motor PD, possibly indicating a more advanced

stage of iRBD [12]. Growing evidence suggests that propagating α-synuclein pathology - probably driving neurodegeneration in PD - impacts lower brainstem nuclei in the medulla and the pons prior to dopaminergic neurons located in the mesencephalon [14,27,28]. This specifically holds true for the arousal promoting noradrenergic locus coeruleus [14,29,30], whereas the serotonergic raphe nuclei seem to be affected only in subpopulations of iRBD subjects [31–33]. These findings underpin the central role of noradrenergic signalling in the regulation of CAP occurrence. Accordingly, CAP rate as well as A index were associated with noradrenergic transporter density independently of motor deterioration as measured with the MDS-UPDRS III in our PD sample. Nevertheless, even though we identified nine PD patients as being levodopa-naïve and we did not observe significant differences between levodopa-naïve patients and patients on levodopa treatment, our study was not designed to properly assess treatment effects on CAP metrics. Levodopa as precursor for various monoamines could potentially also modify noradrenergic signalling, and we cannot rule out treatment effects altering sleep microstructure.

We also observed an association of thalamic and temporal cortical noradrenaline transporter density with CAP rate and A index. The thalamus is a key relay station, in which a plethora of thalamo-cortical connections are converging, which can be selectively enhanced and diminished, eventually modulating the state of consciousness and sleep [34,35]. Strikingly, loss of connectivity in the temporal cortex is a hallmark of the transition from the wake state to sleep and vice versa [36,37]. Hence, loss of noradrenergic function in the thalamus and temporal cortex together with reduced CAP in PD patients might represent an altered ability to (a) react to arousal prompting triggers and (b) to establish states of higher activation during sleep. In line with this, patients also showed a reduced arousal index compared to HC subjects, as shown previously [17].

Reports on patients with mild cognitive impairment (MCI) as a precursor of Alzheimer's disease indicated similar changes of CAP metrics as seen in our study and in iRBD subjects [12,38,39]. Patients with MCI showed reductions in CAP rate and the A index, and a stronger reduction was associated with more rapid cognitive deterioration and conversion to clinical dementia [38,39]. Strikingly, degeneration of the noradrenergic LC is - similar to PD - also an early insult in MCI and Alzheimer's disease and LC cell loss could be associated with cognitive performance in these patients [40,41].

However, limited functional imaging data has been published on brain circuitries engaged in CAP occurrence and modulation. A recent study using functional magnetic resonance imaging techniques reported enhanced activation of a network involving the insula, the middle cingulate gyrus, and the basal forebrain during A phases as recorded with simultaneous electroencephalography [42]. To the best of our knowledge, no other studies have utilized functional imaging data and investigated the relationship between CAP regulatory neurotransmitter systems and potentially involved networks - more work will hopefully shed light on this topic.

In summary, our data indicate that PD patients exhibit changes of microsleep parameters which exceed alterations seen with classic sleep analysis. Specifically, markers of sleep instability were reduced and associated with noradrenergic dysfunction. As noradrenergic function plays a crucial role in maintaining cognitive performance in AD and PD, microsleep disturbances might be another putative link to cognitive disturbances in these disorders.

Disclosure Statement

This research was supported by the Lundbeck Foundation (grant number 6970), the Danish Council for Independent Research, Medical Sciences (grant number 0602-02700), Aarhus University Research Foundation, the Danish Association of Parkinson's Disease (Parkinson-foreningen), the Swiss National Science Foundation (grant number P2SKP3_161812), the Hildegard Henssler-Stiftung, the Koeln Fortune Program / Faculty of Medicine, University of Cologne (grant number 453/2018), the Cologne Clinician Scientist Program (CCSP) / Faculty of Medicine / University of Cologne, funded by the German Research Foundation (DFG, FI 773/15-1) and the Else Kröner-Fresenius-Stiftung (grant number 2019_EKES.02).

Financial disclosure: The authors have no financial disclosures to declare.

Non-financial disclosure: The authors declare no conflicts of interests with regards to this manuscript.

References

1. Pauletto G, Belgrado E, Marinig R, Bergonzi P. Sleep disorders and extrapyramidal diseases: An historical review. *Sleep Med*. 2004;5(2):163-167. doi:10.1016/j.sleep.2003.10.008
2. Lees AJ, Blackburn NA, Campbell VL. The nighttime problems of Parkinson's disease. *Clin Neuropharmacol*. 1988;11(6):512-519. doi:10.1097/00002826-198812000-00004
3. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord*. 2015;30(12):1591-1601. doi:10.1002/mds.26424
4. Del Tredici K, Braak H. To stage, or not to stage. *Curr Opin Neurobiol*. 2020;61:10-22. doi:10.1016/j.conb.2019.11.008
5. Zhang Y, Ren R, Sanford LD, et al. Sleep in Parkinson's disease: A systematic review and meta-analysis of polysomnographic findings. *Sleep Med Rev*. 2020;51. doi:10.1016/j.smrv.2020.101281
6. Peeraully T, Yong MH, Chokroverty S, Tan EK. Sleep and Parkinson's disease: A review of case-control polysomnography studies. *Mov Disord*. 2012;27(14):1729-1737. doi:10.1002/mds.25197
7. Terzano MG, Parrino L, Sherieri A, et al. Atlas, rules, and recording techniques for the scoring of cyclic alternating pattern (CAP) in human sleep. *Sleep Med*. 2001;2(6):537-553. doi:10.1016/S1389-9457(01)00149-6
8. Terzano MG, Parrino L, Fioriti G, Orofiamma B, Depoortere H. Modifications of sleep structure induced by increasing levels of acoustic perturbation in normal subjects. *Electroencephalogr Clin Neurophysiol*. 1990;76(1):29-38. doi:10.1016/0013-4694(90)90055-O
9. Hartmann S, Bruni O, Ferri R, Redline S, Baumert M. Characterization of cyclic

alternating pattern during sleep in older men and women using large population studies. *Sleep*. 2020;43(7). doi:10.1093/sleep/zsaa016

10. Parrino L, Ferri R, Bruni O, Terzano MG. Cyclic alternating pattern (CAP): The marker of sleep instability. *Sleep Med Rev*. 2012;16(1):27-45. doi:10.1016/j.smrv.2011.02.003
11. Dauvilliers Y, Schenck CH, Postuma RB, et al. REM sleep behaviour disorder. *Nat Rev Dis Prim*. 2018;4(1). doi:10.1038/s41572-018-0016-5
12. Melpignano A, Parrino L, Santamaria J, et al. Isolated rapid eye movement sleep behavior disorder and cyclic alternating pattern: Is sleep microstructure a predictive parameter of neurodegeneration? *Sleep*. 2019;42(10). doi:10.1093/sleep/zsz142
13. Andersen KB, Hansen AK, Sommerauer M, et al. Altered sensorimotor cortex noradrenergic function in idiopathic REM sleep behaviour disorder – A PET study. *Park Relat Disord*. 2020;75:63-69. doi:10.1016/j.parkreldis.2020.05.013
14. Knudsen K, Fedorova TD, Hansen AK, et al. In-vivo staging of pathology in REM sleep behaviour disorder: a multimodality imaging case-control study. *Lancet Neurol*. 2018;17(7):618-628. doi:10.1016/S1474-4422(18)30162-5
15. Sommerauer M, Fedorova TD, Hansen AK, et al. Evaluation of the noradrenergic system in Parkinson's disease: An 11 C-MeNER PET and neuromelanin MRI study. *Brain*. 2018;141(2):496-504. doi:10.1093/brain/awx348
16. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov Disord*. 2010;25(15):2649-2653. doi:10.1002/mds.23429
17. Sommerauer M, Imbach LL, Jarallah M, Baumann CR, Valko PO. Diminished event-related cortical arousals and altered heart rate response in Parkinson's disease. *Mov Disord*. 2015;30(6):866-870. doi:10.1002/mds.26165

18. Nahimi A, Kinnerup MB, Sommerauer M, Gjedde A, Borghammer P. Molecular Imaging of the Noradrenergic System in Idiopathic Parkinson's Disease. *Int Rev Neurobiol*. 2018;141:251-274. doi:10.1016/bs.irn.2018.07.028

19. Nahimi A, Sommerauer M, Kinnerup MB, et al. Noradrenergic Deficits in Parkinson Disease Imaged with (11)C-MeNER. *J Nucl Med*. 2018;59(4):659-664. doi:10.2967/jnumed.117.190975

20. Keren NI, Taheri S, Vazey EM, et al. Histologic validation of locus coeruleus MRI contrast in post-mortem tissue. *Neuroimage*. 2015;113:235-245. doi:10.1016/j.neuroimage.2015.03.020

21. Kranz GS, Hahn A, Savli M, Lanzenberger R. Challenges in the differentiation of midbrain raphe nuclei in neuroimaging research. *Proc Natl Acad Sci U S A*. 2012;109(29):E2000. doi:10.1073/pnas.1206247109

22. Moriguchi S, Kimura Y, Ichise M, et al. PET quantification of the norepinephrine transporter in human brain with (S,S)-18F-FMeNER-D2. *J Nucl Med*. 2017;58(7):1140-1145. doi:10.2967/jnumed.116.178913

23. Sommerauer M, Hansen AK, Parbo P, et al. Decreased noradrenaline transporter density in the motor cortex of Parkinson's disease patients. *Mov Disord*. 2018;33(6):1006-1010. doi:10.1002/mds.27411

24. Boulos MI, Jairam T, Kendzerska T, Im J, Mekhael A, Murray BJ. Normal polysomnography parameters in healthy adults: a systematic review and meta-analysis. *Lancet Respir Med*. 2019;7(6):533-543. doi:10.1016/S2213-2600(19)30057-8

25. Terzano MG, Parrino L. Origin and significance of the cyclic alternating pattern (CAP). *Sleep Med Rev*. 2000;4(1):101-123. doi:10.1053/smrv.1999.0083

26. Parrino L, Boselli M, Spaggiari MC, Smerieri A, Terzano MG. Cyclic alternating pattern

(CAP) in normal sleep: Polysomnographic parameters in different age groups. *Electroencephalogr Clin Neurophysiol*. 1998;107(6):439-450. doi:10.1016/S0013-4694(98)00108-4

27. Goedert M, Spillantini MG, Del Tredici K, Braak H. 100 years of Lewy pathology. *Nat Rev Neurol*. 2013;9(1):13-24. doi:10.1038/nrneurol.2012.242

28. Klingelhoefer L, Reichmann H. Pathogenesis of Parkinson disease - The gut-brain axis and environmental factors. *Nat Rev Neurol*. 2015;11(11):625-636. doi:10.1038/nrneurol.2015.197

29. Garcia-Lorenzo D, Longo-Dos Santos C, Ewenczyk C, et al. The coeruleus/subcoeruleus complex in rapid eye movement sleep behaviour disorders in Parkinson's disease. *Brain*. 2013;136(Pt 7):2120-2129. doi:10.1093/brain/awt152

30. Carter ME, Yizhar O, Chikahisa S, et al. Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat Neurosci*. 2010;13(12):1526-1535. doi:10.1038/nn.2682

31. Barber TR, Griffanti L, Muhammed K, et al. Apathy in rapid eye movement sleep behaviour disorder is associated with serotonin depletion in the dorsal raphe nucleus. *Brain*. 2018;141(10):2848-2854. doi:10.1093/brain/awy240

32. Vilas D, Iranzo A, Pont-Sunyer C, et al. Brainstem raphe and substantia nigra echogenicity in idiopathic REM sleep behavior disorder with comorbid depression. *J Neurol*. 2015;262(7):1665-1672. doi:10.1007/s00415-015-7745-0

33. Arnaldi D, Famà F, De Carli F, et al. The role of the serotonergic system in REM sleep behavior disorder. *Sleep*. 2015;38(9):1505-1509. doi:10.5665/sleep.5000

34. Gent TC, Bassetti C LA, Adamantidis AR. Sleep-wake control and the thalamus. *Curr Opin Neurobiol*. 2018;52:188-197. doi:10.1016/j.conb.2018.08.002

35. Spoormaker VI, Schröter MS, Gleiser PM, et al. Development of a large-scale

functional brain network during human non-rapid eye movement sleep. *J Neurosci*. 2010;30(34):11379-11387. doi:10.1523/JNEUROSCI.2015-10.2010

36. González J, Cavelli M, Mondino A, et al. Decreased electrocortical temporal complexity distinguishes sleep from wakefulness. *Sci Rep*. 2019;9(1). doi:10.1038/s41598-019-54788-6

37. Banks MI, Krause BM, Endemann CM, et al. Cortical functional connectivity indexes arousal state during sleep and anesthesia. *Neuroimage*. 2020;211. doi:10.1016/j.neuroimage.2020.116627

38. Carnicelli L, Maestri M, Di Coscio E, et al. A longitudinal study of polysomnographic variables in patients with mild cognitive impairment converting to Alzheimer's disease. *J Sleep Res*. 2019;28(5). doi:10.1111/jsr.12821

39. Maestri M, Carnicelli L, Tognoni G, et al. Non-rapid eye movement sleep instability in mild cognitive impairment: A pilot study. *Sleep Med*. 2015;16(9):1139-1145. doi:10.1016/j.sleep.2015.04.027

40. Kelly SC, He B, Perez SE, Ginsberg SD, Mufson EJ, Counts SE. Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. *Acta Neuropathol Commun*. 2017;5(1):8. doi:10.1186/s40478-017-0411-2

41. Betts MJ, Cardenas-Blanco A, Kanowski M, et al. Locus coeruleus MRI contrast is reduced in Alzheimer's disease dementia and correlates with CSF Aβ levels. *Alzheimer's Dement Diagnosis, Assess Dis Monit*. 2019;11:281-285. doi:10.1016/j.dadm.2019.02.001

42. Kokkinos V, Vulliémoz S, Koupparis AM, et al. A hemodynamic network involving the insula, the cingulate, and the basal forebrain correlates with EEG synchronization phases of sleep instability. *Sleep*. 2019;42(4). doi:10.1093/sleep/zsy259

Figure captions

Figure 1: 11C-MeNER binding and its correlation with CAP metrics.

(A) Left panel: coronal section of averaged 11C-MeNER distribution volume ratio (DVR) of 13 healthy controls (HC, red border) and 27 Parkinson's disease patients (PD, blue border) at the level of the dorsal brainstem as indicated on anatomical T1 image - images are levelled from 0.8 (dark blue) to 1.8 (red). Right panel: Averaged 11C-MeNER DVR in noradrenaline transporter rich regions (red HC subjects, blue PD patients) - all regions but pontine raphe differed significantly ($p < 0.005$). (B) Upper row: dot plots of cyclic alternating pattern (CAP) rate and 11C-MeNER DVR in noradrenaline transporter rich regions; lower row: dot plots of A index and 11C-MeNER DVR in noradrenaline transporter rich regions (red HC subjects, blue PD patients).

Figure 2: Voxel-wise ^{11}C-MeNER correlation to CAP metrics in PD patients.

(A) Correlation of cortical ^{11}C-MeNER binding with cyclic alternating pattern (CAP) rate and (B) with A index.

Tables

Table 1: Clinical and Sleep characteristics of included subjects

	Healthy controls, n = 13	Parkinson's disease patients, n = 27	P value
Age [years]	67.4 ± 6.6	65.5 ± 9.0	0.669[1]
Sex [male / female]	9 / 4	18 / 9	0.871[3]
Body Mass Index [kg/m²]	25.3 ± 2.3	25.6 ± 3.4	0.805[2]
PD-related characteristics			
Disease duration [years]		6.3 ± 4.2	-
Hoehn & Yahr stage		2.2 ± 0.4	-
MDS-UPDRS III, OFF state		35.9 ± 11.1	-
Levodopa equivalent daily dose [mg]		603.0 ± 418.6	-
Subjects without levodopa treatment		9	
Levodopa daily dose [mg]*		525.0 ± 349.0	
Polysomnography characteristics			
Sleep efficiency [%]	82.3 ± 11.0	84.0 ± 9.3	0.613[2]
Sleep cycles	4.5 ± 0.7	4.0 ± 1.0	0.073[1]
Sleep latency N2 [min]	15.0 ± 14.5	16.0 ± 15.6	0.776[1]
Wake [%]	17.7 ± 11.0	15.9 ± 9.3	0.603[2]
N1 [%]	16.2 ± 4.9	12.6 ± 6.2	0.079[2]
N2 [%]	39.2 ± 9.9	39.3 ± 9.5	0.981[2]
N3 [%]	14.6 ± 6.0	13.9 ± 7.2	0.743[2]
REM [%]	12.3 ± 4.6	18.0 ± 9.3	**0.045**[2]
Apnea-Hypopnea Index [/hour]	10.1 ± 7.6	7.5 ± 6.8	0.264[1]
Periodic limb movement index [/hour]	17.6 ± 24.0	17.1 ± 22.5	0.955[1]
Arousal index [/hour]	23.0 ± 4.9	15.2 ± 6.1	**<0.001**[1]
REM sleep behavior disorder [n]	0	13	**<0.001**[3]

*only patients taking levodopa were included

Abbreviations: MDS-UPDRS III, Movement Disorder Society - Unified Parkinson's disease Rating Scale part III; N1-3, non-rapid eye movement sleep stage 1-3, REM, rapid eye movement sleep.

Values are given as mean ± standard deviation, [1] non-parametric Mann-Whitney-Test, [2] parametric Student's t-test, [3] chi-square test.

Table 2: Metrics of cyclic alternating pattern in healthy controls and PD patients

	Healthy controls	PD patients	P value
CAP Rate [%]	46.7 ± 6.6	38.0 ± 11.6	**0.015**[1]
CAP Rate, N1 [%]	22.1 ± 9.7	16.4 ± 11.5	0.132[1]
CAP Rate, N2 [%]	48.4 ± 11.3	37.9 ± 13.0	**0.017**[1]
CAP Rate, N3 [%]	73.4 ± 10.2	56.8 ± 13.8	**<0.001**[1]
Sequence duration [s]	244.0 ± 40.7	193.9 ± 39.5	**<0.001**[2]
Duration A [s]	8.4 ± 1.0	8.6 ± 1.0	0.577[1]
Duration B [s]	26.1 ± 1.8	26.4 ± 2.6	0.754[1]
A index [/hour]	49.0 ± 8.7	40.1 ± 15.4	**0.042**[2]
A1 index [/hour]	36.8 ± 7.1	31.7 ± 13.4	0.204[1]
A2 + A3 index [/hour]	12.2 ± 3.8	8.5 ± 4.3	**0.004**[2]

Abbreviations: CAP, cyclic alternating pattern; N1-3, non-rapid eye movement sleep stage 1-3; PD, Parkinson's disease; s, seconds.

Values are given as mean ± standard deviation, [1] parametric Student's *t*-test, [2] non-parametric Mann-Whitney-Test.

Figure 1

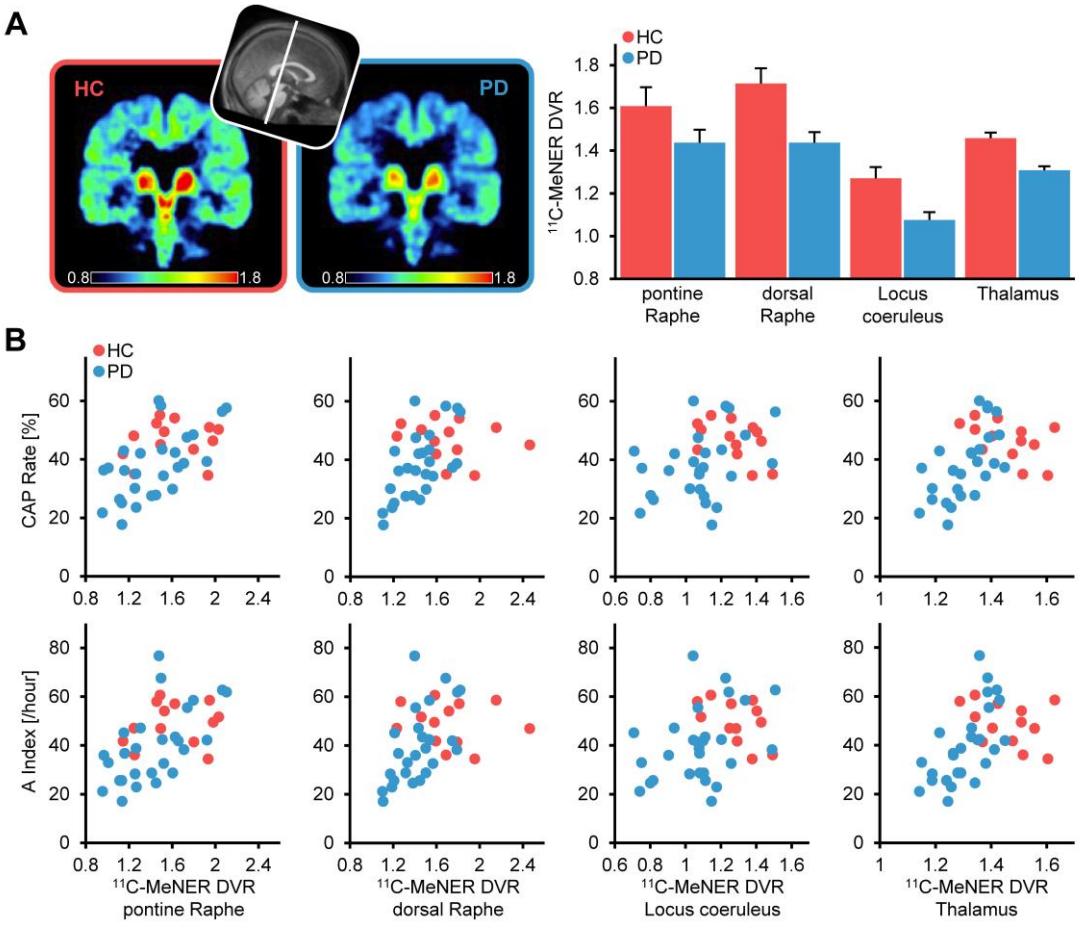

Figure 2