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Prognostic biomarkers in prodromal α-synucleinopathies: DAT binding and REM sleep without atonia

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ABSTRACT

Background Isolated rapid eye movement (REM) sleep behaviour disorder (iRBD) is a prodromal state of clinical α-synucleinopathies such as Parkinson's disease and Lewy body dementia. The lead-time until conversion is unknown. The most reliable marker of progression is reduced striatal dopamine transporter (DAT) binding, but low availability of imaging facilities limits general use. Our prospective observational study aimed to relate metrics of REM sleep without atonia (RWA)—a hallmark of RBD—to DAT-binding ratios in a large, homogeneous sample of patients with RBD to explore the utility of RWA as a marker of progression in prodromal α-synucleinopathies.

Methods DAT single-photon emission CT (SPECT) and video polysomnography (vPSG) were performed in 221 consecutive patients with clinically suspected RBD.

Results vPSG confirmed RBD in 176 patients (162 iRBD, 14 phenoconverted, 45 non-synucleinopathies). Specific DAT-binding ratios differed significantly between groups, but showed considerable overlap. Most RWA metrics correlated significantly with DAT-SPECT ratios (eg, Montreal tonic vs most-affected-region: r=−0.525; p<0.001). In patients taking serotonergic/noradrenergic antidepressants or dopaminergic substances or with recent alcohol abuse, correlations were weaker, suggesting a confounding influence, unlike other possible confounders such as beta-blocker use or comorbid sleep apnoea.

Conclusions In this large single-centre prospective observational study, we found evidence that DAT-binding ratios in patients with iRBD can be used to describe a continuum in the neurodegenerative process. Overlap with non-synucleinopathies and clinical α-synucleinopathies, however, precludes the use of DAT-binding ratios as a precise diagnostic marker. The parallel course of RWA metrics and DAT-binding ratios suggests in addition to existing data that RWA, part of the routine diagnostic workup in these patients, may represent a marker of progression. Based on our findings, we suggest ranges of RWA values to estimate whether patients are in an early, medium or advanced state within the prodromal phase of α-synucleinopathies, providing them with important information about time until possible conversion.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ People can easily obtain information online that describes the acting out of dreams while sleeping as a symptom of isolated rapid eye movement (REM) sleep behaviour disorder (iRBD), which, in turn, can be an early sign of parkinsonism and dementia. Individuals who have this symptom are therefore aware that they might be experiencing an ongoing neurodegenerative process.

WHAT THIS STUDY ADDS

⇒ We provide the first systematic description in a large and homogeneous patient group of the most reliable prognostic marker—striatal dopamine-transporter binding—and report that it correlates moderately with the diagnostic marker of iRBD, namely REM sleep without atonia (RWA).

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our finding that RWA might have prognostic value with regard to disease progression in iRBD patients may (1) help clinicians make better estimates of lead time until conversion; (2) facilitate the recruitment of larger patient populations in trials evaluating the pathophysiology of disease and monitoring treatment effects and (3) emphasise the need for polysomnography as a diagnostic tool in patients with iRBD and improve acceptance among payers of its reimbursement.

INTRODUCTION

Isolated rapid eye movement (REM) sleep behaviour disorder (iRBD) is recognised as a prodromal state of clinical α-synucleinopathies such as Parkinson's disease, Lewy body dementia and multiple system atrophy.[1–4] The neurodegenerative process progresses over a yet unknown period of time, but often precedes the diagnosis of clinical α-synucleinopathies by one or two decades.[4–7] Because the available treatment options are considered symptomatic only,[8] many patients with the diagnosis of iRBD are interested in knowing at which stage of progression they are situated—and thus how much time remains before they manifest neuromotor or cognitive symptoms indicative of parkinsonism or dementia.[9] In addition, the long prodromal interval offers opportunities for trials of early neuroprotective treatment. For these purposes,

several prognostic and therapy monitoring biomarkers are under investigation.[9–11]

The specific binding of FP-CIT in the striatum reflects the local availability of dopamine transporter (DAT) and represents a surrogate marker for the integrity of nigrostriatal dopaminergic neurons projecting from the substantia nigra pars compacta to the striatum.[12] The density of DAT diminishes slowly with healthy ageing,[13] but this process accelerates in individuals with α-synucleinopathies.[14 15] Today, DAT density measured using single-photon emission CT (DAT-SPECT) is considered the most reliable marker for synucleinopathic neurodegeneration, and images determined to be pathological indicate a high risk of short-term phenoconversion to clinical α-synucleinopathies.[3 11 14–17] Nuclear imaging techniques are, however, expensive for health systems and not always readily accessible to patients.

In patients with RBD, REM sleep-associated muscle atonia is impaired. REM sleep without atonia (RWA) is considered the neurophysiological hallmark of RBD and leads to the characteristic acting out of dreams.[18] Only recently, the magnitude of RWA was suggested to be a marker for an advanced prodromal state in patients with RBD, with high RWA scores being predictive of imminent phenoconversion to the clinically manifest phase of α-synucleinopathies.[19 20] Because measuring RWA is relatively inexpensive and part of the standard diagnostic workup in patients with clinical suspicion of RBD, its potential use as a biomarker of disease progression is of great interest to payers and patients alike. Moreover, there are, somewhat surprisingly, scant data on the progression of neurodegeneration within the whole prodromal state from large and homogeneous samples of patients with RBD. The aim of our study was therefore to relate metrics of RWA to DAT-binding ratios in such a sample in order to explore the utility of RWA as a marker of progression to α-synucleinopathies.

MATERIALS AND METHODS
Patients and procedures
In this prospective observational study, we analysed video polysomnography (vPSG) and DAT-SPECT data from all consecutive patients with clinical suspicion of RBD who underwent three-night vPSG and DAT-SPECT between November 2015 and February 2022. For analysis, patients were divided into three groups: (1) those diagnosed with iRBD ('iRBD' group); (2) those with RBD who had already converted to clinical α-synucleinopathies[21] ('RBD-converted' group) and (3) those diagnosed with motor behaviour not stemming from α-synucleinopathies ('Non-syn' group). Conversion to clinical α-synucleinopathies was diagnosed based on neuropsychiatric evaluations including neuromotor and neurocognitive performance using part III of the Unified Parkinson's Disease Rating Scale and the Consortium to Establish a Registry for Alzheimer's Disease battery of tests and its supplements, respectively. All patients in group 2 had developed symptoms of RBD prior to conversion, though their RBD diagnosis was confirmed after clinical conversion using vPSG based on clinical interviews. Based on their medical histories, we excluded patients from the analysis if they were (1) taking Parkinson's medication at the time of vPSG or DAT-SPECT or (2) reported recent alcohol or sedative drug use or withdrawal. We evaluated patients separately if they had (1) taken antidepressants within the past 12 months (ie, because serotonergic effects on RWA are known to persist up to 12 months after discontinuation); (2) were taking dopaminergic agents for restless legs or for depression at the time of examinations; (3) were taking beta-blockers at the time of examinations or (4) had been diagnosed with moderate or severe sleep apnoea.

Video polysomnography
vPSG was performed on three successive nights with a digital recording system (Monet 24-CPU, TMS International, Enschede, The Netherlands), with Rembrandt V.7.5 software (Medcare Automation, Amsterdam, The Netherlands). We regarded the first vPSG night as the adaptation night which, although completely recorded, we usually did not evaluate, the second as the diagnostics night, and the third as the night for initiating treatment (where needed). vPSG included electrooculography, electroencephalography (sample rate 160 Hz, filter settings 0.3–70 Hz, with the 10–20 system derivations F3, F4, C3, C4, O1, O2, each referred to the contralateral mastoid), bipolar surface electromyography (EMG) of m. mentalis and mm. tibiales anterior (sample rate 200 Hz, filter settings 10–120 Hz), ECG, deep-body temperature (rectal thermistor), nasal-oral flow, thoracic and abdominal respiratory effort, oxygen saturation, snoring detection and digitally synchronised video (infrared, 25 fps) and audio recording (44.1 kHz, 16 bits, mono). Biocalibration was performed before the start of each recording to ensure adequate signal quality. Each recording took place in a dark, low-noise, single-bed room.

Scoring
Sleep stages and associated events were always scored by the same experienced scorer (FB) in 30 s epochs of the PSG in accordance with standard American Academy of Sleep Medicine (AASM) criteria. Because the muscle atonia required to score REM sleep is disturbed in individuals with RBD, we modified the scoring of REM sleep according to the methods proposed by the Montreal group.[22 23]

For all patients, we used data from night 2 to evaluate RWA. A minimum of 30 min of REM sleep had to be present. In nine patients, we analysed data from night 1 instead because in four patients there were technical problems during night 2 that did not occur in night 1; in two patients sleep apnoea syndrome (SAS) was more pronounced in night 2; and in three patients there were fewer than 30 min of REM sleep to be analysed in night 2 (6, 14, 25 min in night 2 vs 42, 58, 83 min in night 1, respectively). We had to exclude four patients due to technical problems that occurred during both PSG nights. In addition, to explore the effect of night-to-night variability on RWA metrics we scored the adaptation night of 50 patients (randomly selected).

RWA scoring was performed on all REM epochs, with an automated procedure based on the well-validated Montreal method[22–24] and determined from the surface EMG signal of m. mentalis only. Epochs contaminated by breathing-related RWA events were visually identified and labelled during sleep-stage scoring and excluded from the analysis. The procedure generates two scores, one for tonic ('Montreal tonic') and another for phasic EMG activity ('Montreal phasic'). When tonic EMG with an amplitude at least twice that of the background EMG activity was present for more than 50% of an epoch, that epoch was scored as tonic. The Montreal method uses 20 s epochs for sleep-stage scoring and defines phasic EMG activity as the percentage of 2 s mini-epochs containing EMG events lasting 0.1–10 s, with an amplitude exceeding four times that of the background EMG activity.[23] While using 30 s epochs for sleep-stage scoring, we adapted the Montreal approach and followed the criteria of the Mayo Clinic[25] by defining phasic events as the

percentage of 3 s mini-epochs containing EMG events lasting 0.1–14.9 s, and keeping the original amplitude criterion. In addition, the overall polysomnographic score introduced by Consens *et al*[26] as the average of phasic and tonic EMG activity was calculated. Because this score implies a combination of both types of EMG activity, we refer to it henceforth as 'Montreal overall' for the sake of brevity.

We compared the scores for phasic and tonic EMG activity from the automated procedure to those of the human scorer using a sample of 4148 REM epochs originating from 22 complete nights of 14 patients with RBD. We calculated the agreement (a) between the visual and automated scores and Cohen's kappa (k) for phasic activity (a_{phasic}=91.1%; k_{phasic}=0.800) and tonic activity (a_{tonic}=90.9%; k_{tonic}=0.673), with the results indicating substantial agreement between the two types of scoring.[27]

In addition, the automated procedure generated a simplified RWA measure called Ikelos-RWA, which we routinely use in our clinic to quantify muscle activation in the m. mentalis during REM sleep in RBD and as a parameter to document disease progression during follow-up. In Ikelos-RWA, RWA is defined as any muscle activity lasting longer than 1 s within REM sleep and with an amplitude exceeding four times that of the background EMG activity. Interruptions of RWA with sub-threshold activity shorter than 1 s are included in the score, with the result that transient 'spiky' EMG activity can be detected if multiple such events occur with intervals shorter than one second. Ikelos-RWA is then expressed as a percentage of total time spent in REM sleep. Before the measure is calculated, the EMG signal is automatically checked for quality with regard to interference from external signals (eg, superimposition from power supply, or cross-talk effects from ECG). An extensive description and validation of our Ikelos-RWA algorithm are part of the doctoral thesis of one of the authors (AP) and will be published elsewhere (for preliminary versions see[24 28]).

DAT density measured by DAT-SPECT

Patients underwent DAT-SPECT to evaluate presynaptic DAT binding. After thyroid blocking by oral administration of 1000 mg sodium perchlorate, 130-185 MBq [^{123}I] ioflupane (FP-CIT, Datscan, GE Healthcare) was injected intravenously. The cocaine analogue ioflupane accumulates in the striatum, where it binds with high affinity to the presynaptic DAT protein of the nigrostriatal nerve terminals.[29 30] SPECT images were recorded approximately 4 hours after injection using a GE Healthcare Camera NM/CT 670. SPECT data were reconstructed iteratively using HERMES HybridRecon,[31 32] fitted to a template and analysed automatically using the latest version of BRASS™ ENC-DAT (EARL) (December 2020) with standard settings applied for all patients. The outcome was the specific binding ratio (SBR) for six different striatal regions (right and left caudate, right and left anterior and posterior putamen), using ioflupane uptake in the occipital lobes as the background reference region. These results were then corrected for camera and age dependency and compared (z-score) with a normal collective using 103 SPECT examinations from the European multicentre database of healthy controls for FP-CIT-SPECT (ENC-DAT).[13]

Statistical analysis

We performed all statistical analyses using IBM SPSS Statistics for Windows, V.28.0. (IBM). Pearson correlations were calculated for data that were normally distributed, and Spearman correlations for data that were not. Sex and age were initially included as covariates but were omitted after they failed to show

significant influence (p>0.05). Bonferroni correction was used for multiple comparisons. Group comparisons were analysed using mixed linear models with fixed factors for 'group' (iRBD, RBD-converted and Non-syn) and the random factor 'patient'. Cohen's d effect sizes were calculated for all group comparisons. Receiver operating characteristic analyses were performed to establish cut-off values (using Youden's index) in the SBR of the most affected striatal region, to explore possibilities for distinguishing between patients in the iRBD and RBD-converted groups, or in the Non-syn and iRBD groups.

RESULTS

Of 288 consecutive patients with a suspected diagnosis of RBD who underwent three-night vPSG from November 2015 to February 2022, 221 underwent DAT-SPECT (table 1). vPSG confirmed RBD in 176 of these 221 patients, including 162 who had not converted to clinical α-synucleinopathies, 14 who had phenoconverted (9 Parkinson's disease; 5 Lewy body dementia) and 45 who had sleep-related motor behaviour diagnosed as stemming from factors not related to α-synucleinopathies (eg, NREM parasomnias, respiratory-related movements). RBD diagnoses were made based on data from all three nights of vPSG. Of the 45 patients with Non-syn, we excluded 5 because of anti-IgLON5 disease (n=1), amphetamine use (n=1) or recent initiation of psychotropic substances due to acute illnesses (n=3). Of the 162 patients with iRBD, we excluded 14 for recent general anaesthetic (n=3) or recent chemotherapy, vagotomy, no REM sleep during PSG, unreliable CPAP, intake of cortisone, methadone or anticholinergic, promethazine withdrawal, testosterone injections, levodopa (L-DOPA) or multimorbidity (each n=1). Table 1 presents demographics of the three patient groups and, for generalisability, the demographics of a large multicentre sample.[3]

Figure 1 presents DAT-binding measures for 6 striatal regions in 40 patients who did not have α-synucleinopathies (Non-syn), 148 patients with iRBD and 14 patients with RBD who had already converted to clinical α-synucleinopathies. Of the 6 regions studied in the 148 patients with iRBD, the lowest absolute SBRs were found most often in the posterior putamen (left n=71; right n=69). Compared with a norm population,[13] however, the z-scores of patients with iRBD were diminished more often in the right posterior putamen than in the left (left n=32; right n=74). Table 2 presents statistical comparisons of the three groups of patients and the six regions presented in figure 1, including effect sizes. Significant differences were found between all three groups (p<0.001) with mostly large effect sizes (Cohen's d >0.8).

In the ROC analyses of DAT binding, patients in the iRBD group could be distinguished from the RBD-converted group at a cut-off value of 1.61 (SBR of most affected region, MAR) with 72% sensitivity and 93% specificity and an area under the curve (AUC) of 0.88. In turn, the Non-syn group could be distinguished from the iRBD group at a cut-off value of 2.23 with 70% sensitivity and 68% specificity and an AUC of 0.80.

Visually scored REM sleep time was well above 30 min per night in each subject (total REM sleep scored, mean±SD: 76.8±27.6 min). In 20 patients, not all visually scored REM sleep could be used for the RWA analysis, mostly due to technical artefacts identified by our automatic algorithm, or to epochs labelled by the human scorer as contaminated (total analysed REM sleep: 72.6±28.3 min).

Time between vPSG and DAT-SPECT in patients with iRBD was 47±100 days (mean±SD, n=135; we excluded 9 patients

Table 1 Demographics

			Non-Syn	RBD converted	iRBD with DAT	iRBD with DAT/RWA	iRBD without DAT/RWA confounders*	
Total clinically suspected RBD+DAT-SPECT		N	221					
Non-synucleinopathies		N	45					
Non-syn+factors infl. DAT-binding		N	5					
RBD converted		N		14				Postuma et al,[3] 2019
Total iRBD patients		N			162			1280
Excluded due to:								
>14 months between RWA and DAT		N				9		
Technical problems calculation RWA		N				4		
Other exclusion factors		N			14			
Sample size with DAT-scan		N	40	14	148	135	107	245
Age (in years)		Mean±SD (range)	65±13 (30-84)	73±8 (55-83)	69±7 (53-86)	69±7 (53-85)	70±7 (53-85)	66±8 -
Male		%	85	86	80	81	85	83
DAT/RWA confounders		%	43	36	20	21	–	–
Betablockers		%	25	36	24	25	23	–
SAS		%	–	–	18	20	18	27 (N=1082)
DAT-SPECT path.	(MAR z-value <−2.0)	%	13	93	50	51	50	44 (N=245)†
	(MAR z-value <−1.5)	%	30	100	66	66	64	
	(R.Post. Put. z-value <-2)	%	10	93	41	41	39	
	(R.Post. Put. z-value <−1.5)	%	18	93	56	56	54	
MDS-UPDRS-III		Mean±SD	–	–	2.20±3.19 (N=137)	2.00±2.76 (N=118)	2.14±2.84 (N=98)	6.26±4.94 (N=57)‡ 3.12±3.82 (N=299)§
MMSE <28		%	–	–	14 (N=146)	14 (N=133)	18 (N=105)	39 (N=776)
Olfaction path.¶	(Anosmia)	%	–	–	43 (N=141)	43 (N=122)	47 (N=101)	67 (N=628)
	(Anosmia+hyposmia)	%	–	–	96 (N=141)	95 (N=122)	97 (N=101)	
Constipation**		%	–	–	40 (N=86)	39 (N=79)	53 (N=60)	43 (N=830)

*DAT/RWA confounders included dopaminergic substance, antidepressiva and alcohol abuse. For generalisability, demographics and RBD specifics of the largest existing multicentre sample (Postuma) are given.
†Criteria for abnormal DAT-SPECT differed between single centres.
‡Developed disease.
§Disease free.
¶Sniffin' Sticks.
**SCOPA introduced to test battery in 2018.
DAT, dopamine transporter; iRBD, isolated REM sleep behaviour disorder; MAR, most affected region; MDS-UPDRS-III, Movement Disorders Society-Unified Parkinson Disease Rating Scale; MMSE, Mini Mental State Examination; REM, rapid eye movement; RWA, REM sleep without atonia; SAS, sleep apnoea syndrome; SPECT, single-photon emission CT.

from the correlation analysis because the time between the 2 procedures was ≥14 months, and 4 due to technical problems with establishing RWA). Table 3 presents correlations of RWA-metrics with DAT-SBR and z-values in patients with iRBD who were not taking antidepressants, catecholamine, stimulants or dopaminergic substances and with no recent history of alcohol abuse (n=107). The strongest correlations with DAT-measures were found for Montreal-tonic and Montreal-overall RWA, which were significant for five of six striatal regions, whereas the Montreal-phasic RWA significantly correlated with DAT-measures in only four of six regions with lower correlation coefficients. Partial correlations showed no effect of age and sex. Before Bonferroni correction (n=56 tests), all of the correlations were significant; this remained the case after correction with the exception of the associations for caudate regions. The Ikelos-RWA showed correlations comparable to those of Montreal-tonic and Montreal-overall.

Figure 2 presents correlations between tonic RWA and DAT-SBR and z-values for the MAR, as well as the related scatterplots. The potential confounding factors we measured varied in terms of their effects on the correlations. Beta-blockers and SAS seemed not to have an impact because the correlations were significant when we analysed these patients as separate groups (MAR SBR vs tonic RWA: beta-blockers: r=−0.653, p<0.001; SAS: r=−0.460, p=0.016). Phasic RWA (not shown) did not significantly correlate with DAT measures of MAR (SBR) in the subgroup of patients with SAS (r=−0.296, p=0.134) but did correlate significantly with MAR (SBR) in the beta-blocker

subgroup (r=−0.481, p=0.005). However, the use of noradrenergic or serotonergic antidepressants, dopaminergic agonists or dopaminergic antidepressants, as well as recent alcohol abuse, were associated with weaker, non-significant correlations. As a consequence, to calculate the correlation of DAT binding with different RWA parameters, we excluded patients with these characteristics (figure 3, table 3).

Figure 3 presents scatter plots and correlations between DAT SBR of MAR with 3 measures of RWA (Montreal-tonic, Montreal-phasic and Ikelos) for the 107 patients depicted in figure 2E without confounding antidepressants, dopaminergic agents or alcohol abuse.

Figure 4 presents correlations between DAT SBR of MAR with 2 measures of RWA (Montreal-tonic and Montreal-phasic) for 50 randomly selected iRBD patients where the first night was evaluated. While in this subgroup of patients the correlation of DAT SBR of MAR vs Montreal-tonic is again significant for night 2 (r=−0.476, p<0.001), the adaptation night shows weaker correlations that were not significant.

Time since start of RBD symptoms did not correlate significantly with any DAT or RWA measure (p>0.151).

DISCUSSION

In this prospective observational study of a large sample of patients with suspected RBD, we examined correlations between metrics of RWA and DAT-binding ratios in order to explore the utility of RWA as a marker of progression to α-synucleinopathies.
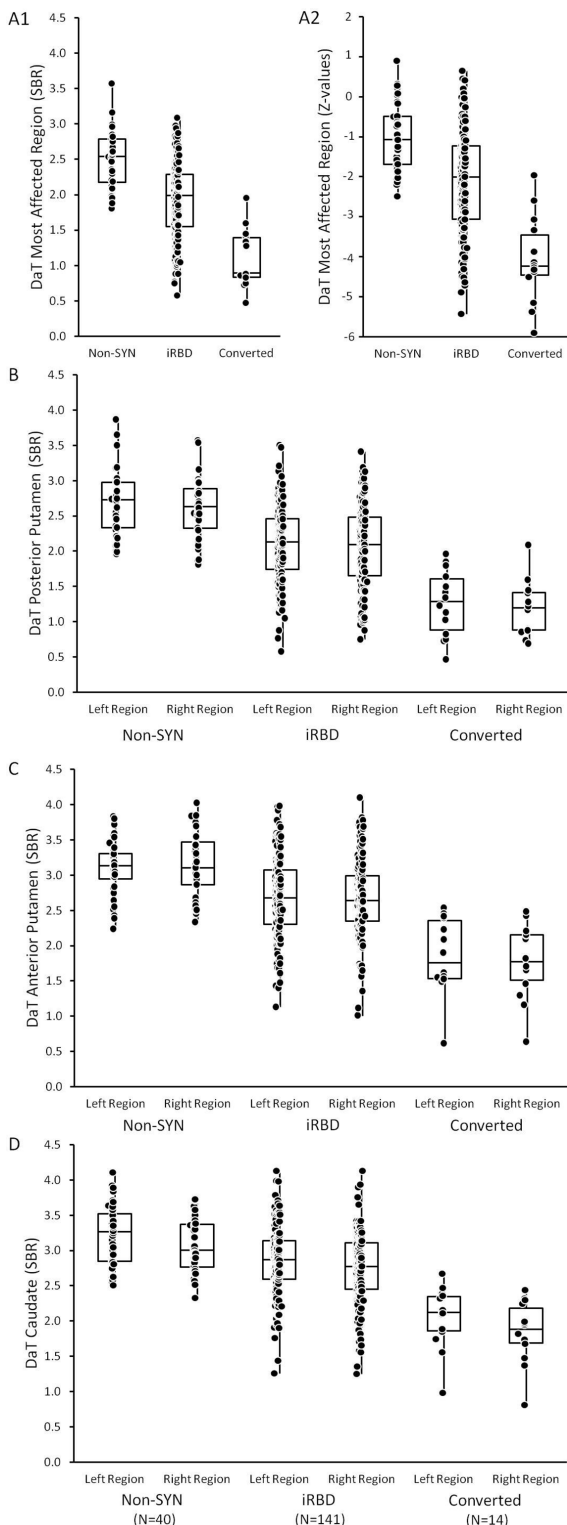
Figure 1 DAT-SPECT in α-synucleinopathies. Shown are DAT-SPECT data for most affected region. (A1) SBR values; (A2) Z-values and six separate regions. (B–D) SBR values in non-synucleinopathies, iRBD and RBD converted patients. Panels show left and right regions of: (B) posterior putamen; (C) anterior putamen; (D) caudate. Box plots show median and 25% quartiles, with whiskers indicating minimum and maximum. Black dots show all individual patients. The three groups of patients showed significant differences in all regions with medium to large effect sizes (see table 2 for statistical comparisons). DAT-SPECT, dopamine transporter single-photon emission CT; iRBD, isolated REM sleep behaviour disorder; REM, rapid eye movement; SBR, specific binding ratio.

We found that RWA is moderately correlated with DaT-binding, and we identified and describe confounding factors.

Across our three groups of patients—those with iRBD, those with RBD who had converted to clinical α-synucleinopathies, and those with motor behaviour not stemming from α-synucleinopathies—there were clear differences in the DAT-binding values, suggesting a continuum from healthy or preclinical states to first prodromal symptoms and then to clinically manifest α-synucleinopathies.[33] At the same time, the substantial overlap in DAT-binding ratios means that we were unable to identify cut-off values for distinguishing clearly between the groups.

When patients receive the devastating diagnosis of an ongoing neurodegenerative process, many want to know how much time they have left before they will develop symptoms. It is therefore crucial for clinicians to be able to estimate the stage at which their patients are located within the prodromal phase of α-synucleinopathies. While DAT imaging is useful in this regard, it is expensive and, depending on the health system, not always readily accessible. In contrast, because vPSG is comparatively inexpensive and part of the routine diagnostic workup in this context, being able to use RWA as a prognostic marker would be highly valuable. The results of our analysis suggest that variations in DAT-binding and RWA follow a parallel course and are substantially correlated, potentially allowing RWA to be used to characterise stages within the prodromal state. Importantly, our data show that the adaptation during the first night has a confounding effect and, therefore, two nights of PSG should be recorded to accurately evaluate RWA. The results of the ROC analysis of DAT-SPECT data suggest that the ability to use DAT-binding values to distinguish between our three groups of patients was rather low, as was the ability to use these values to distinguish among patients within the iRBD group itself. Inspection of our RWA data (using our Ikelos-RWA ranging from 0% to 100%) suggests, however, that it might be helpful to differentiate between three ranges in the prodromal phase of α-synucleinopathies. Most patients with an RWA score below 30% have an SBR above 2 in DAT-SPECT, with only a few having an SBR lower than 1.5 in MAR. This suggests that this group of patients are in an earlier prodromal state. In contrast, most patients with an RWA above 60% have SBR scores below 2.0, indicating a more advanced state. Thus, an RWA up to 30%, up to 60%, and above 60% might indicate early, medium and advanced prodromal states, respectively. The individual speed of progression could be monitored using biomarkers that can be measured in most neurologic practices. We suggest monitoring a combination of prognostic markers, such as DAT-binding—if available—and RWA at the time of diagnosis, with other markers, such as cognition and motor behaviour, on a yearly basis. This information might help clinicians estimate how much time patients have until conversion to clinical α-synucleinopathies, and it could possibly serve as a marker for disease modification in neuroprotective trials.

Assuming that DAT binding and RWA are markers of disease progression that indicate severity,[11 14–17 19 20] the lack of a correlation in our data between self-reported time since occurrence of first RBD symptoms and DAT-binding and RWA scores is worth noting. Of course, the time of symptom onset is a subjective estimate derived from the impression of the patient (or partner), and hence its reliability is questionable. Nevertheless, this observation suggests that the course of disease in α-synucleinopathies is not fully characterised by continuous worsening and may be influenced by factors yet to be determined. This observation is propitious for ongoing research into mechanisms and disease

Table 2 Comparisons depicted in figure 1

		P value	Cohen's d	
Most affected region (SBR)	iRBD versus non-syn	**<0.001**	**1.22**	
	iRBD versus converted	**<0.001**	**1.72**	
	Non-syn versus converted	**<0.001**	**3.43**	
Most affected region (Z-values)	iRBD versus non-syn	**<0.001**	**1.06**	
	iRBD versus converted	**<0.001**	**1.68**	
	Non-syn versus converted	**<0.001**	**3.22**	

		Left region:		Right region:	
		P value	Cohen's d	P value	Cohen's d
Posterior putamen (SBR)	iRBD versus non-syn	**<0.001**	**1.16**	**<0.001**	**1.09**
	iRBD versus converted	**<0.001**	**1.62**	**<0.001**	**1.78**
	Non-syn versus converted	**<0.001**	**3.08**	**<0.001**	**3.52**
Anterior putamen (SBR)	iRBD versus non-syn	**<0.001**	**0.85**	**<0.001**	**0.90**
	iRBD versus converted	**<0.001**	**1.54**	**<0.001**	**1.65**
	Non-syn versus converted	**<0.001**	**2.70**	**<0.001**	**2.75**
Caudate (SBR)	iRBD versus non-syn	**<0.001**	**0.76**	**<0.001**	**0.73**
	iRBD versus converted	**<0.001**	**1.77**	**<0.001**	**1.91**
	Non-syn versus converted	**<0.001**	**2.73**	**<0.001**	**3.04**

		P value	Cohen's d
Posterior putamen (SBR) left versus right	Non-syn	0.232	0.16
	iRBD	0.356	0.05
	Converted	0.372	0.18
Anterior putamen (SBR) left versus right	Non-syn	0.470	<0.01
	iRBD	0.264	0.05
	Converted	0.473	0.13
Caudate (SBR) left versus right	Non-syn	**0.006**	**0.33**
	iRBD	**<0.001**	**0.22**
	Converted	**0.020**	**0.48**

Significant findings are shown in bold.
iRBD, isolated REM sleep behaviour disorder; SBR, specific binding ratio.

modifying treatment in patients with iRBD, including the overall goal of preventing conversion to clinical α-synucleinopathies.

The criteria for scintigraphic evidence of neurodegenerative Parkinson's disease have been described previously.[34] Our findings add evidence in support of earlier observations in patients with α-synucleinopathies, suggesting that a DAT-binding deficit predominates first in the putamen and is even more pronounced in the posterior putamen.[17 33 35 36]

The strength of the correlations between the RWA parameters and DAT-binding measures in our sample differed depending on the Montreal method that was used (ie, tonic, phasic or overall).[22 23 26] The correlation between the combination of

Table 3 Associations of RWA and DaT in iRBD patients without DaT/RWA confounders (N=107)

	Montreal tonic RWA		Montreal phasic RWA		Montreal overall RWA		Ikelos RWA	
	r	P value	r	P value	r	P value	r	P value
Most affected region (SBR)	**−0.525**	**<0.001**	**−0.407**	**0.001**	**−0.497**	**<0.001**	**−0.533**	**<0.001**
Left posterior putamen (SBR)	**−0.461**	**<0.001**	**−0.327**	**0.033**	**−0.428**	**<0.001**	**−0.481**	**<0.001**
Right posterior putamen (SBR)	**−0.493**	**<0.001**	**−0.439**	**<0.001**	**−0.497**	**<0.001**	**−0.515**	**<0.001**
Left anterior putamen (SBR)	**−0.466**	**<0.001**	**−0.342**	**0.018**	**−0.445**	**<0.001**	**−0.465**	**<0.001**
Right anterior putamen (SBR)	**−0.484**	**<0.001**	**−0.398**	**0.001**	**−0.478**	**<0.001**	**−0.482**	**<0.001**
Left caudate (SBR)	−0.314	0.055	−0.291	0.133	**−0.332**	**0.026**	**−0.357**	**0.009**
Right caudate (SBR)	**−0.340**	**0.019**	−0.212	1.000	−0.307	0.074	**−0.331**	**0.028**
Most affected region (Z-values)	**−0.530**	**<0.001**	**−0.429**	**<0.001**	**−0.507**	**<0.001**	**−0.538**	**<0.001**
Left posterior putamen (Z-values)	**−0.472**	**<0.001**	**−0.323**	**0.039**	**−0.433**	**<0.001**	**−0.495**	**<0.001**
Right posterior putamen (Z-values)	**−0.496**	**<0.001**	**−0.439**	**<0.001**	**−0.497**	**<0.001**	**−0.522**	**<0.001**
Left anterior putamen (Z-values)	**−0.454**	**<0.001**	**−0.328**	**0.031**	**−0.432**	**<0.001**	**−0.461**	**<0.001**
Right anterior putamen (Z-values)	**−0.513**	**<0.001**	**−0.424**	**<0.001**	**−0.504**	**<0.001**	**−0.519**	**<0.001**
Left caudate (Z-values)	−0.307	0.074	−0.260	0.387	−0.311	0.062	**−0.349**	**0.013**
Right caudate (Z-values)	**−0.362**	**0.007**	−0.229	0.981	**−0.325**	**0.036**	**−0.354**	**0.010**

(A) Cohen's d effect sizes: 0.2=small, 0.5=medium, 0.8=large. Non-Syn: N=40; iRBD: N=148; Converted: N=14. (B) P values have been adjusted by Bonferroni correction for multiple comparisons for 56 tests.
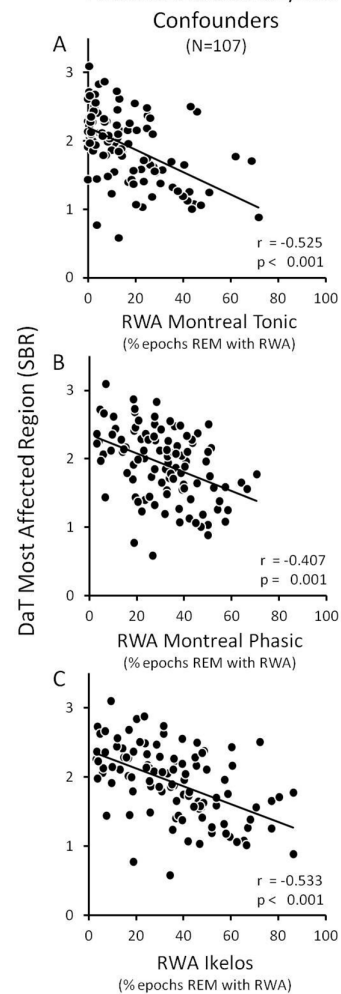Significant effects are shown in bold (p<0.05).
iRBD, isolated REM sleep behaviour disorder; REM, rapid eye movement; RWA, REM-sleep without atonia; SBR, specific binding ratio.
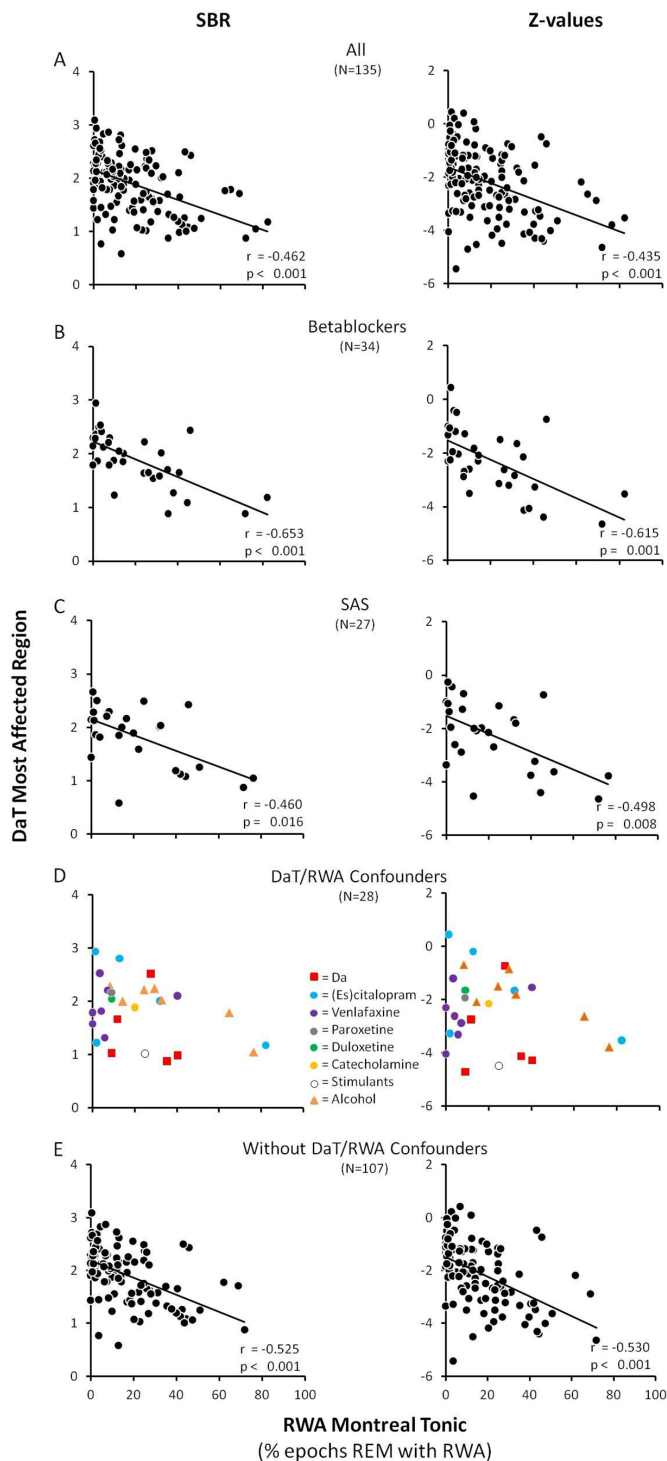
Figure 2 DAT-binding versus RWA in iRBD. Shown are correlations of tonic RWA with DAT-binding in most affected region; left panels show specific binding ratio (SBR); right panels show z-values. (A) All patients; (B) patients who took beta-blockers; (C) patients with sleep apnoea syndrome (SAS); (D) patients who took substances known to influence DAT-binding or RWA (antidepressants, dopaminergic substances (Da), alcohol abuse); (E) all patients without patients defined in (D). DAT, dopamine transporter; iRBD, isolated REM sleep behaviour disorder; REM, rapid eye movement; RWA, REM sleep without atonia.

Figure 3 Correlations of DAT-binding with RWA. Correlations of most affected region DAT special binding ratio (SBR) with three different RWA analyses: (A) Montreal-tonic RWA (B) Montreal-phasic RWA. (C) Ikelos RWA. DAT, dopamine transporter; REM, REM sleep without atonia; RWA, REM sleep without atonia.

phasic and tonic in the category 'Montreal-overall' and DAT-binding was almost identical in value to that for the tonic category alone. Whereas the correlation of phasic RWA with DAT-binding was also significant, measuring phasic RWA did not lead to values that were substantially higher than those obtained using the other methods, which is in line with literature.[19 23] Experimental data as old as the earliest descriptions of continuous loss of REM atonia in cats after pontine lesions,[37] more specifically destruction of the perilocus coeruleus α, imply that the presence of tonic rather than phasic EMG activity was relevant for the reported 'oneiric behaviour'. Emergence of tonic EMG activity in human REM sleep is related to dysfunction of the subcoeruleus (or the human homologue of the perilocus coeruleus α in cats).[38 39] On the pathway of spreading of α-synucleinopathy from lower brainstem to higher structures, the subcoeruleus is ahead of the striato-nigral area. Pervasive damage of the subcoeruleus, and thus its dysfunction with regard to tonic EMG, could therefore reflect the progression from α-synucleinopathy to overt Parkinson's disease.[5] Nevertheless, the role of phasic EMG activity in RWA needs further elucidation before one can assume that it is not related to disease severity in iRBD.

Surprisingly, beta-blockers and sleep apnoea did not seem to have a negative effect on our results. This observation is partly supported by earlier reports[25 40] but requires further evaluation. REM sleep is a highly complex behavioural state. Not
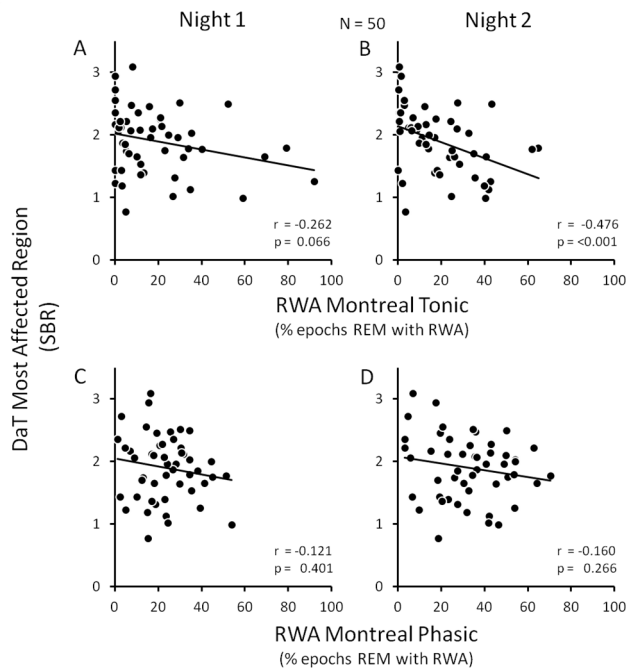
Figure 4 Correlations during the first adaptation night and second night. Correlations of most affected region DAT special binding ratio (SBR) with (A, B) Montreal tonic RWA and (C, D) Montreal phasic RWA for the first night (A, C) and second night (B, D) of those 50 iRBD patients with a scored first PSG night. DAT, dopamine transporter; iRBD, isolated REM sleep behaviour disorder; PSG, polysomnography; REM, rapid eye movement; RWA, REM sleep without atonia.

surprisingly, the influence of anticholinergic, serotonergic, noradrenergic and dopaminergic antidepressants on muscle tone in RBD is complex, as well.[19 40 41] Antidepressants are known to trigger secondary RBD and to increase RWA in patients with iRBD.[40] Our findings add to existing preliminary reports that suggest different effects of antidepressants on the occurrence of RWA, the unmasking of subclinical RBD and the characterisation of higher individual neurodegenerative vulnerability.[40] Systematic evaluation in larger samples is needed to clarify this possible mode of psychotropics' action in RBD.

This study has a number of important limitations, which may suggest avenues for future research. (1) As this is an observational study, observer bias needs to be discussed. Scoring of the two biomarkers evaluated here—RWA and DAT binding—were observer independent (with exception of the prerequisite sleep stage scoring). The results of commonly used visual or semi-automatic scoring of DAT-SPECT images, which are observer dependent, were not included in our evaluation.[42] One strength of the present study is that quantification of both biomarkers was computer based only. (2) As patients with iRBD in our study were not followed up longitudinally, progression over time with eventual risk for phenoconversion was not evaluated. On the other hand, earlier studies have suggested high RWA scores to be predictive of imminent phenoconversion to the clinically manifest phase of α-synucleinopathies.[19 20] The association of DAT-binding ratios and RWA adds to these earlier observations. Final proof of course can only be given in a longitudinal trial. (3) The number of patients with confounding factors was small, and general conclusions regarding the influence of these factors cannot be drawn without confirmation in larger samples. (4) RWA was scored using only EMG input from m. mentalis. It

could be argued that this might entail limitations compared with methods that use combinations of limbs and chin EMG signals. Different methods for RWA scoring have been published while using various definitions of phasic, tonic, 'any' and mixed EMG activity in the chin and/or limbs, but combinations of EMG signals have been proposed primarily to improve diagnostic sensitivity.[43] Diagnostic quality in the current patient cohort is well ensured by using multiple methods such as test batteries, PSG, videometry and DAT-SPECT for each patient and need not be further addressed. Whether additional input from limb EMG might have strengthened or weakened the correlations with DAT-SPECT data in our analysis remains an open question and is an area for future research.

Our study is based on data from the largest single-centre sample of patients with iRBD to be characterised for DaT binding to date. In this context, the major advantage of a single-centre sample is its homogeneity in terms of setting, study protocol, personnel, scanner, imaging protocol and analysis software. This enables a consistent and systematic description of what is currently the most reliable prognostic marker for phenoconversion in prodromal α-synucleinopathies.[3 11 17 35] Moreover, such data facilitate a more precise description of the most vulnerable striatal site in α-synucleinopathies, the posterior part of the putamen. Importantly, the characteristics of our sample—such as age, sex, time since RBD onset, comorbidity and comedication, as well as distribution of RBD-specific prognostic biomarker scores, such as neuromotor ability, cognitive performance, olfaction and constipation—were similar to those of other large groups of iRBD patients.[3] This suggests that our sample can be considered representative of the broader population of patients with iRBD and may serve as a reference.

To conclude, the findings of this prospective observational study of a large and homogeneous sample of consecutive patients with clinical suspicion of RBD suggest that RWA can be regarded as a marker of progression in prodromal synucleinopathies. Beta-blockers and sleep breathing abnormalities do not appear to influence RWA results, and the potentially confounding effects of antidepressant and dopaminergic agents need further study. Because measuring RWA is an essential part of the standard diagnostic workup in patients with clinical suspicion of RBD, it is inexpensive for health systems and more readily available than the more costly nuclear imaging techniques. The information gained can be used to help estimate the lead time until possible conversion to parkinsonism or dementia.

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