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Original Article

ORIGINAL ARTICLE

Automatic analysis of muscular activity in the flexor digitorum superficialis muscles: a fast screening method for rapid eye movement sleep without atonia

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Abstract

Study objectives: To identify a fast and reliable method for rapid eye movement (REM) sleep without atonia (RWA) quantification.

Methods: We analyzed 36 video-polysomnographies (v-PSGs) of isolated REM sleep behavior disorder (iRBD) patients and 35 controls' v-PSGs. Patients diagnosed with RBD had: i) RWA, quantified with a reference method, i.e. automatic and artifact-corrected 3-s Sleep Innsbruck Barcelona (SINBAR) index in REM sleep periods (RSPs, i.e. manually selected portions of REM sleep); and ii) v-PSG-documented RBD behaviors. We quantified RWA with other (semi)-automated methods requiring less human intervention than the reference one: the indices proposed by the SINBAR group (the 3-s and 30-s phasic flexor digitorum superficialis (FDS), phasic/"any"/tonic mentalis), and the REM atonia, short and long muscle activity indices (in mentalis/submentalis/FDS muscles). They were calculated in whole REM sleep (i.e. REM sleep scored following international guidelines), in RSPs, with and without manual artifact correction. Area under curves (AUC) discriminating iRBD from controls were computed. Using published cut-offs, the indices' sensitivity and specificity for iRBD identification were calculated. Apnea-hypopnea index in REM sleep (AHI_{REM}) was considered in the analyses.

Results: RWA indices from FDS muscles alone had the highest AUCs and all of them had 100% sensitivity. Without manual RSP selection and artifact correction, the "30-s phasic FDS" and the "FDS long muscle activity" had the highest specificity (85%) with AHI_{REM} < 15/h. RWA indices were less reliable when AHI_{REM}≥15/h.

Conclusions: If AHI_{REM}<15/h, FDS muscular activity in whole REM sleep and without artifact correction is fast and reliable to rule out RWA.

Statement of Significance

This study shows that, independently from the method used, automatically quantified rapid eye movement (REM) sleep without atonia (RWA) in the flexor digitorum superficialis muscles (FDS) alone can better discriminate patients with and without isolated REM sleep behavior disorder from controls compared to automatic RWA quantified in the mentalis/submentalis muscle alone. Furthermore, the study shows that automatic quantification of RWA in the FDS muscles alone, without manual selection of REM sleep periods nor artifact correction, is useful and reliable to rule out RWA when apnea-hypopnea index in REM sleep is below 15/h. Routine recording of FDS muscular activity in clinical practice will allow ruling out of RWA and make diagnostic procedures faster.

Key words: FDS; RBD; REM sleep without atonia; RWA; SINBAR; upper extremities

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Introduction

Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by loss of muscle atonia during REM sleep and dream enactment [1]. Isolated RBD (iRBD, i.e. RBD not associated with any other disease/condition) constitutes the early stage of an alpha-synucleinopathy (i.e. Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy) [2]. According to the third edition of the International Classification of Sleep Disorders (ICSD-3) [1], demonstration of rapid eye movement (REM) sleep without atonia (RWA) is necessary to establish the diagnosis of RBD. Various manual [3–8] and automatic/semi-automatic [9–18] methods to score RWA have been validated. While most of the proposed methods to score RWA use the chin (i.e. mentalis or submentalis) electromyography (EMG) signal alone, the Sleep Innsbruck Barcelona (SINBAR) group has shown that combining muscular activity in the mentalis and bilateral upper extremities (i.e. flexor digitorum superficialis (FDS)) muscles increases diagnostic accuracy for RBD [6,19,20]. Because of this and of the extensive further validations of the method [6,20–22], the SINBAR montage has been reported in the ICSD-3 as the most current evidence-based method to quantify RWA [1] and recently recommended by international guidelines [23].

The manual quantification of RWA according to the SINBAR recommendations is a time-consuming process. Among the attempts made to automatize RWA scoring [9–18], the company OSG (www.osg.be) has developed, in collaboration with the Sleep Disorders Unit, Department of Neurology, Medical University of Innsbruck (Austria), the semi-automatic integrated BrainRT algorithm to score muscular activity in REM sleep according to the SINBAR recommendations, which has been previously validated [21]. Expert scorers manually identify portions of REM sleep where RWA should be quantified and the automatically identified muscular activities in these portions are manually corrected to remove artifacts, thus avoiding RWA over-quantification [21]. The semi-automated artifact-corrected SINBAR score has been previously used as reference method for RWA quantification in several studies [24–27].

Despite the semi-automatic procedure, the manual selection of the portions of REM sleep for RWA quantification and the manual artifact correction are time-consuming tasks. Therefore, RWA quantification is still cumbersome in everyday clinical practice and alternative faster but still reliable methods are needed. It has been recently shown that the mentalis EMG signal is particularly affected by artifacts and that the higher the apnea-hypopnea index in REM sleep (AHI_{REM}), the higher is the number of artifacts that need to be manually removed in the mentalis muscle [26]. On the other side, it has been shown that FDS muscular activity is minimally affected by artifacts [21,26].

Based on these previous results, we hypothesized that automatically identified FDS muscular activity might be used as a first and fast PSG screening tool to either rule out or to confirm RWA and that it is more precise for this purpose than muscular activity automatically identified in the mentalis. In order to evaluate this hypothesis, the current study presents an analysis and comparison of the diagnostic accuracy of several automatic and semi-automatic RWA indices which require less human intervention than the semi-automatic artifact-corrected RWA quantification proposed by the SINBAR group. Indices are obtained from the FDS muscles alone, the mentalis/submentalis muscle alone, and their combination. The influence of AHI_{REM} on the diagnostic accuracy of these indices is also investigated.

Methods

Subjects

A total of 43 consecutive iRBD patients and 65 consecutive controls (i.e. patients referred to the sleep lab and who were diagnosed with other sleep disorders and did not have either isolated or secondary RBD) were investigated with one full-night video-polysomnography (v-PSG) at the Sleep Disorders Unit, Department of Neurology, Medical University Innsbruck, Austria [24]. Six iRBD patients underwent a second consecutive v-PSG night, as part of regular clinical investigations. Sleep diagnoses were made according to the ICSD-3 criteria [1] and quantification of RWA was performed in all participants with the semi-automated artifact-corrected 3-s SINBAR EMG activity index in REM sleep periods (RSPs, i.e. selected portions of REM sleep for RWA quantification), as described in *Video-polysomnography*. A subject was considered to have RWA if such index was exceeding the cut-off of 31.9%.[6] All iRBD patients had RWA over this cut-off and exhibited RBD behaviors during v-PSG.

Several exclusion criteria were defined for this retrospective study: i) age < 50 years (as iRBD is more frequent over 50 years, and this is also the population that would be most likely screened for risk of alpha-synucleinopathy once neuroprotective treatments will be available); ii) cumulative duration of RSPs < 10 minutes (see *Video-polysomnography* for the exact definition of RSPs); iii) electrical, gain saturation, other technical (such as loose or pulled cables) or ECG interference artifacts in either the mentalis, submentalis or in both FDS EMG channels in at least one REM sleep epoch (this strict criterion was defined to avoid any bias towards any of the RWA quantification methods described in *RWA quantification with different automated and semi-automated methods*); and iv) technical issues in importing v-PSGs in Matlab R2020b (which was used to implement some of the automatic RWA quantification methods described in *RWA quantification with different automated and semi-automated methods*). Figure 1 shows the details of how many subjects and v-PSGs were excluded from the initial cohort. The final cohort included 36 v-PSGs of 31 iRBD patients (i.e. five second-night v-PSGs were included) and 35 v-PSGs of as many controls. Of these controls, 25 had a diagnosis of sleep-disordered breathing with periodic limb movements during sleep (PLMS), three of sleep-disordered breathing, three of restless legs syndrome, and four of PLMS.

This retrospective study was approved by the ethical committee of the Medical University Innsbruck, Austria.

Video-polysomnography

V-PSG included recording of electroencephalography (EEG, with F3, F4, C3, C4, O1, O2, M1, and M2 electrodes), electrooculography (EOG, vertical and horizontal eye movements), EMG recorded at the mentalis (derivation ChinZ-Chin2, according to the American Academy of Sleep Medicine (AASM) manual [28]), submentalis (derivation Chin2-Chin1, according to the AASM manual [28]), bilateral FDS and bilateral tibialis anterior (TA) muscles, and cardiorespiratory signals (single lead electrocardiography (ECG), nasal airflow, tracheal microphone, thoracic and abdominal respiratory movements and transcutaneous oxygen saturation). EMG signals were recorded with sampling frequency of 1000 Hz. Sleep stages and respiratory events were

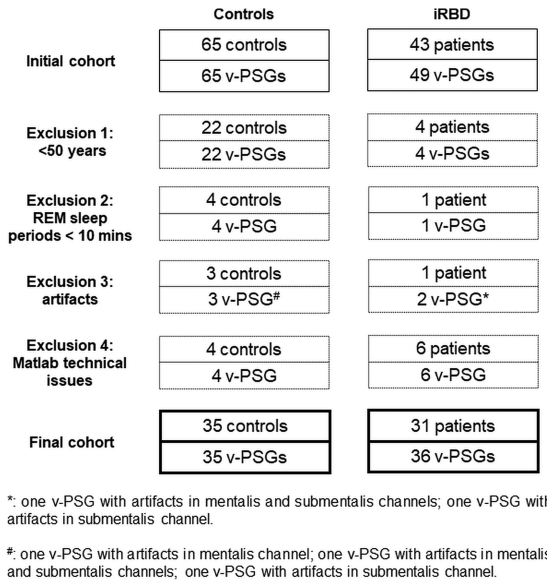

Figure 1. Overview of how many subjects and v-PSGs were excluded from the initial cohort. The different exclusion criteria are reported together with how many subjects and v-PSGs were excluded. *Legend*: iRBD: isolated REM sleep behavior disorder; REM: rapid eye movement; v-PSG: video-polysomnography.

manually scored according to international criteria [28]. PLMS were scored with a validated software [29].

For each v-PSG, RWA was quantified with the validated artifact-corrected semi-automated BrainRT software according to the SINBAR criteria in 3-s mini-epochs included in RSPs [21], following the steps here explained.

First, an expert scorer (AS, blinded to sleep diagnoses) manually selected RSPs in REM sleep on the basis of 3-s mini-epochs. A RSP started at the beginning of the first 3-s mini-epoch including a rapid eye movement and continued until the end of the 3-s mini-epoch occurring right before a mini-epoch including a sleep spindle, K-complex, and/or EEG signs of arousals or after three consecutive minutes without rapid eye movements [3,6]. Figure 2 and Supplementary Figure S1 in the Supplemental Material present an example of start and end of a RSP, respectively. As an example, for the specific patient shown in Figure 2 and Supplementary Figure S1, a total of 194 30-s epochs of REM sleep (i.e. 1h37m) were scored according to the international guidelines [28] and 14 RSPs were manually identified with the method described above. The selected RSPs had a length varying from 42s (i.e. 14 3-s mini-epochs) to 19m42s (i.e. 394 3-s mini-epochs). The cumulative length of the RSPs for this patient was 1h26m03s (i.e. 1721 3-s mini-epochs).

After manual selection of 3-s mini-epochs, EMG signals were then filtered between 50 Hz and 300 Hz and the BrainRT software was run to identify phasic, tonic, and "any" muscular activity in the mentalis muscle and phasic activity in the FDS muscles in REM sleep according to the SINBAR criteria [6,21].

Finally, expert scorers (AH and EH, blinded to sleep diagnoses) visually checked the automatically scored activations in the EMG channels included in RSPs and performed manual artifact correction, which consisted in the manual removal of activations generated by e.g. technical issues, respiration, snoring, ECG, which were not recognized as muscular activity to be considered for RWA quantification [6,21].

The artifact-corrected (AC) 3-s SINBAR EMG activity index in RSPs ($SINBAR_{3s,RSP,AC}$) was calculated as the percentage of 3-s mini-epochs included in RSPs with "any" mentalis and/or phasic activity in the left and/or right FDS muscles, after manual artifact correction. A subject was considered to have RWA if such index was above 31.9% [6].

RWA quantification with different automated and semi-automated methods

In order to investigate our hypothesis, we computed RWA with different automatic and semi-automatic methods requiring less human intervention and being therefore less time-consuming than the reference method (i.e. $SINBAR_{3s,RSP,AC}$). The indices were calculated considering FDS muscular activity alone, mentalis/submentalis muscle activity alone, and their combination. The quantification was performed both in manually selected RSPs as well as in whole REM sleep (i.e. REM sleep scored in 30-s epochs according to international recommendations [28]) and with/without manual artifact correction. With this approach, we could compare the diagnostic performances of different automated and semi-automated RWA quantification methods considering muscular activity in different muscles. Despite TA muscular activity is routinely recorded during v-PSGs, we did not quantify RWA in the TA muscles, as it has been shown that TA muscular activity is less specific for RWA compared to FDS and mentalis muscles [6–8].

RWA indices according to SINBAR recommendations in REM sleep periods. As previously described, the BrainRT software was used to score phasic, "any" and tonic activity in the mentalis muscle and phasic activity in the FDS muscles. Expert scorers performed artifact correction (see *Video-polysomnography*). The software was programmed to identify "any" and tonic activities only in the mentalis muscle and not in the submentalis one, as the mentalis is the derivation recommended by the AASM [28]. For each subject, we calculated the following RWA indices:

- 3-s phasic mentalis EMG activity index with ($phasic\text{-}M_{3s,RSP,AC}$) and without artifact correction ($phasic\text{-}M_{3s,RSP}$): the percentage of 3-s mini-epochs in RSPs with phasic mentalis EMG activity, before and after manual artifact correction, respectively.
- 3-s "any" mentalis EMG activity index with ($any\text{-}M_{3s,RSP,AC}$) and without artifact correction ($any\text{-}M_{3s,RSP}$): the percentage of 3-s mini-epochs in RSPs with "any" mentalis EMG activity, before and after manual artifact correction, respectively.
- 30-s tonic mentalis EMG activity index with ($tonic\text{-}M_{30s,RSP,AC}$) and without artifact correction ($tonic\text{-}M_{30s,RSP}$): the percentage of complete 30-s REM sleep epochs included in RSPs scored as tonic, before and after manual artifact correction, respectively.
- 3-s phasic FDS EMG activity index with ($phasic\text{-}FDS_{3s,RSP,AC}$) and without artifact correction ($phasic\text{-}FDS_{3s,RSP}$): the percentage of 3-s mini-epochs in RSPs with phasic FDS EMG activity, before and after manual artifact correction, respectively. If one FDS channel had electrical, gain saturation, other technical or ECG artifacts, only the other one was used to obtain the $phasic\text{-}FDS_{3s,RSP}$ index.
- 3-s SINBAR EMG activity index with ($SINBAR_{3s,RSP,AC}$) and without artifact correction ($SINBAR_{3s,RSP}$): the percentage of 3-s mini-epochs in RSPs with "any" EMG activity in the mentalis muscle and/or phasic EMG activity in at least one FDS muscle,

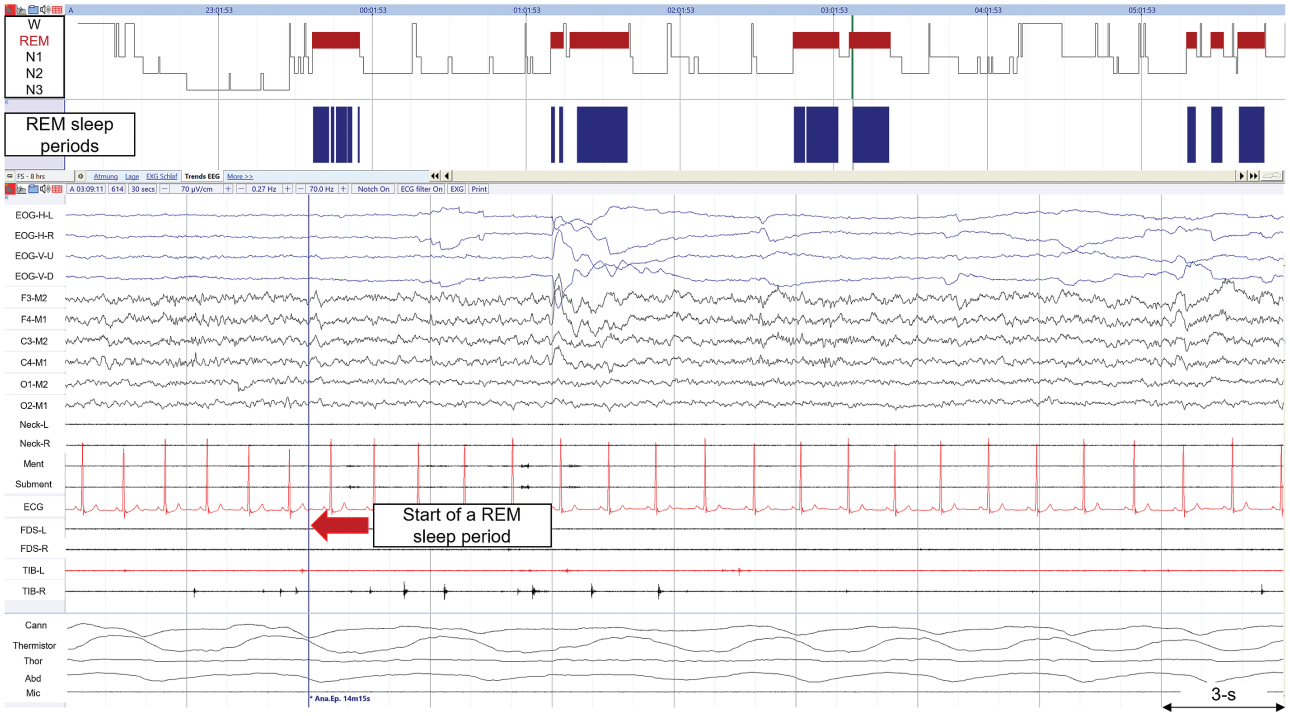

Figure 2. Example of start of a REM sleep period. The REM sleep periods selected for quantification of RWA are shown in blue and it can be seen that they are portions of REM sleep. The start of a REM sleep period is set at the beginning of the first 3-s mini-epoch where a rapid eye movement is present. *Legend*: EOG-H-L: left horizontal electrooculogram (EOG); EOG-H-R: right horizontal EOG; EOG-V-U: vertical upper EOG; EOG-V-D: vertical lower EOG; Neck-L: splenius capitis muscle left; Neck-R: splenius capitis muscle right; Ment: mentalis muscle; Subment: submental EMG; ECG: electrocardiogram; FDS-L: left flexor digitorum superficialis (FDS) muscle; FDS-R: right flexor digitorum superficialis muscle; TIB-L: left anterior tibialis muscle; TIB-R: right anterior tibialis muscle; Cann: cannula; Thor: thorax; Abd: abdomen; Mic: microphone; EMG: electromyogram; REM: rapid eye movement; RWA: REM sleep without atonia.

before and after manual artifact correction, respectively. In the case one FDS channel had electrical, gain saturation, or other technical artifacts, only the other one was used to obtain the $SINBAR_{3s,RSP}$ index. The $SINBAR_{3s,RSP,AC}$ index was used as reference method to quantify RWA (see *Participants*).

RWA indices according to SINBAR recommendations in whole REM sleep. When considering whole REM sleep (i.e. REM sleep scored according to international guidelines [28]), the SINBAR group has validated cut-offs for RWA indices calculated on the basis of 30-s REM sleep epochs [6]. The automatically scored phasic, "any", and tonic activity in the mentalis and phasic activity in the FDS muscles without manual artifact correction were used to compute the RWA indices described below.

- 30-s phasic mentalis EMG activity index ($phasic\text{-}M_{30s,REM}$): the percentage of 30-s REM sleep epochs with at least five 3-s mini-epochs with phasic mentalis EMG activity.
- 30-s "any" mentalis EMG activity index ($any\text{-}M_{30s,REM}$): the percentage of 30-s REM sleep epochs with at least five 3-s mini-epochs with "any" mentalis EMG activity.
- 30-s tonic mentalis EMG activity index ($tonic\text{-}M_{30s,REM}$): the percentage of 30-s REM sleep epochs scored as tonic.
- 30-s phasic FDS EMG activity index ($phasic\text{-}FDS_{30s,REM}$): the percentage of 30-s REM sleep epochs with at least five 3-s mini-epochs with phasic activity in the left and/or right FDS EMG signal. In case of electrical, gain saturation, other technical or ECG artifacts in one FDS channel, only the other one was used for computation.
- 30-s SINBAR EMG activity index ($SINBAR_{30s,REM}$): the percentage of 30-s REM sleep epochs which had at least five 3-s mini-epochs with "any" and/or phasic activity in the left and/or right FDS muscles. Also in this case, in presence of electrical, gain saturation, other technical or ECG artifacts in one FDS muscle, only the other one was used for analysis.

The indices were calculated only without manual artifact correction, because semi-automated artifact-corrected indices in 30-s epochs have not been validated against manual scoring [21]. Manual artifact correction is likely more challenging when scoring RWA in 30-s epochs, due to the inclusion of muscular activity related to arousals, which is automatically excluded from RSPs. For this reason, we did not include artifact-corrected indices in 30-s epochs.

REM atonia index. The REM atonia index (RAI), proposed by Ferri et al.[9,10], is an automatic measure quantifying the level of atonia ranging from 0 (no atonia) to 1 (complete atonia). RAI calculated in the submentalis muscle has been extensively validated to measure RWA and to identify patients with RBD [7–10,30,31]. To calculate RAI for an EMG signal, the following steps are performed [9,10]: i) the EMG signal is filtered between 10 and 100 Hz and a notch filter at 50 Hz is applied; ii) the signal is rectified; iii) the signal is divided in 1-s windows for which the average rectified EMG amplitude is calculated; iv) from each 1-s window average rectified EMG amplitude, the minimum EMG amplitude found in a moving window including the 60 surrounding windows is subtracted, thus obtaining a noise-corrected average rectified EMG amplitude for each 1-s window; v) RAI is calculated as the ratio between the percentage of 1-s windows with noise-corrected average rectified EMG amplitude

≤1μV and the percentage of 1-s windows with noise-corrected average rectified EMG amplitude ≤1μV or >2 μV.

In this work, we implemented this algorithm in Matlab R2020b and we derived the following RAI values for each subject:

- RAI mentalis in RSPs (*RAI-M_{RSP}*) and in whole REM sleep (*RAI-M_{REM}*): the RAI calculated for the mentalis muscle, considering the signal included in RSPs and in whole REM sleep, respectively.
- RAI submentalis in RSPs (*RAI-SM_{RSP}*) and in whole REM sleep (*RAI-SM_{REM}*): the RAI calculated for the submentalis muscle, considering the signal included in RSPs and in whole REM sleep, respectively.
- RAI FDS in RSPs (*RAI-FDS_{RSP}*) and in whole REM sleep (*RAI-FDS_{REM}*): RAI was calculated for both FDS muscles, considering the signals included in RSPs and in whole REM sleep, respectively. The indices were calculated as the average RAI value across the two muscles if both were not affected by saturation or other technical artifacts. In case of one of the two muscles had electrical, gain saturation, other technical or ECG artifacts, only the other muscle was considered.

Short and long muscle activity indices. Mayer et al. proposed an automatic method to identify short (<0.5s) and long (≥0.5s) muscular activity during REM sleep [11]. The short and long muscle activity indices (SMI and LMI, calculated as the frequency of short and long muscle activity per hour of REM sleep, respectively) have been validated for the mentalis and FDS muscles as measures of RWA to identify patients with RBD [11,12]. To automatically score short and long muscular activity in one EMG signal, the following steps are performed [11]: i) the EMG signal is filtered between 10 Hz and 120 Hz; ii) the smoothed (0.025 s) lower and upper envelopes of the EMG signal are obtained; iii) the amplitude signal is calculated as the difference between the upper and lower envelopes; iv) the amplitude signal is smoothed over 200 s with a moving window; v) the threshold curve is obtained as twice the smoothed amplitude signal; vi) muscle activity is identified when the amplitude signal exceeds the threshold curve and clusters of muscle activity at distance <1 s are merged; vii) SMI is calculated as the number of short muscle activity (i.e. with duration <0.5s) per hour of REM sleep and LMI as the number of long muscle activity (i.e. with duration ≥0.5s) per hour of REM sleep.

In this work, we implemented the algorithm in Matlab R2020b and calculated the following values for each subject:

- Indices for the mentalis muscle: short and long muscular activities were identified in the mentalis muscle. The *SMI-M_{RSP}*, *LMI-M_{RSP}*, and their combination (*SMI&LMI-M_{RSP}*) were calculated in RSPs as the number of short, long, and any (i.e. short and long) muscle activity in the mentalis muscle per hour of sleep included in RSPs, respectively. Similarly, these indices were calculated also in whole REM sleep (*SMI-M_{REM}*, *LMI-M_{REM}*, and *SMI&LMI-M_{REM}*).
- Indices for the submentalis muscle: short and long muscular activities were identified in the submentalis muscle. The SMI (*SMI-SM_{RSP}*), LMI (*LMI-SM_{RSP}*), and their combination (*SMI&LMI-SM_{RSP}*) were calculated in RSPs as the number of short, long, and any (i.e. short and long) muscle activity in the submentalis muscle per hour of sleep included in RSPs, respectively. Similarly, these indices were calculated also in whole REM sleep (*SMI-SM_{REM}*, *LMI-SM_{REM}*, and *SMI&LMI-SM_{REM}*).
- Indices for the FDS muscles: muscle activities were identified separately for the left and right FDS muscles and then merged to calculate in RSPs the SMI (*SMI-FDS_{RSP}*), LMI (*LMI-FDS_{RSP}*), and their combination (*SMI&LMI-FDS_{RSP}*) as the number of short, long, and any (i.e. short and long) muscle activity per hour of sleep included in RSPs, respectively. In case of electrical, gain saturation, other technical or ECG artifacts in one FDS muscle, only the other one was used to compute these indices. Similarly, these indices were calculated also in whole REM sleep (*SMI-FDS_{REM}*, *LMI-FDS_{REM}*, and *SMI&LMI-FDS_{REM}*).

Evaluation of the discrimination power of the RWA indices

For each RWA index, we compared with Mann-U-Whitney tests the values obtained for iRBD patients and controls. Furthermore, for each index, we obtained the receiver operating characteristic curve (ROC) and calculated the area under the curve (AUC) to evaluate its discrimination power to distinguish iRBD patients from controls. These analyses were done for all v-PSGs, as well as for only the v-PSGs with AHI_{REM}<15/h and the ones with AHI_{REM}≥15/h. DeLong's test was used to statistically compare the AUC values obtained for the different indices [32,33]. Benjamini-Hochberg procedure was used to correct *p*-values for multiple comparisons (false discovery rate set at 0.05). ROCs, AUCs computations, and DeLong's tests were performed with an open-source Matlab user interface [32,34].

Previous studies have proposed cut-offs to identify RWA for the following indices: phasic mentalis EMG activity index (16.3% for 3-s mini-epochs included in RSPs and 10.6% for 30-s REM sleep epochs in whole REM sleep) [6], "any" mentalis EMG activity index (18.2% for 3-s mini-epochs included in RSPs and 14.5% for 30-s REM sleep epochs in whole REM sleep) [6], tonic mentalis EMG activity index (9.6% for 30-s REM sleep epochs included in RSPs and 8.7% for 30-s REM sleep epochs in whole REM sleep) [6], phasic bilateral FDS EMG activity index (16.8% for 3-s mini-epochs included in RSPs and 7.7% for 30-s REM sleep epochs in whole REM sleep) [6], SINBAR EMG activity index (31.9% for 3-s mini-epochs included in RSPs and 27.2% for 30-s REM sleep epochs in whole REM sleep) [6], RAI in the submentalis muscle (0.8 and 0.9) [10], SMI in the mentalis muscle (90.1/h) [12], LMI in the mentalis muscle (43.1/h) [12], SMI in the FDS muscles (124.3/h) [12] and LMI in the FDS muscles (50.1/h) [12]. Using these cut-offs, we calculated the sensitivity and specificity of these RWA indices for identifying iRBD patients in our study sample. The analysis was done for all v-PSGs, and separately for the v-PSGs with AHI_{REM}<15/h and the ones with AHI_{REM}≥15/h.

Results

Cohort

The demographic and sleep information of the cohort are shown in Table 1, where they are reported for all v-PSGs and separately for v-PSGs with AHI_{REM}<15/h and with AHI_{REM}≥15/h.

RWA indices

The RWA indices are reported in Table 2. When considering all v-PSGs and v-PSGs with AHI_{REM}<15/h, all RWA indices of iRBD

Table 1. Demographic and sleep information of the cohort investigated in the study

	All v-PSGs			V-PSGs with AHI_{REM}<15/h			V-PSGs with AHI_{REM}≥15/h		
Parameter	Cont.	iRBD	*p*-val	Cont.	iRBD	*p*-val	Cont.	iRBD	*p*-val
Number of v-PSGs	35	36	–	20	27	–	15	9	–
Number of subjects	35	31	–	20	23	–	15	9	–
Males/Females	24/11	28/3	**.031**	13/7	22/1	**.010**	11/4	7/2	.808
Age (years)	62 [59–68]	69 [59–73]	.056	63.5 [59–70.5]	70 [62–73]	.116	62 [58–66]	69 [57–75]	.420
Number of patients under antidepressants	6	15	**.007**	4	10	.101	2	5	**.028**
TIB (min)	486 [473–503]	482.5 [470.5–498]	.850	480 [460.5–504]	484 [469–498]	.438	489 [478.5–501]	481 [475–490]	.371
TST (min)	400 [332–439]	415.5 [376.5–443.5]	.367	389 [312.5–429]	412 [378–442]	.208	415 [348.5–450]	419 [361–455.5]	.905
SPT (min)	472 [440–489]	464 [447–484]	.800	468 [430.5–489]	464 [447–486]	.699	476 [463–488.5]	459 [448–481]	.404
Sleep efficiency (%)	83.6 [71.4–90.1]	85.0 [76.2–89.8]	.361	82.8 [67.7–89.8]	85.0 [75.8–89.4]	.420	83.6 [75.9–90]	85.6 [77.3–94.3]	.493
%W (%SPT)	13.9 [8.7–24.1]	11.7 [5.8–17.6]	.154	14.7 [8.8–27.6]	10.8 [6.1–18.7]	.263	11.7 [8.6–20.7]	13.3 [4.8–14.1]	.283
%N1 (%SPT)	14.2 [10.2–20.3]	13.0 [9.1–17.8]	.543	11.2 [8.5–17.3]	13.1 [9.2–17.9]	.445	16.5 [13.2–27.3]	11.6 [8.6–16.1]	.079
%N2 (%SPT)	40.0 [35.9–48.8]	49.0 [35.5–57.7]	**.034**	41.1 [36.0–46.7]	50.7 [35.4–57.5]	.124	38.6 [30.9–54.4]	45.0 [41.2–61.3]	.190
%N3 (%SPT)	7.6 [5.7–17.1]	8.4 [2.8–14.7]	.267	12.6 [6.6–22.5]	8.2 [2.8–11.5]	**.022**	6.5 [2.8–13.6]	14.6 [4.9–17.1]	.387
%REM (%SPT)	14.1 [9.0–19.0]	14.9 [9.7–19.0]	.683	13.3 [9.8–18.6]	15.2 [10.8–20.3]	.471	15.4 [8.3–19.3]	14.4 [8.3–17.3]	.676
REM (min)	65.0 [40.4–91.6]	72.0 [44.8–88.8]	.641	60.0 [42.2–87.6]	72.0 [45.4–97.2]	.349	74.0 [40.0–93.4]	64.0 [42.7–83.0]	.592
AHI (/h)	11.1 [4.2–29.0]	6.9 [3.1–11.7]	**.049**	2.1 [1.7–11.4]	4.8 [2.5–9.5]	.154	38.1 [21.0–55.9]	11.2 [8.2–23.8]	**.037**
AHI_{REM} (/h)	10.1 [1.5–36.0]	6.7 [2.2–15.8]	.345	1.3 [0–6.5]	3.1 [1.2–9.1]	.165	45.3 [27.3–54.6]	22.7 [18.3–32.2]	.079
PLMS index (/h)	19.0 [7.8–46.5]	27.6 [14.2–68.4]	.218	18.2 [8.6–64.5]	36.0 [13.5–70.3]	.378	21.5 [7.3–30.8]	25.0 [15.5–31.9]	.592
# of manually selected 3-s mini-epochs in REM sleep periods	959 [532–1157]	989 [474–1221]	.863	986 [663–1251]	958 [468–1175]	.683	778 [440–1116]	1024 [763–1290]	.551
Cumulative length REM sleep periods (min)	48.0 [26.6–57.9]	49.5 [23.7–61.0]	.863	49.3 [33.2–62.6]	47.9 [23.4–58.8]	.683	38.9 [22.0–55.8]	51.2 [38.2–64.5]	.551
$SINBAR_{3s,RSP,AC}$ (%)	15.7 [9.0–22.2]	63.9 [52.9–74.5]	**<.001**	11.9 [8.9–16.1]	63.6 [52.9–73.0]	**<.001**	22.0 [16.6–28.3]	68.4 [52.3–79.8]	**<.001**

The values are reported as median and [25th percentile–75th percentile] Mann-U-Whitney tests were used to compare all variables, except sex distribution and number of patients under antidepressants. For these two variables, Fisher exact tests were used. Significant *p*-values (<.05) are highlighted in bold.
AC: artifact-corrected; AHI: apnea-hypopnea index, AHI_{REM}: apnea-hypopnea index in REM sleep; Cont: controls; iRBD: patients with isolated REM sleep behavior disorder; N1: non-REM sleep stage 1; N2: non-REM sleep stage 2; N3: non-REM sleep stage 3; *p*-val: *p*-value; PLMS: periodic limb movements during sleep; REM: rapid eye movement sleep; RSP: REM sleep period; SINBAR: Sleep Innsbruck Barcelona; SPT: sleep period time; TIB: time in bed; TST: total sleep time; v-PSG: video-polysomnography; W: wakefulness.

patients were always significantly higher compared to the ones of controls. When considering v-PSGs with AHI_{REM}≥15/h, the values of RWA indices including the FDS muscles were always significantly higher for iRBD patients compared to controls. In contrast, many indices obtained for the mentalis/submentalis muscles alone (i.e. *phasic-*$M_{3s,RSP}$, *any-*$M_{3s,RSP}$, *tonic-*$M_{30s,RSP}$, *RAI-*M_{RSP}, *LMI-*M_{RSP}, *LMI-*SM_{RSP}, *phasic-*$M_{30s,REM}$, *any-*$M_{30s,REM}$, *tonic-*$M_{30s,REM}$, *RAI-*M_{REM}, *RAI-*SM_{REM}, *LMI-*M_{REM}, and *LMI-*SM_{REM}) where not significantly different between iRBD and controls for v-PSGs when AHI in REM sleep was ≥15/h.

AUC values

The values of AUC obtained from the ROC curves to distinguish iRBD patients from controls are reported in Table 3 and shown in Supplementary Figure S2. The *p*-values obtained by using DeLong's test to compare AUCs are shown in Figure 3, when considering all v-PSGs, and in Supplementary Figures S3 and S4 when considering v-PSGs with AHI_{REM}<15/h and with AHI_{REM}≥15/h, respectively. The *SINBAR*$_{3s,RSP,AC}$ index, being the reference method used for quantifying RWA and diagnosing RBD, had always AUC of 1.000, per definition. Detailed analyses of the AUC values are reported below.

All v-PSGs. All RWA indices obtained from the FDS muscles alone (except SMI) had significantly higher AUC values compared to the vast majority of RWA indices calculated including the mentalis/submentalis muscles alone (Figure 3). The *SINBAR*$_{3s,RSP}$ and *SINBAR*$_{30s,REM}$ indices had significantly higher AUC values than most of the indices calculated from the mentalis/submentalis muscles alone. Thus, when considering all v-PSG, RWA indices calculated from FDS muscle only allowed to have significantly better discrimination between iRBD patients and controls than RWA indices obtained from the mentalis/submentalis muscle only.

v-PSGs with AHI_{REM}*<15/h.* The indices obtained from the FDS muscles and the non-artifact corrected SINBAR indices had very high values of AUCs (ranging from 0.987 to 1.000). However, despite the RWA indices obtained from the FDS muscles alone had higher AUC values compared to all RWA indices calculated from the mentalis/submentalis muscle, no significant difference was observed (Supplementary Figure S3). The same was observed for both the non-artifact corrected SINBAR indices. This indicates that, for v-PSG with AHI in REM <15/h, all RWA indices have similar discrimination power.

v-PSGs with AHI_{REM}***≥15/h.*** Supplementary Figure S4 shows that some RWA indices obtained from the FDS muscles alone (i.e. *phasic-*$FDS_{3s,RSP,AC}$, *phasic-*$FDS_{3s,RSP}$, *LMI-*FDS_{RSP}, *SMI&LMI-*FDS_{RSP}, *phasic-*$FDS_{30s,RSP}$, *LMI-*FDS_{REM}, and *SMI&LMI-*FDS_{REM}) had significantly higher AUC values than some of the RWA indices calculated from the mentalis/submentalis muscles alone (i.e. *phasic-*$M_{3s,RSP}$, *RAI-*M_{RSP}, *phasic-*$M_{30s,REM}$, *RAI-*M_{REM}, *RAI-*SM_{RSP}, *RAI-*SM_{REM}), Only SMI calculated in the FDS muscles alone had lower AUC values than the same indices calculated in the mentalis/submentalis muscles (Table 3). The *SINBAR*$_{3s,RSP}$ index had significantly higher AUC value compared to *phasic-*$M_{3s,RSP}$, *phasic-*$M_{30s,REM}$, *RAI-*SM_{RSP}, *LMI-*SM_{RSP}, and *RAI-*SM_{REM} (Supplementary Figure S4). The *SINBAR*$_{30s,REM}$ had significantly higher AUC value than only *phasic-*$M_{30s,REM}$ and *RAI-*SM_{RSP} (Supplementary Figure S4). Therefore, in case of AHI in REM above 15/h, only some indices using FDS muscular activity allow to distinguish iRBD patients from controls significantly better than indices calculated considering muscular activity in the mentalis/submentalis muscle alone.

Additional analyses. In the Supplemental Material (Section S1 and Supplementary Figure S5), additional analyses report: i) differences in AUCs between v-PSGs with AHI_{REM}<15/h and with AHI_{REM}≥15/h; ii) difference in AUCs between the indices computed in RSPs and in whole REM sleep; and iii) differences in AUCs of the SINBAR indices with and without artifact correction. Briefly, such analyses show that: i) higher frequency of apneas and hypopneas in REM sleep generally reduces the discrimination power of RWA indices to identify iRBD patients, but the indices obtained from the FDS muscles alone are more robust than the ones obtained from the mentalis/submentalis muscles alone; ii) all RWA indices did not show significant difference in their discrimination power when computed in RSPs or in whole REM sleep; and iii) the discrimination power of the indices proposed by the SINBAR group did not change significantly when performing or not artifact correction.

Sensitivity and specificity

The values of sensitivity and specificity to identify iRBD patients obtained when using the previously proposed cut-offs are reported in Table 4 and shown in Supplementary Figure S6. The *SINBAR*$_{3s,RSP,AC}$ index, being the reference method used for diagnosis of RBD, had always sensitivity and specificity of 100%. Some detailed analyses are presented below.

Analysis of the performances of different RWA indices. The RWA indices obtained from the FDS muscles alone allowed identifying iRBD patients with 100.0% sensitivity. The specificity of these indices was noninferior to the one of the indices obtained from the mentalis/submentalis muscle alone. The only exception was observed for *SMI-*FDS_{RSP} and *SMI-*FDS_{REM} for v-PSGs with AHI_{REM}<15/h, which had both specificity of 70.0%, which was 10% lower than the lowest specificity of RWA indices obtained from the mentalis/submentalis muscle alone. Among the different RWA indices obtained from the FDS muscles alone, the *phasic-*$FDS_{30s,REM}$ and the *LMI-*FDS_{REM} (both obtained without manual selection of REM sleep periods and artifact correction) had specificity of 85.0% for v-PSGs with AHI_{REM}<15/h. Thus, the *phasic-*$FDS_{30s,REM}$ and the *LMI-*FDS_{REM}, which require only traditional scoring of REM sleep epochs, allowed to identify with high sensitivity and specificity iRBD patients with AHI in REM sleep lower than 15/h.

Both non-artifact corrected SINBAR indices had also 100% sensitivity. For the one calculated in RSPs (i.e. *SINBAR*$_{3s,RSP}$), specificity was high (95%) for v-PSGs with AHI_{REM}<15/h, but low (26.7%) for v-PSGs with AHI_{REM}≥15/h. The index calculated in whole REM sleep (i.e. *SINBAR*$_{30s,REM}$) had low specificity (40% for v-PSGs with AHI_{REM}<15/h and 0% for v-PSGs with AHI_{REM}≥15/h). These results indicate that the non-artifact corrected 3-s SINBAR EMG activity index in RSPs is a reliable RWA quantification method when AHI_{REM}<15/h.

Concerning the indices obtained from the mentalis/submentalis EMG channels only, the artifact-corrected phasic, "any" and tonic EMG activity indices in REM sleep periods were always 100% specific for identification of iRBD patients. For v-PSGs with AHI_{REM}<15/h, the indices obtained from the mentalis/submentalis muscle only had specificity generally higher than 90%, thus indicating that all methods are very specific in case

Table 2. Values of the computed RWA indices

		All v-PSGs			V-PSGs with AHI_{REM}<15/h			V-PSGs with AHI_{REM}≥15/h		
Category	RWA index	Cont.	iRBD	*p*-val	Cont.	iRBD	*p*-val	Cont.	iRBD	*p*-val
According to SINBAR group in REM sleep periods with artifact correction	*phasic-$M_{3s,RSP,AC}$(%)*	3.7 [1.5–8.7]	23.6 [14.2–32.5]	**<.001**	4.2 [1.5–7.6]	23.3 [14.6–32.4]	**<.001**	2.5 [1.5–11.5]	24.9 [12.5–35.4]	**.005**
	any-$M_{3s,RSP,AC}$ (%)	5.2 [1.7–9.0]	27.9 [14.7–46.2]	**<.001**	4.2 [1.5–7.6]	25.3 [15.4–42.4]	**<.001**	7.9 [2.0–13.4]	40.7 [12.5–51.0]	**.012**
	tonic-$M_{30s,RSP,AC}$ (%)	0.0 [0.0–0.0]	2.5 [0.0–8.8]	**<.001**	0.0 [0.0–0.0]	2.4 [0.1–7.8]	**<.001**	0.0 [0.0–1.7]	3.8 [0.0–20.3]	**.028**
	phasic-$FDS_{3s,RSP,AC}$ (%)	7.5 [5.2–16.2]	49.2 [44.5–58.9]	**<.001**	7.5 [5.2–11.2]	49.1 [45.2–58.3]	**<.001**	16.0 [5.5–21.3]	49.3 [43.3–60.6]	**<.001**
	$SINBAR_{3s,RSP,AC}$ (%) [reference]	15.7 [9.0–22.2]	63.9 [52.9–74.5]	**<.001**	11.9 [8.9–16.1]	63.6 [52.9–73.0]	**<.001**	22.0 [16.6–28.3]	68.4 [52.3–79.8]	**<.001**
According to SINBAR group in REM sleep periods without artifact correction	*phasic-$M_{3s,RSP}$ (%)*	13.6 [7.3–26.3]	30.0 [20.8–38.6]	**<.001**	7.5 [6.3–9.8]	29.9 [20.3–38.3]	**<.001**	28.2 [17.1–38.6]	32.1 [23.1–40.8]	.474
	any-$M_{3s,RSP}$ (%)	13.7 [7.3–26.4]	36.7 [21.1–50.0]	**<.001**	7.7 [6.4–10.2]	33.3 [20.4–45.7]	**<.001**	29.0 [17.7–39.1]	47.0 [25.9–52.7]	.095
	tonic-$M_{30s,RSP}$ (%)	0.0 [0.0–1.0]	3.4 [0.3–17.3]	**<.001**	0.0 [0.0–0.0]	2.6 [0.5–13.9]	**<.001**	0.0 [0.0–2.4]	4.5 [0.0–37.7]	.079
	phasic-$FDS_{3s,RSP}$ (%)	7.5 [5.2–16.8]	50.2 [44.5–60.5]	**<.001**	7.5 [5.2–11.2]	50.8 [45.2–60.2]	**<.001**	16.0 [5.5–21.4]	49.3 [43.3–61.2]	**<.001**
	$SINBAR_{3s,RSP}$ (%)	22.0 [13.1–36.0]	67.5 [56.4–76.7]	**<.001**	14.3 [11.7–19.1]	67.4 [56.3–73.9]	**<.001**	36.9 [31.0–46.9]	67.6 [56.3–81.9]	**<.001**
According to SINBAR group in whole REM sleep without artifact correction	*phasic-$M_{30s,REM}$(%)*	8.9 [4.6–25.3]	28.7 [15.1–39.7]	**<.001**	5.3 [2.3–7.6]	27.4 [14.8–39.9]	**<.001**	26.3 [15.1–39.4]	33.3 [15.8–41.0]	.633
	any-$M_{30,REM}$ (%)	9.9 [5.2–32.3]	35.8 [18.8–56.0]	**<.001**	5.9 [2.7–9.0]	33.3 [18.2–50.3]	**<.001**	35.0 [20.2–42.1]	50.7 [23.9–63.9]	.283
	tonic-$M_{30s,REM}$ (%)	0.8 [0.0–2.9]	7.5 [1.8–22.2]	**<.001**	0.0 [0.0–1.3]	5.3 [1.2–19.6]	**<.001**	2.7 [0.8–9.9]	12.5 [1.8–42.4]	.107
	phasic-$FDS_{30s,REM}$ (%)	2.7 [1.4–7.6]	51.8 [44.4–68.2]	**<.001**	2.3 [0.8–5.6]	54.7 [44.0–67.7]	**<.001**	6.4 [1.5–10.0]	50.5 [45.0–75.6]	**<.001**
	$SINBAR_{30s,REM}$ (%)	16.6 [8.7–37.8]	77.2 [63.5–86.5]	**<.001**	9.8 [5.1–15.3]	75.9 [63.1–83.4]	**<.001**	44.7 [31.3–50.0]	82.4 [70.5–92.7]	**.002**
RAI in REM sleep periods	*RAI-M_{RSP} (–)*	0.98 [0.90–0.99]	0.82 [0.26–0.95]	**<.001**	0.99 [0.99–1.00]	0.83 [0.57–0.95]	**<.001**	0.89 [0.56–0.94]	0.34 [0.0–0.95]	.178
	RAI-SM_{RSP} (–)	0.97 [0.90–0.99]	0.76 [0.28–0.92]	**<.001**	0.99 [0.97–1.00]	0.85 [0.37–0.92]	**<.001**	0.86 [0.66–0.96]	0.55 [0.06–0.84]	**.031**
	RAI-FDS_{RSP}(–)	1.00 [0.99–1.00]	0.88 [0.82–0.94]	**<.001**	1.00 [0.99–1.00]	0.88 [0.83–0.94]	**<.001**	0.99 [0.98–1.00]	0.89 [0.80–0.93]	**<.001**
RAI in whole REM sleep	*RAI-M_{REM} (–)*	0.97 [0.90–0.99]	0.73 [0.21–0.93]	**<.001**	0.98 [0.98–0.99]	0.84 [0.51–0.92]	**<.001**	0.9 [0.47–0.93]	0.25 [0.0–0.93]	.209
	RAI-SM_{REM} (–)	0.95 [0.87–0.98]	0.74 [0.26–0.89]	**<.001**	0.98 [0.96–0.99]	0.79 [0.35–0.89]	**<.001**	0.87 [0.62–0.92]	0.46 [0.04–0.84]	.064
	RAI-FDS_{REM}(–)	0.99 [0.99–0.99]	0.89 [0.83–0.94]	**<.001**	0.99 [0.99–1.00]	0.89 [0.83–0.94]	**<.001**	0.99 [0.98–0.99]	0.89 [0.80–0.93]	**<.001**
SMI in REM sleep periods	*SMI-M_{RSP} (/h)*	64.5 [30.2–105.1]	161.7 [119.4–194.3]	**<.001**	33.5 [23.9–64.6]	136.6 [118.9–182.3]	**<.001**	92.0 [67.4–122.0]	204.6 [142.3–230.4]	**.020**
	SMI-SM_{RSP} (/h)	62.8 [37.5–109.7]	162.7 [131.0–206.9]	**<.001**	40.7 [24.6–79.8]	147.3 [122.2–188.2]	**<.001**	97.8 [64.1–119.0]	208.0 [171.4–231.5]	**.004**
	SMI-FDS_{RSP} (/h)	108.7 [70.2–151.4]	214.1 [187.7–242.0]	**<.001**	85.2 [64.9–134.6]	217.0 [187.8–240.9]	**<.001**	133.8 [84.5–223.4]	198.1 [187.7–248.8]	**.023**
SMI in 30-s whole REM sleep	*SMI-M_{REM} (/h)*	57.0 [34.9–101.0]	156.9 [119.7–196.4]	**<.001**	39.0 [27.4–64.9]	140.1 [118.4–179.8]	**<.001**	97.0 [63.8–136.9]	193.4 [145.6–230.7]	**.015**
	SMI-SM_{REM} (/h)	66.3 [42.3–115.6]	161.9 [132.3–211.0]	**<.001**	46.5 [23.6–85.4]	149.3 [126.6–194.1]	**<.001**	103.4 [70.2–123.2]	208.9 [171.8–214.5]	**.008**
	SMI-FDS_{REM} (/h)	110.1 [69.9–139.9]	209.7 [182.4–242.6]	**<.001**	89.2 [66.9–128.2]	215.5 [188.2–239.7]	**<.001**	139.0 [87.0–189.0]	191.3 [172.3–246.7]	**.012**
LMI in REM sleep periods	*LMI-M_{RSP} (/h)*	23.8 [13.2–46.0]	113.9 [73.9–145.0]	**<.001**	13.7 [7.3–19.9]	107.7 [72.8–144.2]	**<.001**	46.3 [38.3–81.3]	125.2 [68.3–163.3]	.107
	LMI-SM_{RSP} (/h)	37.9 [18.8–75.5]	126.6 [89.4–164.3]	**<.001**	23.2 [7.6–32.3]	125.3 [87.9–155.9]	**<.001**	73.2 [47.6–135.1]	138.3 [87.2–170.1]	.152
	LMI-FDS_{RSP} (/h)	24.9 [15.6–54.9]	209.1 [178.8–243.2]	**<.001**	23.6 [15.1–37.0]	209.5 [183.6–240.2]	**<.001**	46.3 [15.6–76.0]	208.7 [146.2–247.4]	**<.001**
LMI in whole REM sleep	*LMI-M_{REM} (/h)*	30.6 [16.1–62.3]	122.4 [75.7–155.5]	**<.001**	18.6 [8.2–29.8]	114.3 [74.2–151.1]	**<.001**	63.1 [40.1–80.5]	124.9 [74.7–168.1]	.056
	LMI-SM_{REM} (/h)	47.6 [22.7–74.1]	120.4 [94.4–178.7]	**<.001**	29.5 [10.0–46.5]	120.0 [97.7–175.6]	**<.001**	75.8 [65.0–133.7]	136.9 [87.3–182.3]	.056
	LMI-FDS_{REM} (/h)	34.0 [19.8–59.8]	206.9 [163.7–229.1]	**<.001**	29.9 [20.0–41.4]	205.0 [170.7–227.0]	**<.001**	51.7 [19.8–78.3]	213.1 [147.7–256.1]	**<.001**
SMI&LMI in REM sleep periods	*SMI&LMI-M_{RSP} (/h)*	90.3 [43.8–139.0]	277.1 [194.6–365.4]	**<.001**	47.2 [37.3–89.4]	242.7 [194.5–329.7]	**<.001**	139.1 [110.8–250.4]	358.0 [244.4–378.8]	**.014**
	SMI&LMI-SM_{RSP}(/h)	106.2 [57.1–192.2]	297.9 [227.5–377.1]	**<.001**	70.6 [29.9–112.1]	260.5 [216.9–375.6]	**<.001**	182.9 [115.7–270.3]	348.0 [302.1–398.3]	**.008**
	SMI&LMI-FDS_{RSP}(/h)	130.3 [89.5–211.8]	435.3 [381.3–475.4]	**<.001**	112.5 [88.0–158.9]	435.0 [384.1–477.6]	**<.001**	197.2 [94.63–312.0]	448.3 [333.8–467.7]	**<.001**
SMI&LMI in whole REM sleep	*SMI&LMIxM_{REM} (/h)*	96.0 [56.1–153.9]	272.4 [194.4–357.8]	**<.001**	58.7 [40.2–96.2]	254.4 [191.9–340.2]	**<.001**	155.9 [110.1–247.2]	345.0 [249.9–386.3]	**.010**
	SMI&LMI-SM_{REM} (/h)	131.3 [66.4–190.0]	292.5 [240.5–387.7]	**<.001**	76.6 [30.2–132.3]	268.7 [236.7–363.5]	**<.001**	180.4 [135.7–250.0]	356.5 [287.5–419.6]	**.007**
	SMI&LMI-FDS_{REM} (/h)	139.2 [98.5–197.7]	422.1 [367.2–461.6]	**<.001**	122.1 [96.3–158.2]	420.8 [393.6–456.8]	**<.001**	200.6 [104.3–259.7]	439.5 [319.9–463.1]	**<.001**

The values are shown as median and [25th percentile–75th percentile]. Statistical analyses were performed with Mann-U-Whitney tests. Significant *p*-values are highlighted in bold.

AC: artifact corrected; AHI_{REM}: apnea-hypopnea index in REM sleep; Cont: controls; iRBD: isolated REM sleep behavior disorder; FDS: flexor digitorum superficialis; LMI: long muscle activity index; M: mentalis; RAI: REM atonia index; REM: rapid eye movement sleep; SM: submentalis; SMI: short muscle activity index; SINBAR: Sleep Innsbruck Barcelona; v-PSG: video-polysomnography.

of AHI in REM sleep lower than 15/h. The highest sensitivity was observed for *LMI-M*$_{REM}$ (96.3%), followed by *LMI-M*$_{RSP}$ (88.9%) and *phasic-M*$_{30s,REM}$ (85.0%). For v-PSGs with AHI$_{REM}$≥15/h, the RWA indices obtained from the mentalis/submentalis muscle only generally showed moderate to high sensitivity values, but low to moderate specificity values.

Additional analyses. In the Supplemental Material (Section S2 and Supplementary Figures S7–S9), additional analyses report: i) differences in sensitivity and specificity between v-PSGs with AHI$_{REM}$<15/h and with AHI$_{REM}$≥15/h; ii) difference in sensitivity and specificity between the indices computed in RSPs and in whole REM sleep; and iii) differences in difference in sensitivity and specificity of the SINBAR indices with and without artifact correction. Briefly, such analyses show that: i) increased frequency of apneas and hypopneas in REM sleep make most of the RWA indices less specific for identification of iRBD patients; ii) the RWA indices obtained from the FDS muscles alone were the ones showing the most similar values of sensitivity and specificity when computed in RSPs or in whole REM sleep; and iii) for the indices proposed by the SINBAR group, artifact correction is fundamental to avoid RWA over-quantification in case of AHI in REM sleep over 15/h, and artifact correction did not change the performances of the phasic FDS EMG activity index when AHI in REM sleep is lower than 15/h, thus demonstrating the robustness of FDS muscular activity to artifacts.

Discussion

Based on the fact that semi-automatic quantification of RWA according to the SINBAR recommendations is a precise, but time-consuming process, the aim of this study was to investigate other (semi)-automated methods with less human intervention to identify a fast but still reliable RWA quantification method. Since FDS muscular activity is minimally affected by artifacts, we hypothesized that automatically identified muscular activity in the FDS muscles alone can be used as a fast screening tool in clinical practice to either rule out or confirm RWA. The main results of our analyses are the following: i) RWA indices calculated from the FDS muscles alone have higher AUC values compared to the RWA indices including the mentalis/submentalis muscle; ii) when using previously proposed cut-offs, the RWA indices obtained from the FDS muscles alone had 100% sensitivity and specificity not inferior to the specificity of RWA indices obtained from the mentalis/submentalis muscle alone; iii) when performing only manual selection of RSPs (but not artifact correction), the 3-s SINBAR EMG activity index had 100.0% sensitivity and 95.0% specificity to identify iRBD patients in case of AHI$_{REM}$<15/h; iv) when performing only traditional scoring of REM sleep (i.e. without additional manual selection of RSPs nor artifact correction), the 30-s phasic FDS EMG activity index (*phasic-FDS*$_{30s,REM}$) and the long muscle activity index (*LMI-FDS*$_{REM}$) allowed to identify iRBD patients with 100.0% sensitivity and 85.0% specificity in case of AHI in REM sleep lower than 15/h.

Based on these findings, we suggest the following step-wise approach to quickly rule out RWA in clinical practice in subjects with AHI$_{REM}$<15/h:

i) Check whether saturation, electrical or other technical and ECG artifacts affect the FDS channels in scored REM sleep;
ii) If at least one FDS channel has none of these artifacts in REM sleep, compute the *phasic-FDS*$_{30s,REM}$ or the *LMI-FDS*$_{REM}$ RWA indices;
iii) If the indices are below the respective previously published cut-offs (i.e. 7.7% for *phasic-FDS*$_{30s;REM}$ and 50.1/h for *LMI-FDS*$_{REM}$), RWA can be ruled out. Otherwise, quantify RWA in REM sleep periods according to the SINBAR recommendations (including manual artifact correction) by combining "any" mentalis and phasic FDS muscular activity to definitely assess RWA [6].

Such approach has two main advantages: i) due to the 100% sensitivity, no patient with RWA is missed; and ii) there is only a relatively little number of false positives subjects, for which RWA can be ruled out by quantifying RWA according to the SINBAR recommendations.

This study is the first to analyze the diagnostic accuracy of FDS muscles when used alone compared to the reference SINBAR method. One previous study compared the diagnostic accuracy of different RWA quantification methods in Parkinson's disease patients when the SINBAR method applied to 30-s epochs in whole REM sleep was used as reference [31], but diagnostic accuracy of the FDS muscles alone was not analyzed in this study. Another previous study compared different automated RWA quantification methods [16], but also in that case no analysis was performed for FDS muscular activity alone, due to absence of FDS recording.

When considering all v-PSGs, the AUC analyses indicate that, independently from the automatic/semi-automatic method used, muscular activity in the FDS muscles alone was generally significantly more discriminative than muscular activity in the mentalis/submentalis muscle alone. When the v-PSGs were separated into the ones with AHI$_{REM}$<15/h and AHI$_{REM}$≥15/h, the RWA indices from the FDS muscles alone had generally higher AUC values compared to the RWA indices obtained from the mentalis/submentalis muscle alone, but significance was only very seldom observed, probably because of the limited amount of v-PSGs included in the analyses. Furthermore, the RWA indices obtained from the FDS muscle alone had generally higher AUC values (even if not significantly higher) than the non-artifact corrected SINBAR EMG activity indices. This indicates that, when not performing manual artifact correction, the FDS muscles alone has higher discrimination power than the combination of mentalis and FDS muscles, probably because of the higher number of artifacts in the mentalis muscle compared to the FDS [26].

Previous validations of the RAI calculated in the submentalis muscle showed that sensitivity to identify iRBD patients ranged between 74.3% and 96% and specificity ranged between 51% and 92.3% when using the cut-off of 0.9 [10,30,35]. When using instead the cut-off of 0.8, the sensitivity ranged between 38.5% and 84%, and the specificity between 81% and 100% [10,30]. When considering RAI calculated in whole REM sleep, our results are within these ranges, thus confirming previous reports of the discrimination ability of RAI. A cut-off for RAI has never been proposed when applied to the FDS muscles, therefore we could not calculate its sensitivity and specificity. However, the AUC values obtained for RAI in the FDS are higher than the ones obtained for the mentalis/submentalis muscle, thus indicating that RAI calculated in the FDS muscles has higher discrimination power than in the mentalis/submentalis muscles to identify iRBD patients.

Table 3. Values of area under the receiver operating characteristic curves to distinguish iRBD patients from controls

Category	RWA index	All v-PSGs	V-PSGs with AHI_{REM}<15/h	V-PSGs with AHI_{REM}≥15/h
According to SINBAR group in REM sleep periods with artifact correction	*phasic-*$M_{3s,RSP,AC}$	0.835 [0.698–0.927]	0.831 [0.668–0.931]	0.852 [0.425–0.992]
	any-$M_{3s,RSP,AC}$	0.885 [0.773–0.955]	0.916 [0.780–0.985]	0.815 [0.434–0.970]
	tonic-$M_{30s,RSP,AC}$	0.789 [0.659–0.868]	0.833 [0.721–0.923]	0.748 [0.514–0.933]
	phasic-$FDS_{3s,RSP,AC}$	0.997 [0.980–1.000]	1.000 [1.000–1.000]	0.993 [0.888–1.000]
	$SINBAR_{3s,RSP,AC}$ *[reference]*	1.000 [1.000–1.000]	1.000 [1.000–1.000]	1.000 [1.000–1.000]
According to SINBAR group in REM sleep periods without artifact correction	*phasic-*$M_{3s,RSP}$	0.782 [0.654–0.883]	0.948 [0.836–0.989]	0.593 [0.342–0.824]
	any-$M_{3s,RSP}$	0.814 [0.687–0.890]	0.952 [0.849–0.988]	0.711 [0.391–0.911]
	tonic-$M_{30s,RSP}$	0.779 [0.668–0.869]	0.844 [0.710–0.927]	0.711 [0.426–0.926]
	phasic-$FDS_{3s,RSP}$	0.997 [0.984–1.000]	1.000 [1.000–1.000]	0.993 [0.899–1.000]
	$SINBAR_{3s,RSP}$	0.978 [0.928–0.996]	1.000 [1.000–1.000]	0.941 [0.680–1.000]
According to SINBAR group in whole REM sleep without artifact correction	*phasic-*$M_{30s,REM}$	0.753 [0.610–0.855]	0.927 [0.798–0.977]	0.563 [0.297–0.812]
	any-$M_{30s,REM}$	0.761 [0.635–0.856]	0.926 [0.829–0.982]	0.637 [0.322–0.883]
	tonic-$M_{30s,REM}$	0.764 [0.639–0.862]	0.861 [0.731–0.940]	0.704 [0.411–0.899]
	phasic-$FDS_{30s,REM}$	0.995 [0.966–1.000]	1.000 [1.000–1.000]	0.993 [0.906–1.000]
	$SINBAR_{30s,REM}$	0.963 [0.863–0.993]	1.000 [1.000–1.000]	0.896 [0.654–1.000]
RAI in REM sleep periods	*RAI-*M_{RSP}	0.799 [0.669–0.887]	0.963 [0.862–1.00]	0.670 [0.367–0.895]
	RAI-SM_{RSP}	0.821 [0.704–0.899]	0.946 [0.847–0.985]	0.770 [0.510–0.919]
	RAI-FDS_{RSP}	0.992 [0.963–1.000]	0.999 [0.977–1.000]	0.989 [0.890–1.000]
RAI in whole REM sleep	*RAI-*M_{REM}	0.795 [0.669–0.884]	0.943 [0.818–0.989]	0.659 [0.342–0.888]
	RAI-SM_{REM}	0.809 [0.690–0.867]	0.927 [0.811–0.976]	0.733 [0.431–0.924]
	RAI-FDS_{REM}	0.988 [0.946–1.000]	0.997 [0.969–1.000]	0.985 [0.824–1.000]
SMI in REM sleep periods	*SMI-*M_{RSP}	0.850 [0.721–0.930]	0.922 [0.751–0.985]	0.793 [0.544–0.952]
	SMI-SM_{RSP}	0.844 [0.712–0.923]	0.867 [0.688–0.959]	0.859 [0.549–0.963]
	SMI-FDS_{RSP}	0.894 [0.777–0.959]	0.981 [0.904–0.998]	0.785 [0.529–0.943]
SMI in whole REM sleep	*SMI-*M_{REM}	0.863 [0.746–0.929]	0.937 [0.790–0.990]	0.807 [0.531–0.948]
	SMI-SM_{REM}	0.846 [0.711–0.928]	0.879 [0.684–0.962]	0.830 [0.527–0.958]
	SMI-FDS_{REM}	0.917 [0.804–0.970]	0.987 [0.926–1.000]	0.815 [0.571–0.951]
LMI in REM sleep periods	*LMI-*M_{RSP}	0.868 [0.753–0.935]	0.967 [0.880–1.000]	0.704 [0.357–0.905]
	LMI-SM_{RSP}	0.846 [0.711–0.919]	0.965 [0.878–0.995]	0.681 [0.422–0.870]
	LMI-FDS_{RSP}	0.996 [0.976–1.000]	1.000 [1.000–1.000]	0.993 [0.914–1.000]

Table 3. Continued

Category	RWA index	All v-PSGs	V-PSGs with AHI_{REM}<15/h	V-PSGs with AHI_{REM}≥15/h
LMI in whole REM sleep	*LMI-M_{REM}*	0.868 [0.764–0.937]	0.963 [0.880–1.000]	0.741 [0.375–0.911]
	LMI-SM_{REM}	0.869 [0.757–0.936]	0.969 [0.866–0.995]	0.741 [0.440–0.926]
	LMI-FDS_{REM}	0.995 [0.968–1.000]	1.000 [1.000–1.000]	0.985 [0.866–1.000]
SMI&LMI in REM sleep periods	*SMI&LMI-M_{RSP}*	0.869 [0.759–0.937]	0.954 [0.814–0.996]	0.807 [0.512–0.954]
	SMI&LMI-SM_{RSP}	0.853 [0.728–0.931]	0.917 [0.765–0.977]	0.829 [0.556–0.963]
	SMI&LMI-FDS_{RSP}	0.982 [0.941–0.994]	1.000 [1.000–1.000]	0.948 [0.772–1.000]
SMI&LMI in whole REM sleep	*SMI&LMI-M_{REM}*	0.882 [0.776–0.947]	0.961 [0.835–1.000]	0.822 [0.528–0.956]
	SMI&LMI-SM_{REM}	0.863 [0.744–0.935]	0.928 [0.805–0.978]	0.837 [0.534–0.963]
	SMI&LMI-FDS_{REM}	0.987 [0.948–0.998]	1.000 [1.000–1.000]	0.948 [0.765–1.000]

The values are shown as point estimates together with the 95% confidence intervals, which were obtained with 1,000 bootstrap replicas.
AC: artifact corrected; AHI_{REM}: apnea-hypopnea index in REM sleep; FDS: flexor digitorum superficialis; LMI: long muscle activity index; M: mentalis; RAI: REM atonia index; REM: rapid eye movement sleep; RSP: REM sleep period; SM: submentalis; SMI: short muscle activity index; SINBAR: Sleep Innsbruck Barcelona; v-PSG: video-polysomnography.

The only study where SMI, LMI, and SMI&LMI were validated showed that for the mentalis muscle the sensitivity and specificity were 85% and 83.3% for SMI, and 75% and 80% for LMI, respectively [12]. When considering the FDS muscles, sensitivity and specificity were 75% and 90% for SMI and 80% and 80% for LMI, respectively [12]. In our cohort, these indices calculated in whole REM sleep had generally lower specificity than in that study. This could be explained by different type of patients included as controls.

In 2012 the SINBAR group proposed the cut-offs for phasic mentalis, "any" mentalis, tonic mentalis, and phasic FDS EMG activity to identify RBD patients with 100% specificity [6]. Our results show that 100% specificity was achieved for the artifact corrected indices obtained for the mentalis muscle only, thus confirming that the previously proposed cut-offs are highly specific to identify patients with RWA. For the phasic FDS EMG activity, 100% sensitivity (but not 100% specificity) was achieved when using the previously validated cut-off. This might seem in contradiction with a previous report, where some iRBD patients had phasic FDS activity below the respective cut-off [20]. However, the cut-offs proposed by the SINBAR group were based on manual scoring of muscular activity and the BrainRT algorithm might be more sensitive than the human eye to recognize short bursts of muscular activity in the FDS muscles. This could explain why the automatic phasic FDS EMG activity index had 100% sensitivity. Our results show that the tonic mentalis EMG activity index was very specific, but not sensitive to identify patients with iRBD. In particular, sensitivity was lower compared to the study where the algorithm was validated [21]. This might be due to the fact that only few iRBD patients had tonic activity and that the cut-off proposed by the SINBAR group was based on a mixed population, including iRBD patients and Parkinson's disease patients with RBD, who had significantly higher tonic activity than iRBD patients [6]. It has been shown that tonic activity increases over time both in iRBD patients [36] and in patients with overt alpha-synucleinopathies [25]. Therefore, the originally proposed cut-off for tonic activity by the SINBAR group might have been influenced by the inclusion of subjects with advanced iRBD and/or Parkinson's disease.

Our analyses allow also to compare the discrimination capabilities of the RWA indices when computed in manually selected RSPs or in whole REM sleep. Compared to scoring RWA in whole REM sleep, RWA scoring in RSPs allows to systematically exclude arousals and to have a more meticulous identification of the areas to be used for RWA quantification. For RAI, SMI, LMI, and SMI&LMI indices, when calculated in whole REM sleep, the AUCs of the RWA indices were not significantly different than when computed in RSPs. When using previously proposed thresholds, the sensitivity and specificity were also similar. These results are in line with a previous comparative study [16]. For the RWA indices proposed by the SINBAR group, both selection of RSPs, as well as manual artifact correction, need to be taken into account when comparing the performances. No significant differences were seen in the AUC values, thus indicating that the indices have similar discrimination capabilities. However, when using previously proposed cut-offs, for the RWA indices calculated considering the mentalis muscle, the manual selection of REM sleep epochs and manual artifact correction increased the specificity for identification of iRBD patients. Instead, the phasic FDS EMG activity index had generally similar performances when calculated in RSPs, in whole REM sleep, and when performing or not manual artifact correction. This further proves that muscular activity in the FDS muscle is a reliable measure of RWA [26], less influenced by artifacts. Finally, it should be further remarked that, in RSPs and for v-PSGs with AHI_{REM}<15/h, the non-artifact corrected 3-s SINBAR EMG activity index had 100% sensitivity and 95% specificity. This indicates that, if automatic methods will be developed to select RSPs, the automatic SINBAR index without artifact correction should be used for fast and precise screening of RWA in patients with AHI_{REM} <15/h.

Table 4. Sensitivity and specificity for discriminating iRBD patients from controls when using previously published thresholds

Category	RWA index	Cut-off	All v-PSGs		V-PSGs with AHI_{REM}<15/h		V-PSGs with AHI_{REM}≥15/h	
			Sens (%)	Spec (%)	Sens (%)	Spec (%)	Sens (%)	Spec (%)
According to SINBAR group in REM sleep periods with artifact correction	*phasic-* $M_{3s,RSP,AC}$	16.3%	69.4 [53.2–82.9]	100.0 [100.0–100.0]	70.4 [50.0–86.2]	100.0 [100.0–100.0]	66.7 [25.0–91.7]	100.0 [100.0–100.0]
	any- $M_{3s,RSP,AC}$	18.2%	69.4 [52.0–82.1]	100.0 [100.0–100.0]	70.4 [50.0–85.2]	100.0 [100.0–100.0]	66.7 [28.6–91.5]	100.0 [100.0–100.0]
	tonic- $M_{30s,RSP,AC}$	9.6%	22.2 [10.0–37.3]	100.0 [100.0–100.0]	22.2 [8.7–39.4]	100.0 [100.0–100.0]	22.2 [0.0–62.5]	100.0 [100.0–100.0]
	phasic- $FDS_{3s,RSP,AC}$	16.8%	100.0 [100.0–100.0]	80.0 [62.8–90.3]	100.0 [100.0–100.0]	90.0 [65.5–100.0]	100.0 [100.0–100.0]	66.7 [39.4–88.3]
	$SINBAR_{3s,RSP,AC}$ *[reference]*	31.9%	100.0 [100.0–100.0]	100.0 [100.0–100.0]	100.0 [100.0–100.0]	100.0 [100.0–100.0]	100.0 [100.0–100.0]	100.0 [100.0–100.0]
According to SINBAR group in REM sleep periods without artifact correction	*phasic-* $M_{3s,RSP}$	16.3%	83.3 [64.9–93.8]	62.9 [45.5–76.9]	81.5 [63.0–92.9]	95.0 [75.8–100.0]	88.9 [41.7–100.0]	20.0 [5.9–49.0]
	any- $M_{3s,RSP}$	18.2%	83.3 [67.9–93.3]	65.7 [46.0–79.4]	81.5 [61.6–92.9]	95.0 [68.8–100.0]	88.9 [31.7–100.0]	20.0 [5.9–47.7]
	tonic- $M_{30s,RSP}$	9.6%	33.3 [19.4–50.0]	94.3 [82.8–100.0]	29.6 [13.6–48.1]	100.0 [100.0–100.0]	44.4 [10.0–80.00]	86.7 [60.4–100.0]
	phasic- $FDS_{3s,RSP}$	16.8%	100.0 [100.0–100.0]	74.3 [58.5–87.2]	100.0 [100.0–100.0]	90.0 [66.8–100.0]	100.0 [100.0–100.0]	53.3 [25.0–77.8]
	$SINBAR_{3s,RSP}$	31.9%	100.0 [100.0–100.0]	65.7 [50.0–81.1]	100.0 [100.0–100.0]	95.0 [68.1–100.0]	100.0 [100.0–100.0]	26.7 [7.7–50.0]
According to SINBAR group in whole REM sleep without artifact correction	*phasic-* $M_{30s,REM}$	10.6%	86.1 [71.0–94.6]	57.2 [40.0–73.8]	85.2 [64.3–95.8]	95.0 [69.5–100.0]	88.9 [40.0–100.0]	6.7 [0.0–37.3]
	any- $M_{30s,REM}$	14.5%	80.6 [64.7–91.4]	60.0 [43.4–76.3]	77.8 [60.0–91.3]	95.0 [70.0–100.0]	88.9 [36.7–100.0]	13.3 [0.0–40.0]
	tonic- $M_{30s,REM}$	8.7%	41.7 [26.9–59.2]	85.7 [70.9–94.9]	37.0 [20.8–58.1]	100.0 [100.0–100.0]	55.6 [18.5–88.9]	66.7 [37.5–86.7]
	phasic- $FDS_{30s,REM}$	7.7%	100.0 [100.0–100.0]	74.3 [55.6–86.1]	100.0 [100.0–100.0]	85.0 [65.0–95.8]	100.0 [100.0–100.0]	60.0 [33.3–83.3]
	$SINBAR_{30s,REM}$	27.2%	100.0 [100.0–100.0]	22.9 [11.1–39.0]	100.0 [100.0–100.0]	40.0 [20.0–63.2]	100.0 [100.0–100.0]	0.0 [0.0–0.0]
RAI in REM sleep periods	$RAI\text{-}SM_{RSP}$	0.8	52.8 [35.5–68.4]	85.7 [71.5–94.6]	48.1 [29.2–66.7]	100.0 [100.0–100.0]	66.7 [33.3–91.0]	66.7 [40.0–86.7]
	$RAI\text{-}SM_{RSP}$	0.9	66.7 [50.0–81.2]	77.1 [57.7–88.4]	63.0 [44.0–80.0]	100.0 [100.0–100.0]	77.8 [40.0–100.0]	46.7 [21.4–73.3]
RAI in whole REM sleep	$RAI\text{-}SM_{REM}$	0.8	55.6 [41.2–73.2]	88.6 [72.0–96.8]	51.9 [32.1–70.0]	100.0 [100.0–100.0]	66.7 [25.0–92.2]	73.3 [47.1–92.4]
	$RAI\text{-}SM_{REM}$	0.9	77.8 [61.6–89.6]	71.4 [54.4–86.5]	77.8 [59.5–90.9]	95.0 [71.6–100.0]	77.8 [29.8–100.0]	40.0 [18.5–68.6]
SMI in REM sleep periods	$SMI\text{-}M_{RSP}$	90.1/h	77.8 [62.1–90.9]	71.4 [54.3–85.0]	77.8 [57.8–90.9]	90.0 [68.3–100.0]	77.8 [37.5–100.0]	46.7 [23.5–76.5]
	$SMI\text{-}FDS_{RSP}$	124.3/h	100.0 [100.0–100.0]	60.0 [42.5–75.6]	100.0 [100.0–100.0]	70.0 [47.4–86.7]	100.0 [100.0–100.0]	46.7 [20.0–72.9]

Table 4. Continued

Category	RWA index	Cut-off	All v-PSGs		V-PSGs with AHI_{REM}<15/h		V-PSGs with AHI_{REM}≥15/h	
			Sens (%)	Spec (%)	Sens (%)	Spec (%)	Sens (%)	Spec (%)
SMI in whole REM sleep	*SMI-M_{REM}*	90.1/h	86.1 [72.9–96.2]	62.9 [45.6–76.6]	85.2 [66.7–96.3]	80.0 [56.3–94.1]	88.9 [48.5–100.0]	40.0 [15.4–66.7]
	SMI-FDS_{REM}	124.3/h	100.0 [100.0–100.0]	57.2 [39.5–72.3]	100.0 [100.0–100.0]	70.0 [45.5–88.9]	100.0 [100.0–100.0]	40.0 [17.6–69.2]
LMI in REM sleep periods	*LMI-M_{RSP}*	43.1/h	86.1 [70.7–94.9]	65.7 [47.1–80.6]	88.9 [72.9–100.0]	95.0 [67.0–100.0]	77.8 [40.0–100.0]	26.7 [7.1–58.9]
	LMI-FDS_{RSP}	50.1/h	100.0 [100.0–100.0]	74.3 [58.5–87.2]	100.0 [100.0–100.0]	90.0 [67.1–100.0]	100.0 [100.0–100.0]	53.3 [26.7–75.0]
LMI in whole REM sleep	*LMI-M_{REM}*	43.1/h	94.4 [81.9–100.0]	65.7 [50.0–82.3]	96.3 [81.6–100.0]	95.0 [73.0–100.0]	88.9 [50.0–100.0]	26.7 [7.7–53.8]
	LMI-FDS_{REM}	50.1/h	100.0 [100.0–100.0]	68.6 [50.0–83.8]	100.0 [100.0–100.0]	85.0 [62.0–95.5]	100.0 [100.0–100.0]	46.7 [21.4–73.3]

The values are shown as point estimates together with the 95% confidence intervals, which were obtained with 1,000 bootstrap replicas.
AC: artifact corrected; AHI_{REM}: apnea-hypopnea index in REM sleep; FDS: flexor digitorum superficialis; LMI: long muscle activity index; M: mentalis; RAI: REM atonia index; REM: rapid eye movement sleep; RSP: REM sleep period; SM: submentalis; SMI: short muscle activity index; SINBAR: Sleep Innsbruck Barcelona; v-PSG: video-polysomnography.

RWA indices were generally less specific for v-PSGs with AHI_{REM}≥15/h than with AHI_{REM}<15/h. Considering also our previous findings that the number of artifacts in the mentalis muscle increases linearly with AHI_{REM} [26], our results further substantiate recent recommendations stating to perform RWA quantification only when AHI_{REM}<15/h [23]. Therefore, positive airway pressure treatment should be sought in order to avoid over-quantification and less reliable RWA quantification, as well as to avoid possible mimics of RBD due to movements related to respiratory events [37].

Recording of FDS muscular activity is still not routinely performed in clinical practice, despite many studies have shown its diagnostic utility in the context of RBD [6,19,20]. Our results demonstrate another additional practical benefit of recording muscular activity in the FDS muscles, as automatic FDS analysis would allow not to miss any patient with RWA. It should be pointed out that a visual analysis of the FDS muscles is always recommended before calculating the automated indices. Despite rare, excessive fragmentary myoclonus as well as respiration artifacts might affect the FDS channels.

The proposed fast screening using automatic analysis of FDS muscular activity might be particularly useful in the context of identification of patients with isolated RWA (iRWA). This is relevant because increasing reports are showing that iRWA likely represents the prodromal stage of iRBD and therefore a very early stage of alpha-synucleinopathies [2,38–40].

However, our findings should not be interpreted in the strict sense that the RWA quantification should be performed only considering FDS muscular activity. The combination of mentalis and FDS muscular activity in REM sleep periods should still be considered as the gold standard for a definite identification of RWA. Furthermore, RWA quantification in the mentalis/submentalis muscle is relevant, as muscular activity in the mentalis/submentalis has been proven to increase over time [36] and to be a marker of conversion to overt alpha-synucleinopathies [41–43], while no study has evaluated whether muscular activity in the FDS muscles can also be considered useful as biomarker of progression and/or conversion to an overt alpha-synucleinopathies.

Our study has a number of limitations. First, we included a similar amount of v-PSG of iRBD patients and controls, however, iRBD patients and controls are not equally represented in the typical population of a sleep lab, due to the relatively low prevalence of RBD [44]. Second, we included only patients with iRBD and none with RBD associated to overt alpha-synucleinopathies, narcolepsy, or other diseases. The utility of muscular activity quantification in the FDS alone as a screening method for RWA quantification should be evaluated in the future in a study population representative of a typical sleep lab cohort, as well as including patients with RBD associated with other pathologies. In particular, the findings here reported should be validated in patients with RBD associated with overt alpha-synucleinopathies. Third, we implemented the automatic RAI, SMI, LMI, and SMI&LMI by following the descriptions of the algorithms described in the original publications [9–11], but we did not have access to the original code of the methods. Thus, there might be small implementation differences between the methods here used and the original described methods. Fourth, significant difference on gender distribution as well as on antidepressant intake between iRBD patients and controls was found. While we decided to have age-matched groups and only patients over 50 years due the importance of a

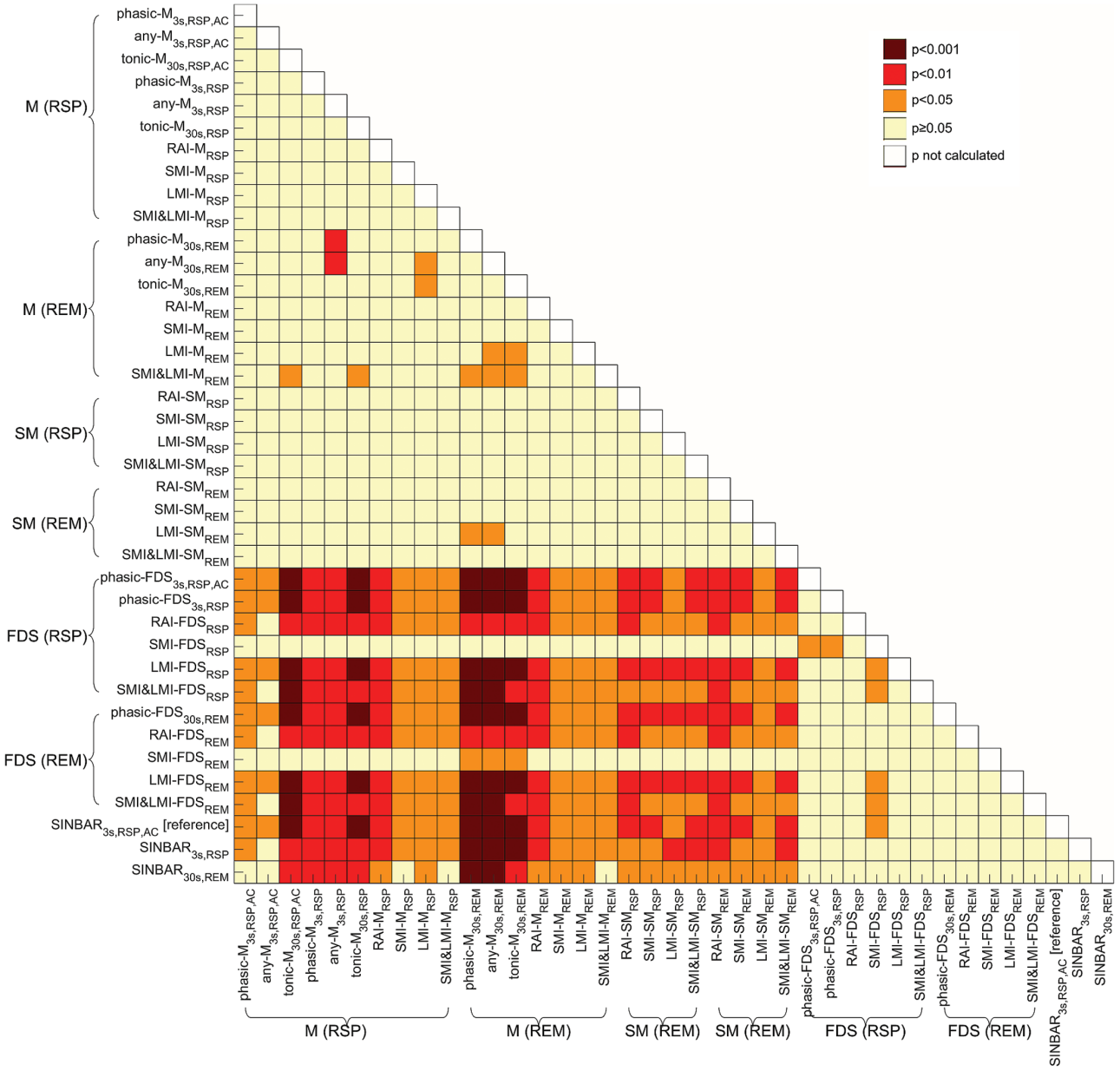

Figure 3. P-values obtained for pairwise comparison of area under the receiver operating characteristic curve values. All-v-PSGs were included. The color code used is shown in the legend. Dark red = $p < .001$; red = $p < .01$; orange = $p < .05$; light yellow = $p \geq .05$; white = p not calculated. *Legend*: AC: artifact corrected; AHI_{REM}: apnea-hypopnea index in REM sleep; FDS: flexor digitorum superficialis; LMI: long muscle activity index; M: mentalis; RAI: REM atonia index; REM: rapid eye movement sleep; RSP: REM sleep period; RWA: REM sleep without atonia; SM: submentalis; SMI: short muscle activity index; SINBAR: Sleep Innsbruck Barcelona; v-PSG: video-polysomnography.

correct diagnosis of iRBD in such population, we did not aim at having gender and antidepressant-matched groups. This is because higher male prevalence is usually seen in iRBD cohorts [45] and because the role of antidepressants in RBD patients in the context of progression to an overt alpha-synucleinopathy is still not clear [2].

In conclusion, our study shows that automatic analysis of muscular activity in the FDS muscles (i.e. performed in whole REM sleep and without artifact correction) can be used as a first screening tool to rule out RWA for subjects with AHI_{REM}<15/h. Automatic RWA quantification is instead not recommended for subjects with AHI_{REM}≥15/h. However, the final RWA quantification as part of a certain RBD diagnosis should still be performed combining FDS and mentalis muscular activity in RSPs and carefully correcting for possible artifacts. Our results underline the importance of including recording of FDS muscular activity in the clinical routine, as it can provide a fast and preliminary measure to rule out RWA and make diagnostic procedures faster.

Supplementary material

Supplementary material is available at *SLEEP* online.

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References

1. American Academy of Sleep Medicine. *The International Classification of Sleep Disorders (ICSD-3)*. 3rd ed. Darien, IL: American Academy of Sleep Medicine, ed.. 2014.
2. Högl B, *et al*. Idiopathic REM sleep behaviour disorder and neurodegeneration—an update. *Nat Rev Neurol*. 2018;**14**(1):40–55.
3. Lapierre O, *et al*. Polysomnographic features of REM sleep behavior disorder: development of a scoring method. *Neurology*. 1992;**42**(7):1371–1374.
4. Montplaisir J, *et al*. Polysomnographic diagnosis of idiopathic REM sleep behavior disorder. *Mov Disord*. 2010;**25**(13):2044–2051.
5. Bliwise DL, *et al*. Quantification of electromyographic activity during sleep: a phasic electromyographic metric. *J Clin Neurophysiol*. 2006;**23**(1):59–67.
6. Frauscher B, *et al*.; SINBAR (Sleep Innsbruck Barcelona) Group. Normative EMG values during REM sleep for the diagnosis of REM sleep behavior disorder. *Sleep*. 2012;**35**(6):835–847. doi:10.5665/sleep.1886
7. McCarter SJ, *et al*. Diagnostic thresholds for quantitative REM sleep phasic burst duration, phasic and tonic muscle activity, and REM atonia index in REM sleep behavior disorder with and without comorbid obstructive sleep apnea. *Sleep*. 2014;**37**(10):1649–1662. doi:10.5665/sleep.4074
8. McCarter SJ, *et al*. Diagnostic REM sleep muscle activity thresholds in patients with idiopathic REM sleep behavior disorder with and without obstructive sleep apnea. *Sleep Med*. 2017;**33**:23–29.
9. Ferri R, *et al*. A quantitative statistical analysis of the submentalis muscle EMG amplitude during sleep in normal controls and patients with REM sleep behavior disorder. *J Sleep Res*. 2008;**17**(1):89–100.
10. Ferri R, *et al*. Improved computation of the atonia index in normal controls and patients with REM sleep behavior disorder. *Sleep Med*. 2010;**11**(9):947–949.
11. Mayer G, *et al*. Quantification of tonic and phasic muscle activity in REM sleep behavior disorder. *J Clin Neurophysiol*. 2008;**25**(1):48–55.
12. Guttowski D, *et al*. Validation of semiautomatic scoring of REM sleep without atonia in patients with RBD. *Sleep Med*. 2018;**46**:107–113.
13. Frandsen R, *et al*. Analysis of automated quantification of motor activity in REM sleep behaviour disorder. *J Sleep Res*. 2015;**24**(5):583–590.
14. Kempfner J, *et al*. Rapid eye movement sleep behavior disorder as an outlier detection problem. *J Clin Neurophysiol*. 2014;**31**(1):86–93.
15. Kempfner J, *et al*. Early automatic detection of Parkinson's disease based on sleep recordings. *J Clin Neurophysiol*. 2014;**31**(5):409–415.
16. Cesari M, *et al*. Comparison of computerized methods for rapid eye movement sleep without atonia detection. *Sleep*. 2018;**41**(10). doi:10.1093/sleep/zsy133
17. Cesari M, *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.
18. Cesari M, *et al*. External validation of a data-driven algorithm for muscular activity identification during sleep. *J Sleep Res*. 2019;**28**(6):e12868.
19. Iranzo A, *et al*.; SINBAR (Sleep Innsbruck Barcelona) Group. Usefulness of the SINBAR electromyographic montage to detect the motor and vocal manifestations occurring in REM sleep behavior disorder. *Sleep Med*. 2011;**12**(3):284–288.
20. Fernández-Arcos A, *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). doi:10.1093/sleep/zsx025
21. Frauscher B, *et al*. Validation of an integrated software for the detection of rapid eye movement sleep behavior disorder. *Sleep*. 2014;**37**(10):1663–1671. doi:10.5665/sleep.4076
22. Figorilli M, *et al*. Diagnosing REM sleep behavior disorder in Parkinson's disease without a gold standard: a latent-class model study. *Sleep*. 2020;**43**(7). doi:10.1093/sleep/zsz323
23. Cesari M, *et al*. Video-polysomnography procedures for diagnosis of rapid eye movement sleep behavior disorder (RBD) and the identification of its prodromal stages: Guidelines from the International RBD Study Group. *Sleep*. Published online October 25, 2021. doi:10.1093/sleep/zsab257.
24. Waser M, *et al*. Automated 3D video analysis of lower limb movements during REM sleep: a new diagnostic tool for isolated REM sleep behavior disorder. *Sleep*. 2020;**43**(11). doi:10.1093/sleep/zsaa100
25. Sringean J, *et al*. Rapid eye movement sleep behavior disorder and rapid eye movement sleep without atonia are more frequent in advanced versus early Parkinson's disease. *Sleep*. 2021;**44**(9). doi:10.1093/SLEEP/ZSAB067
26. Cesari M, *et al*. Flexor digitorum superficialis muscular activity is more reliable than mentalis muscular activity for rapid eye movement sleep without atonia quantification: a study of interrater reliability for artifact correction in the context of semiautomated scoring. *Sleep*. Published online 2021;44(9). doi:10.1093/sleep/zsab094
27. Heidbreder A, *et al*. Video-polysomnographic findings after acute COVID-19: REM sleep without atonia as sign of CNS pathology? *Sleep Med*. 2021;**80**:92–95.
28. Berry RB, *et al*. *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications: Version 2.6*. Darien, IL: American Academy of Sleep Medicine; 2020.
29. Stefani A, *et al*. Validation of a leg movements count and periodic leg movements analysis in a custom polysomnography system. *BMC Neurol*. 2017;**17**(1):42.
30. Ferri R, *et al*. Comparison between an automatic and a visual scoring method of the chin muscle tone during rapid eye movement sleep. *Sleep Med*. 2014;**15**(6):661–665.
31. Figorilli M, *et al*. Comparison between automatic and visual scorings of REM sleep without atonia for the diagnosis of REM sleep behavior disorder in Parkinson disease. *Sleep*. 2017;**40**(2). doi:10.1093/sleep/zsw060
32. Sun X, *et al*. Fast implementation of DeLong's algorithm for comparing the areas under correlated receiver operating characteristic curves. *IEEE Signal Process Lett*. 2014;**21**(11):1389–1393.
33. DeLong ER, *et al*. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;**44**(3):837–845.
34. Sun X. DeLong User Interface. Published Sept 01, 2015. Accessed Jun 01, 2021. https://github.com/PamixSun/DeLongUI.

35. Ferri R, *et al*. Night-to-night variability of automatic quantitative parameters of the chin EMG amplitude (Atonia Index) in REM sleep behavior disorder. *J Clin Sleep Med*. 2013;**9**(3):253–258.
36. Iranzo A, *et al*. Excessive muscle activity increases over time in idiopathic REM sleep behavior disorder. *Sleep*. 2009;**32**(9):1149–1153. doi:10.1093/sleep/32.9.1149
37. Iranzo A, *et al*. Severe obstructive sleep apnea/hypopnea mimicking REM sleep behavior disorder. *Sleep*. 2005;**28**(2):203–206. doi:10.1093/sleep/28.2.203
38. Stefani A, *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*. 2015;**11**(11):1273–1279.
39. Barone DA, *et al*. Autonomic dysfunction in isolated rapid eye movement sleep without atonia. *Clin Neurophysiol*. 2015;**126**(4):731–735.
40. Eisensehr I, *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**(5):507–512. doi:10.1093/sleep/26.5.507
41. Postuma RB, *et al*. Severity of REM atonia loss in idiopathic REM sleep behavior disorder predicts Parkinson disease. *Neurology*. 2010;**74**(3):239–244.
42. McCarter SJ, *et al*. REM sleep muscle activity in idiopathic REM sleep behavior disorder predicts phenoconversion. *Neurology*. 2019;**93**(12):e1171–e1179.
43. Nepozitek J, *et al*. Simultaneous tonic and phasic REM sleep without atonia best predicts early phenoconversion to neurodegenerative disease in idiopathic REM sleep behavior disorder. *Sleep*. 2019;**42**(9). doi:10.1093/sleep/zsz132
44. Cicero CE, *et al*. Prevalence of idiopathic REM behavior disorder: a systematic review and meta-analysis. *Sleep*. 2021;**44**(6). doi:10.1093/sleep/zsaa294
45. Zhang Y, *et al*. Polysomnographically measured sleep changes in idiopathic REM sleep behavior disorder: a systematic review and meta-analysis. *Sleep Med Rev*. 2020;**54**:101362.