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Rapid eye movement (REM) sleep behavior disorder (RBD) is a frequent non-motor symptom of Parkinson's disease (PD) and a potential early marker of synucleinopathy-related neurodegeneration. While striatal dopaminergic dysfunction in PD-RBD has been extensively studied, the role of extrastriatal monoaminegic alterations -particularly serotonergic - remains poorly understood.

In this study, 155 PD patients underwent [123I]FP-CIT SPECT imaging to assess striatal and extrastriatal tracer binding, reflecting dopaminergic and serotonergic function, respectively. Probable RBD was identified using a validated single-question screening tool with high sensitivity and specificity. Patients with probable RBD (RBD+, n=44) were compared to those without (RBD−, n=111) using voxel-wise and region-of-interest analyses, controlling for age, sex, disease duration, motor and non-motor symptom severity, and cognitive function.

No significant differences were observed in striatal dopamine transporter binding. However, RBD+ patients showed significantly higher extrastriatal binding in the prefrontal cortex ($pFWE<0.05$), suggesting a role for altered serotonergic neurotransmission in PD-RBD pathophysiology.

Key words: Parkinson's disease, RBD, SPECT, serotonin

Increased frontal serotonergic function in Parkinson's disease patients with self-reported REM sleep behavior disorder

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Abstract

Rapid eye movement (REM) sleep behavior disorder (RBD) is a frequent non-motor symptom of Parkinson's disease (PD) and a potential early marker of synucleinopathy-related neurodegeneration. While striatal dopaminergic dysfunction in PD-RBD has been extensively studied, the role of extrastriatal monoaminegic alterations -particularly serotonergic - remains poorly understood. In this study, 155 PD patients underwent [^{123}I]FP-CIT SPECT imaging to assess striatal and extrastriatal tracer binding, reflecting dopaminergic and serotonergic function, respectively. Probable RBD was identified using a validated single-question screening tool with high sensitivity and specificity. Patients with probable RBD (RBD+, n=44) were compared to those without (RBD−, n=111) using voxel-wise and region-of-interest analyses, controlling for age, sex, disease duration, motor and non-motor symptom severity, and cognitive function. No significant differences were observed in striatal dopamine transporter binding. However, RBD+ patients showed significantly higher extrastriatal binding in the prefrontal cortex ($pFWE<0.05$), suggesting a role for altered serotonergic neurotransmission in PD-RBD pathophysiology.

Introduction

Rapid eye movement (REM) sleep behavior disorder (RBD) is a condition characterized by the enactment of dreams and complex motor behaviors, such as kicking, laughing, or crying during REM sleep[1]. Unlike in normal REM sleep, individuals with RBD have an absence of the typical muscle atonia. This disorder is closely associated with synucleinopathic neurodegeneration observed in Parkinson's disease (PD), dementia with Lewy bodies, multiple system atrophy and pure autonomic failure[1]. RBD can emerge as an early warning sign for these conditions, manifesting many years before the onset of motor or cognitive decline[2]. As such, RBD not only serves as a specific marker of synucleinopathies but also appears to demonstrate predictive power[2].

In the context of brain dopaminergic function, idiopathic RBD (iRBD) patients have been found to have reduced striatal dopamine transporter (DAT) uptake when compared to healthy controls, suggesting the role of iRBD as a premotor symptom of synucleinopathy leading to dopaminergic degeneration[3,4]. However, investigations into DAT activity among RBD-positive and RBD-negative PD patients have shown conflicting results. Some studies have suggested decreased DAT binding in the striatum in PD-RBD compared to PD patients without RBD[5-7], while other studies have shown no differences in striatal DAT between PD patients with or without RBD[8,9] , with the possibility of later decline in DAT binding.[10]

Beyond dopamine, several other neurotransmitters systems, including cholinergic, noradrenergic and serotonergic systems, are involved in PD pathophysiology[11]. Among these, emerging evidence suggests that serotonergic function may have particular relevance to RBD in PD. The serotonergic system has a modulatory effect of sleep-wake cycle[12], acute RBD can be induced by selective serotonin reuptake inhibitors (SSRIs) and selective serotonin and noradrenalin reuptake inhibitors (SNRIs)[13], and 5-hydroxytryptophan (5-HTP), a precursor of serotonin and melatonin, may ameliorate RBD symptoms in PD[14], further implicating the serotonergic system in the underlying mechanism of RBD.

A limited number of neuroimaging studies with relatively small sample sizes have specifically examined serotonin function in PD in relation to RBD. A positron emission tomography (PET) study using the serotonin transporter (SERT) ligand [^{11}C]DASB, focusing on the raphe nucleus and striatum, reported no differences in SERT binding between RBD-positive and RBD-negative PD patients (n = 11 vs 24)[15]. Another study, using [^{123}I]FP-CIT SPECT to examine the brainstem and thalamus, similarly found no differences in SERT function between iRBD patients and healthy controls (n = 20 vs 23)[16]. However, a separate PET study with [^{11}C]DASB demonstrated reduced SERT activity in the striatum, raphe nuclei and hypothalamus in PD patients with sleep disturbances compared to those without sleep dysfunction (n = 14 vs 14), although this study did not specifically compare RBD+ and RBD- patients[17]. The previous studies predominantly restricted analyses to subcortical brain regions.

Given the limited number of neuroimaging studies exploring serotonergic function in PD with RBD, particularly in cortical regions, and the potential importance of serotonin in the treatment and pathophysiology of RBD, we conducted a larger-scale investigation compared to previous studies. This study utilized [^{123}I]FP-CIT SPECT, a monoaminergic tracer commonly used to study DAT binding in the striatum, while focusing on its potential to reflect SERT binding[18] in cortical regions using voxel-wise image analysis.

Results

Demographics

Of the 155 patients included, 44 (28.4%) were classified as having self-reported probable RBD (RBD+), while 111 (71.6%) were without RBD (RBD-). RBD+ patients had longer motor symptom duration compared to RBD- patients (Table 1). No significant differences were observed between the groups in terms of age, sex, Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS) motor scores, total Non-Motor Symptoms Scale (NMSS) scores, Beck Depression Inventory (BDI) scores, use of dopaminergic or antidepressant medications, levodopa equivalent daily dose (LEDD), or use of alcohol, coffee, or nicotine. Cognitive performance, as measured by the Mini-Mental State Examination (MMSE), was significantly lower in RBD+ patients compared to RBD- patients (Table 1).

Region of Interest Analyses

In the region of interest (ROI) analyses, left caudate Specific binding ratios (SBRs) were lower at a trend level in RBD+ patients compared to RBD- patients (Table 1). However, no significant differences in striatal SBRs were observed between the groups after controlling for the effects of covariates (Table 2). The results remained unchanged when motor symptom duration was included as an additional covariate in the model.

Voxel-Wise Analyses

Whole-brain voxel-wise analyses revealed significantly increased [^{123}I]FP-CIT binding in the frontal cortex of RBD+ patients compared to RBD- patients, after adjusting for age, sex and MDS-UPDRS Part III motor scores (FWE-corrected $P<0.05$, Figure 1). The finding remained significant when MMSE and NMSS scores were included as additional covariates. The mean SBR within the identified frontal cluster was 9.2% higher in RBD+ patients (mean [SD] 1.17 [0.15]) compared to RBD- patients (1.08 [0.12]). No significant group differences in striatal [^{123}I]FP-CIT binding were observed in the voxel-wise analysis.

Discussion

The findings of this study demonstrate an association between self-reported RBD and increased monoaminergic binding in frontal brain regions, suggesting that alterations in frontal serotonin neurotransmission play a role in the pathophysiology of PD-related RBD. The absence of significant differences in striatal DAT binding between RBD+ and RBD− patients suggests that, at least beyond the initial motor symptom onset, dopaminergic dysfunction may not be the primary contributor to RBD in PD. These results support the growing view that non-dopaminergic systems, particularly serotonergic pathways in cortical regions, may play a critical role in the development of RBD.

Previous research on dopaminergic dysfunction in PD patients with RBD has yielded inconsistent findings. While some studies have reported reduced striatal DAT binding in RBD+ patients, these results have often been associated with greater motor impairment in the RBD+ group, raising the possibility that the observed reductions in DAT binding may reflect disease severity rather that a specific link to RBD[5]. Moreover, some of these differences have not remained significant after adjusting for confounding factors such as age, sex, and disease duration, or after correction for multiple comparisons[6,19]. Data from the Parkinson's Progression Markers Initiative (PPMI) have also been mixed, with some studies reporting mildly decreased striatal DAT binding in RBD+ patients at baseline [20,21], while others have found no group differences – consistent with our current findings[9,10]. These inconsistencies demonstrate the complexity of interpreting dopaminergic alterations in PD-RBD and suggest that changes in dopaminergic pathways may be subtle and dependent on study design, sample characteristics, and methodological approaches.

Although no differences in striatal DAT binding were observed between RBD+ and RBD- patients, a clear increase in tracer binding was observed in a region which encompassed several frontal cortical areas. Although FP-CIT is not a specific SERT tracer, we interpret this extrastriatal finding to reflect mainly SERT in the frontal region. This interpretation is supported by earlier studies suggesting that extrastriatal [^{123}I]FP-CIT reflects SERT density, as FP-CIT has moderate affinity to SERT[18,22,23]. In PD, SERT levels are generally reduced in the raphe nuclei, striatum, thalamus, and hypothalamus, with more pronounced motor symptoms correlating with greater

reductions in SERT activity[24]. In contrast, PD patients with depression have been reported to show increased SERT activity in the limbic system and raphe nuclei[24]. However, studies specifically examining serotonergic function in PD patients with RBD remain scarce. Kotagal et al. investigated SERT function in PD-RBD using PET with the SERT ligand [^{11}C]DASB and found no significant differences in brainstem or striatal SERT binding between PD patients with and without RBD. In contrast, their study showed widespread cholinergic denervation in multiple brain regions among PD-RBD patients[15]. Findings from studies of iRBD without PD are similarly inconsistent. A study using [^{123}I]FP-CIT SPECT to assess the relationship between SERT binding and iRBD reported no differences between iRBD patients and controls. This study focused only on brainstem and thalamic regions, excluding cortical areas, which may have contributed to the negative findings[16]. In contrast, a PET study using the SERT ligand [^{11}C]DASB in iRBD patients found reduced SERT densities in the neocortex, insula and putamen compared to control subjects[25]. However, the control group in this study was younger than the iRBD group, potentially confounding the results. Considering the findings in iRBD, it is important to note that the present study examined PD patients rather than comparing iRBD patients to healthy controls. This distinction is crucial when interpreting differences across studies, as the underlying neurodegenerative processes in PD could significantly change the role of SERT in the pathophysiology of RBD.

Increased SERT function in frontal regions could be associated with multiple features of RBD and sleep regulation, as serotonin plays an important role in maintaining wakefulness and preventing REM sleep[26]. Serotonergic dysfunction has been implicated in RBD pathophysiology, as evidenced by the observation that antidepressants, particularly SSRIs and SNRIs, may cause REM sleep without atonia (RSWA) or trigger RBD. This suggests that elevated serotonin activity could contribute to the disruption of normal REM sleep mechanisms. Further studies should be carried out investigate the structure of sleep and RBD characteristics in relation to frontal SERT changes.

There are certain limitations in the present study. First, RBD was identified using a single-question screening tool rather than polysomnography, which is the gold standard for RBD diagnosis. However, the screening tool used in this study has

been validated in a multicenter setting, demonstrating a sensitivity of 93.8% and a specificity of 87.2%[27]. To account for this limitation, we classified cases as *self-reported* rather than *confirmed* RBD, and further studies incorporation full polysomnography are needed to validate these findings. Second, the patient cohort in this study may not fully represent broader PD population, as all participants underwent [^{123}I]FP-CIT SPECT imaging due to initial diagnostic uncertainty. This selection criterion could introduce a bias toward atypical and diagnostically challenging cases. Finally, some patients characterized as non-RBD at the time of assessment may develop RBD in over time, as RBD symptoms can emerge later in the disease course. Longitudinal follow-up studies are required to assess the potential conversion of non-RBD patients to RBD+ status and to better understand the progression of serotonergic changes in PD.

In conclusion, this study provides evidence of increased frontal SERT availability in PD patients with self-reported RBD, suggesting a role for altered serotonergic neurotransmission in the pathophysiology of RBD in PD. These findings raise the possibility that serotonergic changes may contribute to, or compensate for, sleep-related dysfunction. Further longitudinal and mechanistic studies are needed to clarify the functional significance of frontal serotonergic alterations and their relationship to clinical features of PD-RBD.

Methods

Patients and study design

Between 2014 and 2019, patients undergoing [^{123}I]FP-CIT SPECT imaging due to clinically uncertain parkinsonism or tremor at Turku University Hospital or Helsinki University Hospital in Finland were recruited for this study (NMDAT study; ClinicalTrials.gov identifier: NCT02650843). The study cohort has been comprehensively described in previous reports[28-31]. All participants underwent clinical examination 3-4 hours before imaging. These evaluations included a neurological clinical examination with a clinical interview, MDS-UPDRS part III[32], MMSE[33], BDI[34], and NMSS[35]. All investigators had completed the MDS-UPDRS Training Program and Exercise certification.

Final PD diagnoses were confirmed through chart review by two movement disorder specialists 3-8 years after imaging, taking into account clinical progression, phenotype, treatment response and imaging findings. Patients with an MMSE score <18 (n=1) or incomplete imaging data (n=2) were excluded from the study. Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki, and the study was approved by the local ethics committee.

RBD Assessment

The presence of self-reported RBD was assessed using the validated single-question screen for RBD (RBDQ1)[27]. The screening question asks: "*Have you ever been told, or suspected yourself, that you seem to 'act out your dreams' while asleep (for example, punching, flailing your arms in the air, making running movements, etc.)?*". Participants provide a simple "yes" or "no" response. The RBDQ1 has been validated against polysomnogram in a multicenter, well-characterized clinical cohort, with a demonstrated sensitivity of 94% and specificity of 87% for detecting probable RBD[27].

Image acquisition and preprocessing

Patients received an intravenous injection of 185 MBq of [^{123}I]FP-CIT three hours prior to SPECT imaging. To minimize radiation exposure to the thyroid gland, patients were pretreated with either potassium perchlorate (250-300 mg) or

potassium iodine (130 mg) 30-60 min before the radiotracer injection. The imaging was performed using eight different SPECT/CT devices, all calibrated prior to the study using a striatal phantom (Radiology Support Devices, Inc., Long Beach, CA, USA) to ensure comparability across systems. Calibration procedures followed the guidelines provided by Hermes Medical Solutions[36,37]. SPECT data were reconstructed using the 3D OSEM algorithm with corrections for attenuation, collimator response and scatter applied (HybridRecon Neurology, version 1.3, Hermes Medical Solutions AB, Stockholm, Sweden). The acquisition and reconstruction protocols adhered to the European Associaton of Nuclear Medicine (EANM) recommendations across all devices[38].

Region of Interest Analyses

Reconstructed SPECT images were analyzed using the BRASS semiautomated analysis software (version 2.6, Hermes Medical Solutions, Stockholm, Sweden), incorporating scanner-specific corrections derived from prior calibrations. SBRs were calculated for six volumes of interest (VOIs): left and right caudate, anterior putamen and posterior putamen. The occipital cortex served as the reference area, and the SBRs were computed using the formula:

$SBR = (VOI_{caudate\ or\ putamen} - VOI_{occipital})/VOI_{occipital}$ [38].

Voxelwise analyses

Voxelwise analyses were conducted using general linear model implemented in Statistical Parametric Mapping (SPM12, https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) software to investigate associations between RBD status and both striatal and extrastriatal [^{123}I]FP-CIT binding. The SBR images realigned in the BRASS analysis were normalized to Montreal Neurological Institute (MNI) space using an in-house MNI space [^{123}I]FP-CIT template, as described previously[39]. The search volume in the voxelwise analyses was restricted to brain regions showing specific binding. Age, sex and UPDRS-III score were used as nuisance covariates. To ensure that the results are not driven by other non-motor symptoms or cognitive impairment, the analyses were confirmed by using MMSE score and NMSS score as additional covariates to the model. Cluster-level family-wise error (FWE) corrected P-values less than 0.05 were considered significant.

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics (version 29, SPSS Inc., Chicago, Illinois, USA). The normality of the variables was assessed a combination of visual inspection of histograms and the Shapiro-Wilk test. Differences between patients with and without probable RBD were analyzed using the Mann-Whitney U-test for continuous variables and either the Chi-square test or Fisher's exact test for categorical variables, as appropriate. Analysis of covariance (ANCOVA) was used to control the effects of covariate variables in SBRs. Like in voxelwise analyses, age, sex, MDS-UPDRS motor score, MMSE score and NMSS total score were used as covariates. Normality of residuals in ANCOVA models were checked and skewed residuals were met for three SBRs (right anterior putamen, left and right posterior putamen). To obtain normality, ANCOVA models were done using log transformed SBRs. Results remain unchanged and to allow easier interpretation results for untransformed SBRs are shown. Additional analyses were performed adding motor symptom duration to the model as well.

Table 1. Demographic and clinical characteristics of the sample studied. Values are median [IQR] or n (%).

		RBD + **(n=44)**	**RBD –** **(n=111)**	**p-value**
Demographics	Age (years)	67.5 [15]	66.0 [17]	0.19[1]
	Sex (m/f)	26/18	48/63	0.075[2]
Motor symptoms	MDS-UPDRS motor score	31.0 [13]	34.0 [21]	1.0[1]
	Motor symptom duration (mo)	24.0 [43]	15 [19]	0.014[1]
	Hoehn and Yahr scale	2.0 [0]	2.0 [1]	0.15[1]
Non-motor symptoms	NMSS total score	46.0 [54]	36.0 [46]	0.055[1]
	Cardiovascular incl. falls	0.0[3]	0.0 [3]	0.97[1]
	Sleep / fatigue	10.5 [13]	6.0 [15]	0.15[1]
	Mood / cognition	3.0 [11]	2.0 [12]	0.77[1]
	Perceptual problems	0.0 [0]	0.0 [0]	0.72[1]
	Attention / Memory	3.0 [7]	1.0 [4]	0.060[1]
	Gastrointestinal tract	1.0 [7]	0.0 [4]	0.26[1]
	Urinary	8.0 [20]	5.0 [11]	0.030[1]
	Sexual function	0.0 [6]	0.0 [4]	0.29[1]
	Miscellaneous	8.0 [13]	4.0 [12]	0.29[1]
	BDI score	7.0 [7]	6.0 [9]	0.16[1]
	MMSE score	27.0 [3]	28.0 [3]	0.003[1]
Medications	Dopaminergic drug users	17 (38.6)	30 (27.0)	0.16[2]
	Levodopa users	11 (25.0)	18 (16.2)	0.21[2]
	LEDD	0.0 [200]	0.0 [75]	0.23[1]
	Clonazepam users	3 (6.8)	0 (0.0)	0.022[3]
	Melatonin users	3 (6.8)	1 (0.9)	0.069[3]
	Antidepressant users	4 (9.1)	16 (14.4)	0.37[2]
Psychoactive substances	Nicotine users	7 (15.9)	8 (7.2)	0.13[3]
	Alcohol (drinks/week)	1.0 [4]	1.0 [4]	0.84[1]
	Coffee (cups/day)	3.0 [3]	3.0 [3]	0.73[1]
	Right caudate	1.92 [0.70]	2.11 [0.80]	0.063[1]
	Left caudate	1.81 [0.92]	2.14 [0.80]	0.012[1]

Striatal DAT binding	Right anterior putamen	1.33 [0.73]	1.67 [0.92]	0.048[1]
	Left anterior putamen	1.31 [0.79]	1.61 [0.84]	0.058[1]
	Right posterior putamen	0.88 [0.52]	0.97 [0.61]	0.49[1]
	Left posterior putamen	0.89 [0.60]	0.90 [0.59]	0.63[1]

[1]Mann-Whitney U-test

[2]Pearson's Chi-squared test

[3]Fisher's exact test

RBD=REM sleep behavior disorder, m=male, f=female, mo=months, NMSS=Non-Motor Symptoms Scale, BDI=Beck depression inventory, MMSE=Mini-Mental State Examination, LEDD=levodopa equivalent daily dose, DAT=dopamine transporter.

Table 2. ANCOVA analysis with DAT binding as dependent variable.

Dependent variable	Independent variable	RBD+ Adjusted[1] mean (SE)	RBD- Adjusted[1] mean (SE)	Adjusted[1] mean difference for RBD (95% CI)	P value
Right caudate	RBD	2.01 (0.10)	2.15 (0.06)	-0.14 (-0.37 to 0.09)	0.23
Left caudate	RBD	2.05 (0.10)	2.24 (0.06)	-0.19 (-0.42 to 0.03)	0.095
Right anterior putamen	RBD	1.51 (0.10)	1.73 (0.06)	-0.21 (-0.45 to 0.02)	0.075
Left anterior putamen	RBD	1.50 (0.09)	1.65 (0.05)	-0.15 (-0.36 to 0.06)	0.15
Right posterior putamen	RBD	1.03 (0.09)	1.13 (0.06)	-0.10 (-0.32 to 0.13)	0.40
Left posterior putamen	RBD	0.99 (0.07)	0.96 (0.04)	0.03 (-0.14 to 0.20)	0.70

[1]Age, sex, MDS-UPDRS motor score, MMSE score and NMSS total score were used as covariates in ANCOVA.

DAT=Dopamine transporter, RBD=REM sleep behavior disorder, MDS-UPDRS=Movement Disorder Society's Unified Parkinson's Disease Rating Scale, MMSE=Mini-Mental State Examination, NMSS=Non-Motor Symptoms Scale

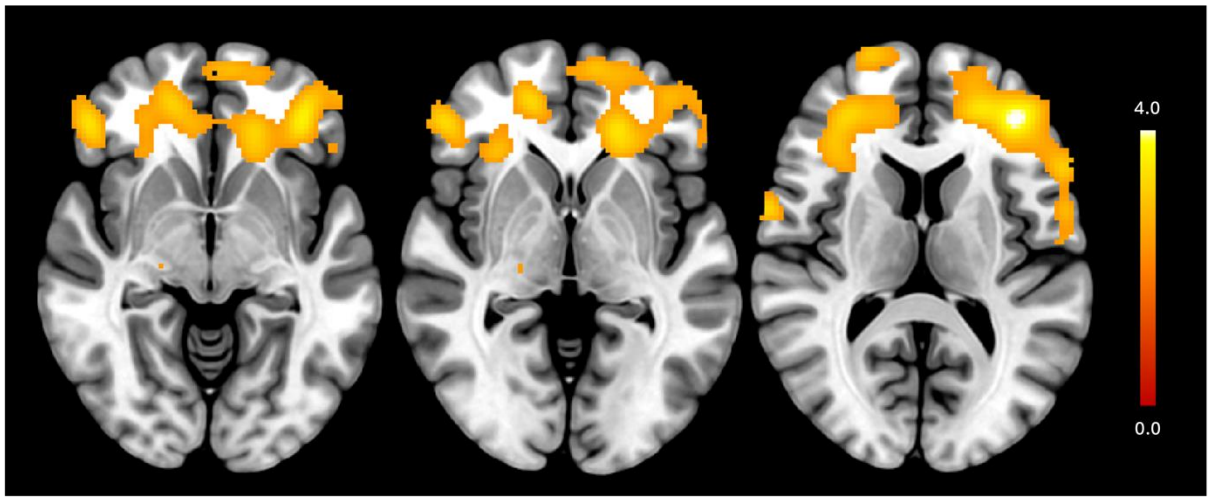

Figure 1. Increased [^{123}I]FP-CIT binding in patients with probable REM sleep behavior disorder (RBD+) compared to those without RBD (RBD-). The image displays regions showing significantly higher binding in RBD+ patients, with results thresholded at cluster-level FWE-corrected P<0.05. No regions showed significantly lower binding in RBD+ patients relative to RBD- patients.

Data availability: The data that supports the findings of this study are available from the corresponding author upon reasonable request.

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