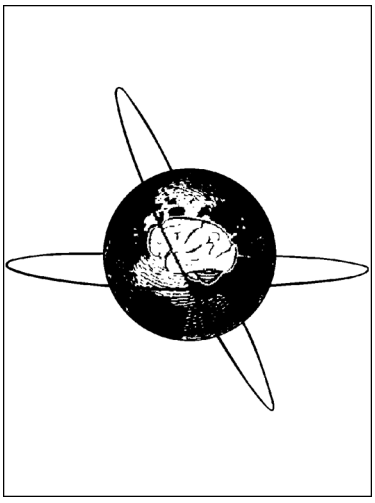
Polysomnographic phenotype of isolated REM sleep without atonia

Journal Pre-proofs



Polysomnographic phenotype of isolated REM sleep without atonia

Femke Dijkstra, Mineke Viaene, Ilse De Volder, Erik Fransen, Patrick Cras, David Crosiers

PII: S1388-2457(20)30415-6
DOI: https://doi.org/10.1016/j.clinph.2020.07.005
Reference: CLINPH 2009313

To appear in: *Clinical Neurophysiology*

Received Date: 15 January 2020
Revised Date: 23 June 2020
Accepted Date: 16 July 2020

Please cite this article as: Dijkstra, F., Viaene, M., De Volder, I., Fransen, E., Cras, P., Crosiers, D., Polysomnographic phenotype of isolated REM sleep without atonia, *Clinical Neurophysiology* (2020), doi: https://doi.org/10.1016/j.clinph.2020.07.005

Polysomnographic phenotype of isolated REM sleep without atonia

Femke Dijkstra[1,2,3*], Mineke Viaene[3], Ilse De Volder[1,2,4,5], Erik Fransen[6], Patrick Cras[1,2,7], David Crosiers[1,2,7]

[1] Department of Neurology, Antwerp University Hospital, Wilrijkstraat 10, 2650 Edegem, Belgium

[2] Faculty of Medicine and Health Sciences, Translational Neurosciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

[3] Laboratory for Sleep disorders and Department of Neurology, St.-Dimpna Regional Hospital, J.-B. Stessensstraat 2, 2440 Geel, Belgium

[4] Department of Psychiatry, Antwerp University Hospital, Wilrijkstraat 10, 2650 Edegem, Belgium

[5] Multidisciplinary Sleep Disorders Center, Antwerp University Hospital, Wilrijkstraat 10, 2650 Edegem, Belgium

[6] StatUa Center for Statistics, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

[7] Institute Born-Bunge, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

Correspondence to:

F. Dijkstra

Department of Neurology, Antwerp University Hospital

Wilrijkstraat 10, 2650 Edegem, Belgium

Tel.: +32 3 821 30 00

E-mail: Femke.Dijkstra@uza.be

Institution where work was performed:

Laboratory for Sleep disorders, St Dimpna Regional Hospital, Geel, Belgium.

Highlights

- Rapid eye movement (REM) density is increased in isolated REM sleep without atonia (RSWA).
- Periodic limb movements of sleep are increased in isolated RSWA.
- Heart rate variability is normal in isolated RSWA.

Keywords:

REM sleep without atonia; Parkinson's disease; Polysomnography; REM density; Heart rate variability; Periodic limb movements of sleep.

Abstract

Objective: Isolated REM sleep without atonia (iRSWA) is regarded as a prodromal phase of REM sleep behavior disorder and synucleinopathies. In iRSWA patients, we investigated the polysomnographic characteristics that are known to be altered in (prodromal) Parkinson's disease (PD): periodic limb movements of sleep [PLMS] (increased), REM density (reduced), and heart rate variability ([HRV] (reduced).

Methods: We compared video-polysomnographic studies of 49 iRSWA subjects with 41 controls. RSWA and PLMS were scored visually. REM density (REM/hour) and HRV were calculated automatically.

Results: We found a higher median total (15.90 vs 7.20;p=0.001), REM (21.80 vs 11.0;p<0.001) and non-REM (11.75 vs 5.72;p=0.027) PLMS index, and a higher mean REM density (342.45 vs 275.96;p=0.010) in the iRSWA group, with a significant positive correlation between RSWA severity and these variables (r=0.39;p<0.00, r=0.48;p<0.001, r=0.24;p=0.021, r=0.28;p=0.012). We found no significant difference in HRV between groups.

Conclusions: Our results suggest an association between RWSA and REM density and PLMS, but not HRV. The positive correlation between these variabilities may imply overlapping pathophysiological processes.

Significance: The evidence of higher REM density and normal HRV weakens the hypothesis that iRWSA is a prodromal PD stage. An alternative interpretation is, however, that REM density and HRV change during caudal-rostral neurodegeneration.

1. Introduction

Parkinson's disease (PD) is a movement disorder, characterized by an asymmetric hypokinetic-rigid syndrome and resting tremor (Jankovic 2008). Before the onset of motor symptoms and PD diagnosis, patients frequently suffer for several years from non-motor symptoms in a prodromal phase (Berg et al. 2015). Recognition of this prodromal phase is important for early diagnosis as well as for patient selection for neuroprotective trials.

Autonomic dysfunction and sleep disturbances are prodromal PD symptoms (Berg et al. 2015), both of which can cause abnormalities in polysomnography (PSG). The most specific and most investigated prodromal PD sleep disorder is idiopathic/isolated rapid eye movement (REM) sleep behavior disorder (iRBD) (Al-Qassabi et al. 2017), which is a parasomnia characterized by loss of atonia during REM sleep and dream enacting behavior (DEB), usually together with vivid and violent dreams (American Academy of Sleep Medicine 2014). Up to 91% of the iRBD patients will develop PD or a related disorder within 15 years after RBD onset (Iranzo et al. 2014). Isolated REM sleep without atonia (iRSWA) on PSG without DEB is regarded as a prodromal phase of iRBD and subsequently of an underlying synucleinopathy (e.g. PD) (Högl et al. 2018). iRSWA has been less investigated, but in the few previous studies iRSWA patients showed several other prodromal PD biomarkers such as hyposmia, substantia nigra hyperechogenicity, and decreased striatal tracer uptake on ^{123}I-Ioflupane Single Photon Emission Computed Tomography (Eisensehr et al. 2003; Stefani et al. 2016). Extensive follow-up studies to confirm this hypothesis, however, are lacking.

In this study, we investigate the polysomnographic profile of iRSWA patients with the focus on other PSG parameters that are known to be altered in PD and prodromal PD, such as periodic limb movements of sleep ([PLMS], increased in PD and RBD) (Sixel-Döring et al. 2011b), REM density (reduced in PD) (Schroeder et al. 2016) and heart rate variability [HRV]

(reduced in PD) (Kallio et al. 2004; Sauvageot et al. 2011; Palma et al. 2013b; Sorensen et al. 2013; Pyatigorskaya et al. 2016). Abnormalities of these parameters in iRSWA patients may support the hypothesis of iRSWA as a prodromal PD biomarker. Furthermore, differences in these parameters between iRSWA patients may help to differentiate iRSWA in the context of neurodegeneration from incidental RSWA. Finally, the association between RSWA and other PSG parameters may give more insights into the possibly overlapping underlying (patho)physiologies.

2. Materials and methods

2.1 Population

We performed a prospective age- and gender-matched case-control study between subjects with iRSWA and subjects with preserved REM atonia on PSG in the sleep department of the General Hospital Geel (Belgium) between September 2016 and September 2019. Details of the study have been described elsewhere (Dijkstra et al. 2019b)**.** In short, all data were collected as part of the routine sleep lab assessment and included age, gender, medical history, medication use, body mass index (BMI) and PSG parameters. The presence of depressive symptoms with the Beck Depression Inventory-II (BDI-II) (Beck et al. 1996) and the history of dream enacting behavior (DEB) was assessed with the RBD1Q questionnaire (Postuma et al. 2012) and RBD screening questionnaire (Stiasny-Kolster et al. 2007). Subjects with possible secondary RSWA were excluded according to the following exclusion criteria: present or past use (< 3 months ago) of antidepressants, antipsychotics, beta-blockers, melatonin or dopaminergic medication; moderate or severe OSA (AHI ≥15); narcolepsy; a history of central neurologic or psychiatric disorders; a history of synucleinopathy or RBD (with DEB on polysomnography or a history of DEB). Subjects whose clinical history was unknown were also excluded from the analysis. The control group

consisted of subjects with preserved REM atonia during polysomnography. We used the same exclusion criteria for the iRSWA group as for the control group.

2.2 Video-polysomnography

A classic full-night video-PSG was performed (Brainnet-Morpheus, MEDATEC, Belgium) with 2 electro-oculogram channels, 3 EMG channels (submental muscle and bilateral anterior tibial muscle), electroencephalogram channels (bilateral prefrontal, central and occipital leads with a reference lead to mastoids), 2 nasal airflow channels, 2 respiratory effort channels, 1 oximetry channel, 1 snoring detector channel, 1 electrocardiography channel, 1 pulse transit time channel, and a position and light detector, all with American Academy of Sleep Medicine (AASM) recommended setup specifications (Berry et al. 2017). All PSGs were visually analysed by a neurologist specialized in sleep disorders. Sleep stage classification, arousals, limb movements, and respiratory events were scored according to the AASM manual (Berry et al. 2017). DEB was scored according to the RBD severity scale (Sixel-Döring et al. 2011a) on video analyses. Subjects with complex motor behaviors or vocalizations were scored as DEB positive.

2.3 RSWA scoring

We only included subjects with > 15 minutes of REM sleep, and the total REM sleep was analysed. RSWA was scored in the submental muscle and bilateral anterior tibial muscles. RSWA was visually scored in 30-second epochs in every PSG by both a sleep specialist (MV) and researcher (FD). Only PSG that were scored as RSWA positive by both scorers were included. RSWA was scored according to the AASM manual (Berry et al. 2017): 1. Increased tonic activity, measured on the submentalis EMG channel and defined as an increase of ≥50% in baseline EMG amplitude (measured during nonREM sleep) or ≥10 μV during ≥50 percent of a REM epoch. 2. Increased phasic activity, measured on all EMG channels, and defined as

all muscle activity during 0,1-5 seconds that exceeded 4 times the background EMG activity in ≥50% of 3 second mini-epochs within a 30 second REM epoch. All EMG activity due to respiratory events, snoring, arousals, or PLMS was excluded from analysis. Patients with indefinable REM tonus due to severe artefacts on EMG channels were also excluded from analysis. Total RSWA, tonic RSWA, and phasic RSWA percentage were calculated as the percentage of REM epochs that show (tonic or phasic) RSWA, as a measure of RSWA severity.

2.4 Periodic limb movements of sleep

PLMS are repetitive limb movements during sleep, usually involving the lower limbs with dorsal extension of the big toe. PLMS were scored visually on both anterior tibial EMG channels, according to the World Association of Sleep Medicine standards (Zucconi et al. 2006) that defines a PLMS as an event between 0.5-10 seconds with an EMG increase ≥ 8 μV above resting baseline during sleep. The event is only scored as a PLMS as part of a series of ≥ 4 PLMS and when the period between 2 PLMS is between 5-90 seconds. A leg movement that overlapped or occurred < 0.5 seconds after an apnea was not scored as PLMS. PLMS were expressed as a PLMS index (number of PLMS/hour) of total sleep time, of REM sleep (measured in all REM sleep epochs) and of NREM sleep.

2.5 REM density

REM density is the number of rapid eye movements (REMs) per hour of REM sleep. REMs were recorded from both eyes with two electro-oculogram channels on PSG. Both channels were visually checked for severe artefacts (when positive, the subject was excluded from analysis) as well as to confirm REM sleep. REM density was calculated automatically in all REM sleep epochs by dividing the number of REMs by the hours of total REM sleep with

Brainnet-morpheus (MEDATEC, Belgium) software, which identifies a REM when the amplitude on the recording is conjugal or exceeding 15 μV during REM sleep.

2.6 Heart rate variability

HRV is the variation in time between two successive heartbeats. HRV is driven by both sympathetic and parasympathetic components of the autonomic nervous system (Shaffer and Ginsberg 2017), and changes in HRV are considered a biomarker for autonomic dysfunction.

We analysed 5-minute epochs of electrocardiogram (ECG) on PSG during wakefulness (at rest), NREM sleep stage 2 and REM sleep. All epochs were visually checked for the presence of apneas, arousals, PLMS, ectopic heartbeats, complex movements, or artefacts on the ECG channel. Only epochs without these confounders were included for analysis. Subjects with a history of cardiac disease or subjects on medications that affect heart rate were excluded from the analysis. The 5-minute epochs of wakefulness were extracted from the pre-sleep period. To investigate within-subject variability of HRV across the night during sleep, we compared two 5-minute epochs from two different sleep cycles in 30 subjects (15 subjects with iRSWA and 15 control subjects). The first epoch and last epoch that fulfilled the inclusion criteria for both REM sleep and NREM sleep stage 2 were included. Secondly, we compared the differences in HRV between iRSWA subjects and control subjects. Because some of the HRV parameters showed significant within-subject variability over the night, we included the last 5-minute epochs that fulfilled the inclusion criteria of REM sleep (since RBD is most pronounced during the last REM cycle) and the first 5-minute epoch of NREM stage 2 that met the inclusion criteria before the included REM sleep epoch.

Data were exported from Brainnet-morpheus (MEDATEC, Belgium) into BrainRT

(OSG, Belgium), where the 5-minute epochs were extracted and uploaded into Kubios software version 3.1.0.1 (University of Eastern Finland, Kuopio, Finland) for HRV analysis. Since HRV can be expressed through many different parameters (Shaffer and Ginsberg 2017) and considering our limited sample size, we only included the most commonly used HRV parameters and those altered in PD and RBD for analysis. These parameters are from both the time-domain and the frequency-domain.

Time-domain incides measure direct HRV during recording and include the normal-to-normal (NN) heart beat interval and its standard deviation (SDNN) (Shaffer and Ginsberg 2017). Frequency-domain measurement reflects the fluctuations within the NN time series, expressed by power spectrum density, including high frequency (HF) bands (0.15-4 Hz), low frequency (LF) bands (0.04-0.15 Hz) and very low (VLF) frequency bands (0.003-0.04 Hz. Finally, we analysed the LF/HF ratio, which is a surrogate for sympathetic/parasympathetic balance (Shaffer and Ginsberg 2017). All analyses were performed with preprocessing through threshold-based artefact correction for the time-domain analyses, as described in the Kubios user guide (Tarvainen et al. 2019). We used both threshold-based artefact correction and detrending (with prior smoothening with a cut-off frequency of 0,035 Hz) for the frequency-domain analyses. Frequency components were then separated using a Fast Fourier Transformation for the frequency analyses.

2.7 Statistics

Differences in mean REM density between both groups were calculated with a general linear model, adjusted for age, gender and BDI-II score. We included the BDI-II score as a co-variate, since REM density is known to be associated with depression, and we found a significant positive correlation between BDI-II score and REM density (Pearson's correlation coefficient adjusted for age and gender, r=0.23, p=0.037). Since the PLMS indices are not

normally distributed, differences in PLMS indices between groups were analysed with a (non-parametric) Mann-Whitney U test.

Most HRV parameters are not normally distributed. For the intra-subject HRV variability analysis, we used the Wilcoxon signed-rank test to test for within-subject differences between the 2 epochs for both NREM sleep and REM sleep. To test whether the change in HRV over the 3 stages differs between the iRSWA group and control group, we fitted linear mixed models with the HRV parameter value as the outcome and fixed the effects of the stage (categorical), group and interaction between stage and group, as well as a random intercept for each individual. For the non-normally distributed parameters, testing was based upon logtransformed values. The significance of the fixed effects was tested using an F-test with Kenwardroger correction for degrees of freedom. Linear mixed models were then fitted with only the main effect terms of stage and group included as fixed effects. To further study which stages are different from one another for the HRV parameters, we carried out a post-hoc analysis with Tukey correction for multiple hypothesis testing. Finally, to test for differences in HRV parameters per stage between groups, a Mann-Whitney U test was used.

Differences in clinical variables and other PSG variables between groups were calculated with a univariate general linear model for continuous variables, adjusted for age and gender, and a Fisher's exact test for categorical variables.

Correlations between total, tonic, and phasic RSWA severity and REM density were calculated with Spearman's partial correlation coefficient, adjusted for age, gender, and BDI-II. Correlations between total, tonic, and phasic RSWA severity and PLMS index (total during REM and NREM sleep) were calculated with Spearman's partial correlation coefficient, adjusted for age and gender.

The linear mixed-model analyses were performed in R statistics. The remaining analyses were performed with SPSS (IBM SPSS Statistics for Mac, Version 26.0. Armonk, NY: IBM Corp.)

2.8 Ethical approval

This study used data collected for clinical purposes, and no additional interventions (for research purposes) were performed. All subjects provided written informed consent for using their clinical data for scientific research. The Medical Ethics Committee of the General Hospital Geel approved the study.

3. Results

All clinical and several polysomnographic parameters are summarized in Table 1. We included 49 iRSWA subjects (mean age 43.8 (±13.0) years, 67% male) and 41 control subjects (mean age 44.6 (±10.8), 59 % male).

The distribution of total PLMS index and PLMS index of REM sleep in both groups is shown in Figure 1. The median total PLMS index was 15.90 (7.10-26.80) PLMS/hour in the iRSWA group and 7.20 (3.50-12.50) PLMS/hour in the control group ($p=0.001$, $U=615.5$). The median PLMS index of REM sleep was 21.80 (10.70-38.90) PLMS/hour in the iRSWA group and 11.0 (1.25-19.40) PLMS/hour in the control group ($p<0.001$, $U=555.0$). The median PLMS index of NREM sleep was 11.75 (3.32-28.72) PLMS/hour in the iRSWA group and 5.72 (1.05-12.74) PLMS/hour in the control group ($p=0.027$, $U=732.0$). Furthermore, we found a significant positive correlation between total PLMS index and both total ($r=0.39$, $p<0.001$) and phasic ($r=0.40$, $P<0.001$) RSWA severity, and a non-significant positive correlation between total PLMS index and RSWA tonic severity ($r=0.186$, $p=0.080$). A significant positive correlation between PLMS index of REM sleep and total ($r=0.48$,

p<0.001), phasic (r=0.50, P<0.001), and tonic RSWA (r=0.27, P=0.012) severity was also found. Finally, we found a significant positive correlation between NREM PLMS index and both total (r=0.24, p=0.021) and phasic (r=0.25, P=0.020) RSWA severity, and a non-significant positive correlation between NREM PLMS index and RSWA tonic severity (r=0.167, p=0.116).

The distribution of REM density in both groups is shown in Figure 1. Mean REM density was 342.45 (±111.59) REM/hour in the iRSWA group and 275.96 (±87.08) REM/hour in the control group (p=0.010). Furthermore, we found a significant positive correlation between REM density and both total (r=0.28, p=0.012) and phasic (r=0.30, p=0.007) RSWA severity. A non-significant positive correlation between REM density and RSWA tonic severity was found (r=0.10, p=0.100).

The results of the HRV intra-subject variability analyses are summarized in Table E-1 (Supplementary Material). Although the majority of HRV parameters showed no significant intra-subject variability during different sleep cycles, NN in both NREM stage 2 (Z score -2.705, p=0.007) and REM sleep (Z score -3.959, p<0.001) were significantly lower in the last sleep cycle. The longitudinal analysis results are summarized in Table 2, Table E-2, and Figure E-1 (Supplementary Material). The majority of HRV parameters showed a statistically significant change across the different stages of wakefulness, NREM sleep stage 2, and REM sleep (Table 2, Table E-2, Figure E-1): most clearly a reduced NN interval during sleep (most pronounced in NREM sleep), and (from the frequency-domain measures) a relatively low LF/HF ratio in NREM sleep and a relatively high LF/HF ratio in REM sleep. These changes, however, did not differ between the 2 groups (Table 2). The results of the HRV analyses in each stage are summarized in Table 3. Median NN intervals were smaller in the iRSWA group than in the control group during all stages of sleep, but the difference was not

statistically significant. We also found no significant difference in any of the other HRV parameters between groups.

Regarding the other polysomnographic variables analysed, the total sleep time and percentage of different sleep stages were similar in both groups. We found a higher arousal index in the iRSWA group (24.4 ±7.9/hour vs. 0.5±7.3/hour, p=0.021) and a higher total AHI index and REM AHI index in the control group (4.3±3.5/hour vs 6.0±3.7/hour, p= and 4.6±4.3/hour vs. 7.8±8.0/hour, p=0.001).

4. Discussion

Our study describes different polysomnographic parameters in patients with iRSWA, with the focus on the polysomnographic patterns known to be altered in patients with PD and iRBD. We observed a higher PLMS index and REM density in the iRSWA group, with a significant positive correlation between RSWA severity and total PLMS index, REM PLMS index, NREM PLMS index and REM density. We could not demonstrate a significant difference in HRV parameters between groups. This is, to our knowledge, the first study investigating the polysomnographic phenotype of iRSWA.

4.1 Periodic limb movements of sleep

We observed a higher PLMS index in patients with iRSWA, both during total sleep (median of 15.90 PLMS/hour, p=0.001) and during both REM sleep (median of 21.80 PLMS/hour, p=0.001) and NREM sleep (median of 11.75 PLMS/hour, p=0.027). PLMS are associated with several disorders such as restless legs syndrome, sleep apnea, narcolepsy, and cardiovascular disease (Hornyak et al. 2006; Huang et al. 2019). The neurophysiology of PLMS is only partially understood, and there is a complex involvement of spinal, supraspinal and autonomic influences (Ferri et al. 2017). PLMS in RBD and PD are considered to be

caused by dopamine deficiency (Happe et al. 2003). PLMS are increased in PD patients with RBD (Sixel-Döring et al. 2011b) as well as in iRBD patients (Fantini et al. 2002); and PLMS index predicts early phenoconversion to PD in iRBD patients (Schenck et al. 1996). In PD patients without RBD, PLMS indices are usually not increased in the early stage (Wetter et al. 2001), but increase with disease severity (Young et al. 2002).

Our findings of higher PLMS indices in patients with iRSWA are in line with the hypothesis of iRSWA as a prodromal PD phase. Furthermore, our results of a positive correlation between REM PLMS index and RSWA severity also suggest a direct relation between RSWA and PLMS. Lack of motor inhibition during REM sleep may cause both increase in REM tonus and REM PLMS. The positive correlation between NREM PLMS index and RSWA severity suggests that this motor inhibition may not only be restricted to REM sleep. Alternatively, there might be a causal relation between both variables, in which RSWA causes PLMS or vice versa. The previous finding that patients with periodic limb movement disorder show lower chin muscle activity during REM sleep compared to RBD patients (Cesari et al. 2019), however, suggests that there are also differences in motor activity pathophysiology between RSWA and PLMS.

4.3 REM density

We found a higher REM density in the iRSWA group (mean 342.45 REM/hour, p=0.010) and a significant positive correlation between RSWA severity and amount of REM density (r=0.28, p=0.012). REM density is a measure of the frequency of REMs during REM sleep and is expressed as the number of REMs per hour of REM sleep. REMs and REM density are hypothesized to be induced by ponto-geniculo-occipital potentials, termed PGO waves (Peigneux et al. 2001; Gott et al. 2017). Previous studies suggested a correlation between REM density and dreamed visual imaginary (Hong et al. 1997), as well as an

association between REM density and sleep need (Lucidi et al. 1996). REM density is known to be increased in psychiatric disorders, especially major depression (Foster et al. 1976; Palagini et al. 2013) and post-traumatic stress disorder (Mellman et al. 1995; Kobayashi et al. 2007), and in narcolepsy (Vanková et al. 2001; Dauvilliers et al. 2007), while reduced in neurodegenerative diseases such as PD (Schroeder et al. 2016) and Alzheimer's disease (Shinno and Ishikawa 2016). Conflicting results have been reported on the amount of REM density in RBD (Schenck et al. 1987; Tachibana et al. 1991; Lapierre and Montplaisir 1992; Dauvilliers et al. 2007).

Our results showing increased REM density in iRSWA patients are not in line with the reduced REM density found in PD. There are two possible explanations for these findings. First of all, RSWA may be a nonspecific marker for prodromal PD, and the majority of patients included may have RSWA outside the context of neurodegeneration. The positive correlation between REM density and phasic RSWA severity ($r=0.30$, $P=0.007$) - but not tonic severity - supports this hypothesis, since tonic RSWA severity has been reported to be the strongest predictor of conversion to Lewy body disease (Postuma et al. 2010a; Fernández-Arcos et al. 2017). This hypothesis is of particular interest, because the amount of REM density might be useful to differentiate RSWA due to neurodegeneration (low REM density) from incidental RSWA (high REM density) on PSG.

An alternative interpretation of our results is, that REM density in (prodromal) PD patients might change during caudal to rostral neurodegeneration. REM density might be increased in the beginning, through disinhibition at pontomedullary level similar to REM sleep atonia disinhibition (Dijkstra et al. 2019a), but when neurodegeneration progresses, REM density finally might be reduced by cortical influences on the PGO waves. Previous studies on ocular movements in PD patients showed that the impaired ocular movements in PD indeed seem to be part of executive dysfunction driven by the cortex rather than the

brainstem (Gorges et al. 2016; Vintonyak et al. 2017). One case study also reported a decrease of REM density after treatment with levodopa (Miyamoto et al. 2001), which might suggest a pharmacologic influence on the reduction of REM density in PD patients as well.

Future (retrospective) studies should focus on REM density in definite prodromal PD patients to evaluate whether REM density is already decreased at this stage. Future studies should also focus on longitudinal follow-up of iRSWA patients to assess whether the amount of REM density in those patients can predict phenoconversion to Lewy body disease.

4.4 Heart rate variability

We found a wide distribution of results in all the different HRV parameters within both groups. Within subjects, all HRV parameters changed during the different stages of wakefulness, NREM sleep stage 2, and REM. Our results show a decrease in NN interval during sleep (most pronounced in NREM sleep). The frequency-domain measures show a relatively low LF/HF ratio in NREM sleep, suggesting parasympathetic dominance, and a relatively high LF/HF ratio in REM sleep, suggesting sympathetic dominance. A minority of HRV parameters (especially the NN interval) also significantly changed between 2 sleep cycles across the night. These changes are in line with the current literature regarding normal HRV (Stein and Pu 2012; Kontos et al. 2020).

However, we found no significant difference between groups in any of the HRV parameters, neither per sleep stage nor across the night. A reduction in HRV is considered a biomarker for autonomic dysfunction (Shaffer and Ginsberg 2017). Several previous studies consistently reported a change in HRV in PD patients, with lower variability in time-domain parameters (both in NN and its standard deviation [SDNN]), as well as lower VLF and LF band values of the frequency-domain parameters (Kallio et al. 2004; Sauvageot et al. 2011; Palma et al. 2013b; Sorensen et al. 2013; Pyatigorskaya et al. 2016), reflecting both

sympathetic and parasympathetic nervous system dysfunction. The changes have been described in wakefulness (Palma et al. 2013b; Sorensen et al. 2013; Pyatigorskaya et al. 2016) and during different sleep stages (Kallio et al. 2004; Sauvageot et al. 2011; Palma et al. 2013b; Sorensen et al. 2013; Pyatigorskaya et al. 2016) and are hypothesized to originate from the medulla oblongata (Pyatigorskaya et al. 2016). HRV further decreases during motor progression of PD (Palma et al. 2013b).

In prodromal PD, HRV has been investigated using different approaches. Two epidemiologic studies with large sample cohorts retrospectively investigated HRV in subjects that later developed PD, and found lower heart rate (Palma et al. 2013a) as well as lower SDNN and its root mean square (Alonso et al. 2015) in those subjects. A couple of studies found lower SDNN, VLF, and LF bands in iRBD patients during wakefulness (Postuma et al. 2010b; Sorensen et al. 2013). These findings are in line with the HRV abnormalities in PD, but less pronounced, suggesting that HRV is already affected in prodromal PD to a lesser extent.

Barone et al. published, to our knowledge, the only study investigating HRV in iRSWA patients, and reported lower SDNN, LF band, and HRV power during wakefulness (Barone et al. 2015). Our study could not replicate these findings. Although the median NN interval of the iRSWA group was lower during wakefulness and all sleep stages when compared to the median NN interval of the control group, this difference between the two groups was only a trend that neared significance. The median LF band during wakefulness and NREM sleep was also lower in the iRSWA group, but again non-significant. The discrepancies between both studies might be due to methodological and statistical differences (for example parametric vs. non-parametric testing). HRV is also known to be influenced by many other different variables, such as age, respiration, hormones, and both mental and physical stress (Shaffer and Ginsberg 2017); we could not control for these factors, and they

may have had confounding influences. Furthermore, the wide range in HRV parameters in iRSWA patients suggest heterogeneity between iRWSA subjects and, as discussed in the REM density section, this finding might indicate that RSWA in general is a less specific biomarker for prodromal PD than RBD.

4.5 Limitations

Our study has several limitations. First of all, previous studies showed higher specificity for phasic RSWA when measured on the flexor digitorum superficialis muscle instead of the anterior tibial muscle. In case of suspicion of RBD a PSG with arm EMG recordings is recommended (Frauscher et al. 2012). As iRSWA is usually a co-incidental finding, we could not predict which patients needed additional arm electrodes, and a classical video-PSG with only tibialis anterior recordings was performed in all patients. In consequence, we may have missed a small percentage of phasic RSWA. Furthermore, we cannot exclude the possibility that, due to the presence of fragmentary myoclonus, with criteria overlapping with RSWA, a small percentage of false-positive phasic RSWA on the anterior tibial muscle. Fragmentary myoclonus sometimes occurs in REM sleep and can therefore mimic phasic activity (Berry et al. 2017).

Secondly, different visual and automatic scoring methods for RSWA are available and, until now, there has been no consensus on the best scoring method (Dijkstra et al. 2019a). We used the AASM scoring manual criteria (Berry et al. 2017), but other methods of RSWA scoring may have led to a different selection of iRSWA patients.

Thirdly, we based our selection of PSG parameters altered in PD on previous research, while a positive control group with PD subjects or definite prodromal PD subjects would have allowed direct comparison.

Finally, our control group had a slightly higher AHI during total sleep and REM sleep, and the iRSWA group had a higher PLMS index. For the HRV analyses we analysed 5-minute epochs without apneas, but we cannot exclude a more subtle confounding effect of apneas or PLMD on HRV between groups.

4.6 Conclusions

In conclusion, our study suggests an association between RSWA and both PLMS and REM density, but not HRV, and the positive correlations between these variables may suggest an overlapping (patho)physiology. Our results of a higher REM density and normal HRV in the iRSWA group do not mirror the PSG profiles of PD patients and RBD patients: these findings therefore weaken the hypothesis that iRWSA is a prodromal stage of PD. An alternative interpretation of our results is, however, that REM density and HRV change during the caudal-to-rostral progression of neurodegeneration. Longitudinal studies are required to evaluate whether the amount of REM density in iRSWA patients can predict phenoconversion to Lewy body disease (with the hypothesis that a low REM density increases the risk of phenoconversion and a high REM density decreases the risk).

Acknowledgments

We would like to thank all employees of the Laboratory for Sleep disorders at the General Hospital in Geel for their help with data collection. We would also like to thank Prof. Dr. L. Yperzeele and Dr. T. Reynders, both from University Hospital Antwerp, for their help with the heart rate variability analyses.

Disclosure statement

All authors: no financial or non-financial disclosures.

Funding

We thank "Move for Parkinson", a patient support group, and Rotary Belgium for their participation in funding the project. Funding sources were not involved in research, protocol development and manuscript editing.

References

Alonso A, Huang X, Mosley TH, Heiss G, Chen H. Heart rate variability and the risk of Parkinson disease: The Atherosclerosis Risk in Communities study. Ann Neurol. 2015;77:877–83.

Al-Qassabi A, Fereshtehnejad S-M, Postuma RB. Sleep Disturbances in the Prodromal Stage of Parkinson Disease. Curr Treat Options Neurol. 2017;19:22.

American Academy of Sleep Medicine. International Classification of Sleep Disorders. Darien, IL; 2014. (Third Edition).

Barone DA, Ebben MR, Samie A, Mortara D, Krieger AC. Autonomic dysfunction in isolated rapid eye movement sleep without atonia. Clin Neurophysiol. 2015;126:731–5.

Beck AT, Steer R, Borwn GK. Manual for the Beck Depression Inventory-II. Psychological corperation San AntonioTX. 1996;

Berg D, Postuma RB, Adler CH, Bloem BR, Chan P, Dubois B, et al. MDS research criteria for prodromal Parkinson's disease. Mov Disord. 2015;30:1600–11.

Berry RB, Brooks R, Gamaldo CE, Hardin SM, Lloyd RM, Quan SF et al. for the American Academy of Sleep Medicine. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Darien, IL: American Academy of Sleep Medicine; 2017. (Version 2.4.).

Dauvilliers Y, Rompré S, Gagnon J-F, Vendette M, Petit D, Montplaisir J. REM sleep characteristics in narcolepsy and REM sleep behavior disorder. Sleep. 2007;30:844–9.

Cesari M, Christensen JAE, Sixel-Döring F, Trenkwalder C, Mayer G, Oertel WH, et al. Validation of a new data-driven automated algorithm for muscular activity detection in REM sleep behavior disorder. J Neurosci Methods. 2019;312:53–64.

Dijkstra F, Van den Bossche K, de Bruyn B, Reyn N, Viaene M, De Volder I, et al. REM sleep without atonia and the relation with Lewy body disease. Parkinsonism Relat Disord. 2019a;67:90-98

Dijkstra F, Viaene M, Crosiers D, De Volder I, Cras P. Frequency and characteristic features of REM sleep without atonia. Clin Neurophysiol. 2019b;130:1825–32.

Eisensehr I, Linke R, Tatsch K, Kharraz B, Gildehaus JF, Wetter CT, et al. Increased muscle activity during rapid eye movement sleep correlates with decrease of striatal presynaptic dopamine transporters. IPT and IBZM SPECT imaging in subclinical and clinically manifest idiopathic REM sleep behavior disorder, Parkinson's disease, and controls. Sleep. 2003;26:507–12.

Fantini ML, Michaud M, Gosselin N, Lavigne G, Montplaisir J. Periodic leg movements in REM sleep behavior disorder and related autonomic and EEG activation. Neurology. 2002;59(12):1889–94.

Fernández-Arcos A, Iranzo A, Serradell M, Gaig C, Guaita M, Salamero M, et al. Diagnostic Value of Isolated Mentalis Versus Mentalis Plus Upper Limb Electromyography in

Idiopathic REM Sleep Behavior Disorder Patients Eventually Developing a Neurodegenerative Syndrome. Sleep. 2017;40(4).

Ferri R, Koo BB, Picchietti DL, Fulda S. Periodic leg movements during sleep: phenotype, neurophysiology, and clinical significance. Sleep Med. 2017;31:29–38.

Foster FG, Kupfer DJ, Coble P, McPartland RJ. Rapid eye movement sleep density. An objective indicator in severe medical-depressive syndromes. Arch Gen Psychiatry. 1976;33:1119–23.

Frauscher B, Iranzo A, Gaig C, Gschliesser V, Guaita M, Raffelseder V, et al. Normative EMG values during REM sleep for the diagnosis of REM sleep behavior disorder. Sleep. 2012;35:835–47.

Gorges M, Müller H-P, Lulé D, Pinkhardt EH, Ludolph AC, Kassubek J, et al. The association between alterations of eye movement control and cerebral intrinsic functional connectivity in Parkinson's disease. Brain Image and Behav. 2016;10:79–91.

Gott JA, Liley DTJ, Hobson JA. Towards a Functional Understanding of PGO Waves. Front Hum Neurosci. 2017:11:89

Happe S, Pirker W, Klösch G, Sauter C, Zeitlhofer J. Periodic leg movements in patients with Parkinson's disease are associated with reduced striatal dopamine transporter binding. J Neurol. 2003;250:83–6.

Högl B, Stefani A, Videnovic A. Idiopathic REM sleep behaviour disorder and neurodegeneration — an update. Nat Rev Neurol. 2018;14:40–55.

Hong CC, Potkin SG, Antrobus JS, Dow BM, Callaghan GM, Gillin JC. REM sleep eye movement counts correlate with visual imagery in dreaming: a pilot study. Psychophysiology. 1997;34:377–81.

Hornyak M, Feige B, Riemann D, Voderholzer U. Periodic leg movements in sleep and periodic limb movement disorder: Prevalence, clinical significance and treatment. Sleep Med Rev. 2006;10:169–77.

Huang T-C, Tseng P-T, Wu M-N, Stubbs B, Carvalho AF, Lin P-Y, et al. Periodic limb movements during sleep are associated with cardiovascular diseases: A systematic review and meta-analysis. J Sleep Res. 2019;28:e12720.

Iranzo A, Fernández-Arcos A, Tolosa E, Serradell M, Molinuevo JL, Valldeoriola F, et al. Neurodegenerative Disorder Risk in Idiopathic REM Sleep Behavior Disorder: Study in 174 Patients. Plos One. 2014;9:e89741.

Jankovic J. Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry. 2008;79:368–76.

Kallio M, Suominen K, Haapaniemi T, Sotaniemi K, Myllylä VV, Astafiev S, et al. Nocturnal cardiac autonomic regulation in Parkinson's disease. Clin Auton Res. 2004;14:119–24.

Kobayashi I, Boarts JM, Delahanty DL. Polysomnographically measured sleep abnormalities in PTSD: a meta-analytic review. Psychophysiology. 2007;44:660–9.

Kontos A, Baumert M, Lushington K, Kennedy D, Kohler M, Cicua-Navarro D, et al. The Inconsistent Nature of Heart Rate Variability During Sleep in Normal Children and Adolescents. Front Cardiovasc Med. 2020;7:19.

Lapierre O, Montplaisir J. Polysomnographic features of REM sleep behavior disorder: development of a scoring method. Neurology. 1992;42:1371–4.

Lucidi F, Devoto A, Violani C, De Gennaro L, Mastracci P, Bertini M. Rapid eye movements density as a measure of sleep need: REM density decreases linearly with the reduction of prior sleep duration. Electroencephalogr Clin Neurophysiol. 1996;99:556–61.

Mellman TA, David D, Kulick-Bell R, Hebding J, Nolan B. Sleep disturbance and its relationship to psychiatric morbidity after Hurricane Andrew. Am J Psychiatry. 1995;152:1659–63.

Miyamoto M, Miyamoto T, Takekawa H, Kubo J, Hirata K. Parasomnia as an occasion for the diagnosis of Parkinson's disease. Psychiatry Clin Neurosci. 2001;55:273–4.

Palagini L, Baglioni C, Ciapparelli A, Gemignani A, Riemann D. REM sleep dysregulation in depression: state of the art. Sleep Med Rev. 2013;17:377–90.

Palma J-A, Carmona-Abellan M-M, Barriobero N, Trevino-Peinado C, Garcia-Lopez M, Fernandez-Jarne E, et al. Is cardiac function impaired in premotor Parkinson's disease? A retrospective cohort study. Mov Disord. 2013a;28:591–6.

Palma J-A, Urrestarazu E, Alegre M, Pastor MA, Valencia M, Artieda J, et al. Cardiac autonomic impairment during sleep is linked with disease severity in Parkinson's disease. Clin Neurophysiol. 2013b;124:1163–8.

Peigneux P, Laureys S, Fuchs S, Delbeuck X, Degueldre C, Aerts J, et al. Generation of rapid eye movements during paradoxical sleep in humans. Neuroimage. 2001;14:701–8.

Postuma RB, Arnulf I, Hogl B, Iranzo A, Miyamoto T, Dauvilliers Y, et al. A single-question screen for rapid eye movement sleep behavior disorder: A multicenter validation study. Mov Disord. 2012;27:913–6.

Postuma RB, Gagnon JF, Rompré S, Montplaisir JY. Severity of REM atonia loss in idiopathic REM sleep behavior disorder predicts Parkinson disease. Neurology. 2010a;74:239–44.

Postuma RB, Lanfranchi PA, Blais H, Gagnon J-F, Montplaisir JY. Cardiac autonomic dysfunction in idiopathic REM sleep behavior disorder. Mov Disord. 2010b;25:2304–10.

Pyatigorskaya N, Mongin M, Valabregue R, Yahia-Cherif L, Ewenczyk C, Poupon C, et al. Medulla oblongata damage and cardiac autonomic dysfunction in Parkinson disease. Neurology. 2016;87:2540–5.

Sauvageot N, Vaillant M, Diederich NJ. Reduced sympathetically driven heart rate variability during sleep in Parkinson's disease: a case-control polysomnography-based study. Mov Disord. 2011;26:234–40.

Schenck CH, Bundlie SR, Mahowald MW. Delayed emergence of a parkinsonian disorder in 38% of 29 older men initially diagnosed with idiopathic rapid eye movement sleep behaviour disorder. Neurology. 1996;46:388–93.

Schenck CH, Bundlie SR, Patterson AL, Mahowald MW. Rapid eye movement sleep behavior disorder. A treatable parasomnia affecting older adults. JAMA. 1987;257:1786–9.

Schroeder LA, Rufra O, Sauvageot N, Fays F, Pieri V, Diederich NJ. Reduced Rapid Eye Movement Density in Parkinson Disease: A Polysomnography-Based Case-Control Study. Sleep. 2016;39:2133–9.

Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. Front Public Health. 2017;5:258.

Shinno H, Ishikawa I. Alterations in Rapid Eye Movement Sleep Parameters Predict for Subsequent Progression from Mild Cognitive Impairment to Alzheimer's Disease. J Alzheimers Dis Parkinsonism. 2016;6:218.

Stein PK, Pu Y. Heart rate variability, sleep and sleep disorders. Sleep Med Rev. 2012;16(1):47–66.

Sixel-Döring F, Schweitzer M, Mollenhauer B, Trenkwalder C. Intraindividual Variability of REM Sleep Behavior Disorder in Parkinson's Disease: A Comparative Assessment Using a New REM Sleep Behavior Disorder Severity Scale (RBDSS) for Clinical Routine. J Clin Sleep Med. 2011a;7:75–80.

Sixel-Döring F, Trautmann E, Mollenhauer B, Trenkwalder C. Associated factors for REM sleep behavior disorder in Parkinson disease. Neurology. 2011b;77:1048–54.

Sorensen GL, Mehlsen J, Jennum P. Reduced sympathetic activity in idiopathic rapid-eye-movement sleep behavior disorder and Parkinson's disease. Auton Neurosci. 2013;179:138–41.

Stefani A, Gabelia D, Högl B, Mitterling T, Mahlknecht P, Stockner H, et al. Long-Term Follow-up Investigation of Isolated Rapid Eye Movement Sleep Without Atonia Without Rapid Eye Movement Sleep Behavior Disorder: A Pilot Study. J Clin Sleep Med. 2016;11:1273.

Stiasny-Kolster K, Mayer G, Schäfer S, Möller JC, Heinzel-Gutenbrunner M, Oertel WH. The REM sleep behavior disorder screening questionnaire—A new diagnostic instrument. Mov Disord. 2007;22:2386–93.

Tachibana N, Sugita Y, Terashima K, Teshima Y, Shimizu T, Hishikawa Y. Polysomnographic characteristics of healthy elderly subjects with somnambulism-like behaviors. Biol Psychiatry. 1991;30:4–14.

Tarvainen MP, Lipponen J, Niskanen J-P, Ranta-aho PO. Kubios HRV user guide version 3.3. 2019;

Vanková J, Nevsímalová S, Sonka K, Spacková N, Svejdová-Blazejová K. Increased REM density in narcolepsy-cataplexy and the polysymptomatic form of idiopathic hypersomnia. Sleep. 2001;24:707–11.

Vintonyak O, Gorges M, Müller H-P, Pinkhardt EH, Ludolph AC, Huppertz H-J, et al. Patterns of Eye Movement Impairment Correlate with Regional Brain Atrophy in Neurodegenerative Parkinsonism. Neurodegener Dis. 2017;17:117–26.

Wetter TC, Brunner H, Högl B, Yassouridis A, Trenkwalder C, Friess E. Increased alpha activity in REM sleep in de novo patients with Parkinson's disease. Mov Disord. 2001;16:928–33.

Young A, Home M, Churchward T, Freezer N, Holmes P, Ho M. Comparison of sleep disturbance in mild versus severe Parkinson's disease. Sleep. 2002;25:573–7.

Zucconi M, Ferri R, Allen R, Baier PC, Bruni O, Chokroverty S, et al. The official World Association of Sleep Medicine (WASM) standards for recording and scoring periodic leg movements in sleep (PLMS) and wakefulness (PLMW) developed in collaboration with a task force from the International Restless Legs Syndrome Study Group (IRLSSG). Sleep Med. 2006;7:175–83.

Figure Legend

Figure 1. Boxplots showing the distribution with the median and interquartile range in both groups for A. Total periodic movement of sleep (PLMS) index, B. Rapid eye movement (REM) PLMS index, C. non-REM PLMS index and D. REM density. P values are shown (Mann-Whitney U test for A, B and C. General Linear Model, adjusted for age, gender and Beck Depression Inventory-II for D).

Tables

	iRSWA group (n=49)	*Control group (n=41)*	*P value*
Age (years)	43.8 (±13.0)	44.6 (±10.8)	0.743
Gender (M/F)**	33/16	24/17	0.388
Total sleep time (min)	409.9 (±85.1)	380.6 (±85.4)	0.101
N1 (%)	2.7 (±2.6)	3.5 (±3.7)	0.231
N2 (%)	44.1 (±17.0)	41.9 (±13.3)	0.509
N3 (%)	32.9 (±14.0)	37.1 (±16.8)	0.169
REM sleep (%)	23.9 (±12.6)	20.1 (±7.0)	0.109
Total PLMS/hour*	15.90 (7.1-26.80)	7.20 (3.50-12.50)	**0.001**
REM PLMS/hour*	21.80 (10.70-38.90)	11.0 (1.25-19.40)	**<0.001**
NREM PLMS/hour*	11.75 (3.32-28.72)	5.72 (1.05-12.74)	**<0.001**
REM/hour	342.45 (±111.59)	275.96 (±87.08)	**0.010**
Total AHI (n/hour)	4.3 (±3.5)	6.0 (±3.7)	**0.011**
AHI during REM (N/hour)	4.6 (±4.3)	7.8(±8.0)	**0.001**
Total RSWA (%)	11.4 (±12.4)	0 (±0)	-
Tonic RSWA (%)	10.9 (±12.4)	0 (±)	-
Phasic RSWA (%)	3.7 (±8.0)	0 (±0)	-
Arousal index (N/hour)	24.4 (±7.9)	20.5 (±7.3)	0.021
Sleep time in supine body position (%)	38.0 (±24.2)	40,8 (±28,1)	0.587
RLS (no/yes)**	38/11	38/4	0.083
BDI-II score	11.7 (±8.2)	9.4 (±8.2)	0.154
BMI (kg/m^2)	27.8 (±5.3)	27.3 (±5.4)	0.513

Table 1. Clinical and polysomnographic parameters of isolated rapid eye movement (REM) sleep without atonia (iRSWA) subjects and control subjects. Means are given (± standard deviations) or * medians (interquartile range). **Gender and Restless legs syndrome (RLS) are given in numbers. M: male, F: female. PLMS: periodic limb movement of sleep. REM PLMS: periodic limb movement of REM sleep. NREM PLMS: periodic limb movement of non-REM sleep. RSWA: REM sleep without atonia. AHI: apnea/hypopnea index. BMI: body

mass index. BDI: Beck Depression Inventory. P values are shown, with a significance level set at $P<0.05$. Significant P values are shown in bold.

HRV parameter	*P group/stage interaction*	*P Group*	*P stage*
NN	0.332	0.390	**<0.001**
SDNN	0.283	0.457	**<0.001**
VLF	0.442	0.558	**<0.001**
LF	0.148	0.261	**<0.001**
HF	0.062	0.110	**<0.001**
LF/HF ratio	0.737	0.802	**<0.001**

Table 2. The linear mixed model analysis of heart rate variability. Time-domain analyses included the normal to normal RR interval (NN) and its standard deviation (SDNN). Frequency-domain analyses included the very low frequency bands (VLF), low frequency bands (LF), high frequency bands (HF) and LF/HF ratio. P*group/stage interaction* indicates the P value of difference between isolated rapid eye movement (REM) sleep without atonia (iRSWA) subjects and control subjects across the different stages of wakefulness, nonREM (NREM) sleep and REM sleep. P*groups* indicates the P value of differences in the HRV parameters between groups and P*stage* indicates the P value of difference between the stages of wakefulness, nonREM (NREM) sleep and REM sleep. Significant P values are shown in bold.

HRV parameter	*iRSWA group*	*Control group*	U	P value
Wake	N=33	N=28		
NN (ms)	897.5 (826.1-958.8)	951.5 (892.2-1010.6)	347	0.096
SDNN (ms)	46.2 (33.8-57.5)	41.5 (36.8-58.1)	445	0.806
VLF (ms^2)	58.9 (29.8-88.4)	67.8 (29.3-132.9)	387	0.283
LF (ms^2)	620.4 (275.9-1058.4)	651.6 (295.6-1141.2)	421	0.560
HF (ms^2)	401.4 (149.4-836.9)	261.2 (135.3-562.1)	417	0.522
LF/HF ratio	1.3 (0.8-2.4)	2.0 (0.9-3.9)	399	0.368
NREM sleep	N=31	N=28		
NN (ms)	1015.2 (896.9-1128.6)	1084.1 (980.8-1084.1)	322	0.089
SDNN (ms)	47.1 (35.9-67.0)	48.0 (37.0-61.8)	429	0.946
VLF (ms^2)	71.8 (44.6-108.0)	73.9 (32.8-158.6)	403	0.646
LF (ms^2)	492.7 (333.7-824.0)	720.4 (319.9-1038.8)	409	0.712
HF (ms^2)	620.0 (168.0-1173.3)	645.8 (251.3-1247.2)	418	0.860
LF/HF ratio	0.8 (0.6-1.6)	1.0 (0.5-2.2)	424	0.886
REM sleep	N=31	N=27		
NN (ms)	985.7 (908.4-1105.0)	1045.9 (937.0-1116.5)	385	0.610
SDNN (ms)	63.2 (51.9-81.1)	55.0 (41.4-73.0)	334	0.192
VLF (ms^2)	120.3 (120.3-195.0)	158,2 (65.0-177.7)	394	0.710
LF (ms^2)	970.9 (478.0-1784.4)	649.2 (403.0-1272.0)	311	0.096
HF (ms^2)	559,0 (188.0-1156.3)	269.4 (117.1-547.6)	319	0.123
LF/HF ratio	1.8 (1.0-4.4)	1.9 (1.0-4.3)	395	0.722

Table 3. The heart rate variability between-groups analyses. Differences in heart rate variability (HRV) between isolated rapid eye movement (REM) sleep without atonia (iRSWA) subjects and control subjects per stage. Time-domain analyses included the normal to normal RR interval (NN) and its standard deviation (SDNN). Frequency-domain analyses included the very low frequency bands (VLF), low frequency bands (LF), high frequency bands (HF) and LF/HF ratio. Medians (interquartile range) are given. U and P values of the

Mann-Whitney U test are shown.