



Long-Term Follow-up Investigation of Isolated Rapid Eye Movement Sleep Without Atonia Without Rapid Eye Movement Sleep Behavior Disorder: A Pilot Study

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Study Objectives: Idiopathic rapid eye movement (REM) sleep behavior disorder (RBD) is a harbinger of synuclein-mediated neurodegenerative diseases. It is unknown if this also applies to isolated REM sleep without atonia (RWA). We performed a long-term follow-up investigation of subjects with isolated RWA.

Methods: Participants were recruited from 50 subjects with isolated RWA who were identified at the sleep laboratory of the Department of Neurology at the Medical University of Innsbruck between 2003 and 2005. Eligible subjects underwent follow-up clinical examination, polysomnography, and assessment of neurodegenerative biomarkers (cognitive impairment, finger speed deficit, impaired color vision, olfactory dysfunction, orthostatic hypotension, and substantia nigra hyperechogenicity).

Results: After a mean of 8.6 ± 0.9 y, 1 of 14 participating subjects (7.3%) progressed to RBD. Ten of 14 RWA subjects (71.4%) were positive for at least one neurodegenerative biomarker. Substantia nigra hyperechogenicity and presence of mild

cognitive impairment were both present in 4 of 14 subjects with isolated RWA. Electromyographic activity measures increased significantly from baseline to follow-up polysomnography ("any" mentalis and both anterior tibialis muscles: 32.5 ± 9.4 versus $52.2 \pm 16.6\%$; $p = 0.004$).

Conclusion: This study provides first evidence that isolated RWA is an early biomarker of synuclein-mediated neurodegeneration. These results will have to be replicated in larger studies with longer observational periods. If confirmed, these disease findings have implications for defining at-risk cohorts for Parkinson disease.

Keywords: biomarker, EMG activity, polysomnography, REM sleep behavior disorder, SINBAR

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Rapid eye movement (REM) sleep without atonia (RWA) is the polysomnographic (PSG) hallmark of REM sleep behavior disorder (RBD), a condition characterized by repeated dream-enactment behaviors potentially resulting in injury of the patient or the bedpartner.¹ A correct diagnosis of RBD has prognostic implications as long-term follow-up investigations of idiopathic RBD (iRBD) cohorts have shown, that up to 80% of subjects will eventually develop a synuclein-mediated neurodegenerative disease.^{2–4}

RWA is defined as sustained muscle activity in REM sleep in the chin electromyography (EMG) and/or excessive transient muscle activity during REM sleep in the chin or limb EMG.⁵ Previous studies quantified RWA in order to define cutoff values and to allow a correct diagnosis of RBD.^{6–8} The SINBAR (Sleep Innsbruck Barcelona) group proposed cutoff values of 15% for phasic, 18% for "any", and 9.6% for tonic chin EMG activity.⁷

RWA can also be incidentally observed in PSGs of patients with no history of dream-enacting behavior or relevant visible movements in video-PSG (v-PSG). Whether the proposed EMG cutoff values⁷ are also suitable for the definition of RWA is not definitely resolved. Normative values exceeding the 90th percentile resulted in similar theoretical cutoff values for

BRIEF SUMMARY

Current Knowledge/Study Rationale: Isolated rapid eye movement (REM) sleep without atonia (RWA) might be an even earlier stage of synuclein-mediated neurodegeneration than REM sleep behavior disorder (RBD). To date, no data on isolated RWA progression over time are available.

Study Impact: This is the first long-term follow-up study in subjects with isolated RWA. We provide first evidence for neurodegeneration in isolated RWA, as shown by a progression of RWA over time, a positivity in neurodegenerative biomarkers, and a reported conversion rate to RBD in isolated RWA subjects.

RWA.⁸ In addition, a recent study of our group demonstrated that at least some of the subjects with isolated RWA meet the quantitative EMG criteria for RBD diagnosis.⁹

Although it has been proposed that RWA is a precursor of RBD and—by implication—a potential prodromal manifestation of neurodegenerative synucleinopathies, no study so far investigated its long-term course. The hypothesis that RWA is indeed a potential neurodegenerative biomarker is further supported by its progressive course in patients with RBD,¹⁰ as well as the finding that the loss of REM atonia predicts the occurrence of future neurodegenerative disease.¹¹ It is unknown,

however, whether neurodegenerative biomarkers such as olfactory dysfunction,^{2,12–16} orthostatic hypotension,^{13,17–20} impaired color vision,^{13,15} neurocognitive deficits,^{21–24} or substantia nigra (SN) hyperechogenicity²⁵ also associate with isolated RWA.

The aim of this study was to perform the first long-term follow-up evaluation of patients with an isolated finding of RWA, in order to examine the proportion of RWA subjects who will develop *de novo* RBD with or without parkinsonism, perform an investigation of neurodegenerative biomarkers in RWA, and assess RWA progression.

METHODS

Selection of the Cohort of Subjects with Isolated RWA

All subjects were recruited from an existing cohort of 50 subjects with an isolated finding of qualitative RWA diagnosed at the sleep laboratory, Department of Neurology, Medical University of Innsbruck, between the years 2003 and 2005. Information on the original cohort as well as detailed inclusion and exclusion criteria are described elsewhere.⁹ RWA was defined according to current diagnostic criteria¹ as an increased amount of phasic or tonic EMG activity in either the chin or extremities in the absence of both a history suggestive for RBD and RBD-specific behavioral manifestations as documented in the v-PSG.

The major inclusion criterion was a follow-up interval ≥ 7 y. Exclusion criteria were presence of any relevant psychiatric or neurological disease, and use of medication acting on the central nervous system or potentially inducing RBD. Eligible patients were invited for a follow-up visit. Patients who had at follow-up PSG an apnea-hypopnea index (AHI) > 15 /h were excluded. Patients with sleep-related breathing disorders (SRBD) under effective treatment with nasal continuous positive airway pressure (nCPAP) therapy were included.

This study was approved by the local ethical committee of Innsbruck Medical University. All participants granted written informed consent prior to study participation.

Clinical Follow-Up Visit

All eligible RWA subjects underwent a clinical visit between 2012 and 2014. It comprised a detailed history including RBD-specific questionnaires (RBD single question screen, Innsbruck RBD inventory),^{26,27} and questions focused on environmental RBD risk factors.²⁸ Neurological examination, including the unified Parkinson disease (PD) rating scale motor score (UPDRS III), was performed by two trained raters.

Assessment of Neurodegenerative Biomarkers

All participants underwent a test battery of neurodegenerative biomarkers including the Montreal Cognitive Assessment scale (MoCA),²² the Purdue pegboard test,¹³ the Farnsworth-Munsell 100 (FM-100) Hue color vision test,²⁹ the University of Pennsylvania smell identification test (UPSIT),³⁰ the orthostatic standing test, and a transcranial sonography.

MoCA

This test is a validated scale demonstrated to be more sensitive for cognitive impairment in iRBD than the Mini-Mental

State Examination.²² For assessment of mild cognitive impairment, the cutoff was set at 26.²²

Purdue Pegboard Test

This test is one of hand dexterity, motor speed, and finger-eye coordination. Subjects performed separately for each hand and then bimanually different items, consisting of transferring a series of pins from a dish into corresponding holes, with three 30-sec trials for each item. The outcome measure was the average number of pins placed in the three conditions.¹³

FM-100 Hue Color Vision Test

Each subject was first tested for color blindness using 24 pseudoisochromatic cards. Only subjects without color vision blindness can perform the FM-100 Hue test.²⁹ Both color blindness and color vision tests were performed under a standard light of D65 (UnityColor Light2go, Torso-Verlag, Wertheim and Herbert Waldmann GmbH & Co. KG, Villingen-Schwenningen). The color vision test consists of 85 sortable colored discs spanning the color spectrum, divided into four components (red-yellow, yellow-green, green-blue, and blue-red). For each component, there are two anchor discs of different colors. The examiner scrambled the discs, and asked the subjects to place them in the correct order. The total error score was calculated using the Farnsworth-Munsell test scoring software. An error score $> 125\%$ of the age-expected average was considered indicative of color vision deficit.¹⁵

UPSIT

Odor discrimination was assessed with the 40-item UPSIT (UPSIT-40),³⁰ consisting of 40 scratch-and-sniff pads. Patients were instructed to choose the correct odor from four options. The cutoff score for olfactory dysfunction was set at 80% of the sex- and age-expected average.¹³

Orthostatic Standing Test

After a 10 min resting time in the supine position, the participants were asked to stand up and remain in standing position for 5 min. Orthostatic hypotension was defined as drop of systolic blood pressure ≥ 20 mmHg and/or diastolic blood pressure ≥ 10 mmHg.³¹

Transcranial Sonography

This procedure was performed using a 2.5-MHz transducer (Logiq 7; General Electric, Milwaukee, WI, USA). The penetration depth was 16 cm, the dynamic range 45–50 dB. Image brightness and time gain were adapted as needed for best visualization. The examination was performed from both sides using the temporal approach. Areas of echogenicity were manually encircled on digitally stored images, and the total area of echogenic signals in the SN region was measured as described elsewhere.³² Hyperechogenicity was defined as an area of echogenic signal ≥ 0.18 cm² on at least one side.³³

Baseline and Follow-Up V-PSG

All subjects underwent v-PSG according to current standards.⁵ V-PSG consisted of electrooculography, electroencephalography (F3, F4, C3, C4, O1, O2, M1 and M2 electrodes), cardiorespiratory recording [single channel

Table 1—Polysomnographic parameters of the study sample with isolated RWA at baseline and follow-up.

| Sleep Parameters | Baseline, Mean ± SD | Follow-up, Mean ± SD | p |
|-----------------------------|---------------------|----------------------|-------------------|
| TIB, min | 481.9 ± 11.7 | 481.1 ± 22.7 | 0.901 |
| TST, min | 420.3 ± 31.1 | 391.6 ± 60.2 | 0.068 |
| Sleep efficiency, % | 87.3 % ± 7.2 | 81.6 % ± 12.7 | 0.077 |
| Wake after sleep onset, min | 42.8 ± 30 | 71.4 ± 40.9 | 0.010 |
| N1 (% of SPT) | 10.4 ± 4.7 | 12.1 ± 3.9 | 0.230 |
| N2 (% of SPT) | 47.1 ± 6.8 | 46.9 ± 8 | 0.895 |
| N3 (% of SPT) | 10.4 ± 8.6 | 16.3 ± 21 | 0.364 |
| REM (% of SPT) | 22.5 ± 5.6 | 14.4 ± 5.3 | < 0.001 |
| Sleep onset latency, min | 16.5 ± 9.9 | 19.9 ± 26.3 | < 0.001 |
| REM sleep latency, min | 73.4 ± 25.8 | 92.7 ± 37.5 | 0.080 |
| REM periods | 4.1 ± 0.8 | 3.7 ± 1.1 | 0.212 |
| 3-s REM mini epochs | 1865.8 ± 441.7 | 1214.1 ± 500.8 | < 0.001 |
| 30-s REM epochs | 165.3 ± 37.4 | 108.5 ± 48.8 | 0.001 |
| AHI | 4.5 ± 4.1 | 2.1 ± 2.2 | 0.047 |
| PLMS index | 14.2 ± 12.5 | 22 ± 27.2 | 0.172 |
| during NREM | 16 ± 15.4 | 24.2 ± 30.6 | 0.217 |
| during REM | 8.7 ± 12.4 | 13.4 ± 16.6 | 0.341 |

Significant p values after Bonferroni correction for multiple comparisons are represented by bold text. AHI, apnea-hypopnea index; NREM, nonrapid eye movement; PLMS, periodic leg movements during sleep; REM, rapid eye movement; SD, standard deviation; SPT, sleep period time; TIB, time in bed; TST, total sleep time.

electrocardiography, recording of nasal air flow (thermocouple), nasal pressure cannula, tracheal microphone, thoracic and abdominal respiratory movements (piezo), transcutaneous oxygen saturation], EMG of the mental, submental, both anterior tibialis (AT), and both flexor digitorum superficialis (FDS) muscles, and time-synchronized digital videography. The video was recorded with an infrared camera (Elbex Inc. EX series, Regensburg, Germany).

Sleep was scored according to American Academy of Sleep Medicine (AASM) criteria⁵ with the allowance to score REM sleep despite excessive chin EMG activity.^{7,8} REM sleep onset was determined by the occurrence of the first REM in the electrooculographic channel, and the end of REM sleep when either no REM was detected in 3 consecutive min or an arousal, K-complexes, or spindles were observed.^{7,8} V-PSG during REM sleep was carefully looked through for the presence of movement or vocalizations suggestive for RBD.¹

Periodic leg movements in sleep (PLMS) were scored according to AASM criteria⁵ and indices were calculated for NREM, REM, and total sleep. EMG activity during REM sleep in RWA subjects at baseline and follow-up PSG and in controls was manually analyzed by one trained scorer, who was blind to patient status and time point of investigation. Before analysis, EMG activity during REM sleep was accurately checked for artifacts; all mini epochs containing artifacts preventing correct scoring were excluded from analysis. PLMS during REM sleep were identified by their characteristic morphology and periodicity, and excluded from quantitative analysis of phasic EMG activity.^{7,8} EMG activity during REM sleep was manually quantified according to SINBAR criteria⁷ in the mentalis and both AT muscles at baseline and also in both FDS muscles at follow-up PSG. Each EMG channel was analyzed separately. Percentages of phasic, tonic, and “any” EMG activities were calculated.

Statistics

IBM SPSS 21 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analysis. Data were tested for normal distribution using the Shapiro-Wilk test. Descriptive statistics are given as numbers (percentages) as well as means ± standard deviations, as data are normally distributed. Independent and dependent *t*-tests, as applicable, were performed. Any p values less than 0.05 were considered significant. In case of multiple comparisons, Bonferroni correction was performed, and p values were set accordingly.

RESULTS

RWA Subjects

Fourteen subjects (13 men) of the initial isolated RWA sample of 50 patients participated in this study; 36 subjects did not participate, as they fulfilled exclusion criteria (*n* = 15), denied study participation (*n* = 13), or were lost to follow-up (*n* = 8). Baseline RWA percentages for “any”, phasic and tonic mentalis EMG activity, phasic EMG activity in both AT, and their combination did not differ between RWA subjects who participated and those who did not participate (data not shown, all *p* > 0.05).

The mean age of the investigated isolated RWA subjects at follow-up was 62.5 ± 12.8 y, and the mean follow-up interval was 8.6 ± 0.9 y. The primary reasons for baseline PSG examination were suspected SRBD (*n* = 5, 35.7%), control of nCPAP therapy in SRBD (*n* = 5, 35.7%), excessive daytime sleepiness (*n* = 3, 21.4%) and insomnia (*n* = 1, 7.1%).

Table 1 provides the sleep parameters at baseline and follow-up PSG. AHI and PLMS indices did not differ across both investigations after Bonferroni correction (AHI: *p* = 0.047, PLMS: all *p* > 0.05).

Table 2—Assessment of neurodegenerative biomarkers in the subjects with isolated RWA.

| Neurodegenerative Biomarker | N Assessed | N Positive (%) | Mean ± SD |
|-----------------------------|-----------------|----------------|-------------|
| MoCA score | 14 | 4 (28.6) | 26.7 ± 2.8 |
| Purdue Pegboard | 14 | n.a | 12.1 ± 2.1 |
| Color vision error score | 13 | 1 (7.7) | 48 ± 53.4 |
| UPSIT | 14 | 4 (28.6) | 30.9 ± 4.8 |
| Orthostatic hypotension | 14 | 0 (0) | n.a. |
| SN echogenic area | 13 ^a | 4 (33.3) | 0.15 ± 0.08 |

^aOne patient had no bone window so that echogenicity could not be determined. MoCA, Montreal Cognitive Assessment; n.a., not applicable; RWA, rapid eye movement sleep without atonia; SD, standard deviation; SN, substantia nigra; UPSIT, University of Pennsylvania smell identification test.

Table 3—REM-related EMG activity measures in the subjects with isolated RWA at baseline and follow-up.

| EMG Activity % | N Over SINBAR Cutoff (%) | | | Mean ± SD | | |
|-------------------------------|--------------------------|-----------|--------------|------------|-------------|-------------------|
| | Baseline | Follow-up | p | Baseline | Follow-up | p |
| “Any” mentalis | 8 (57.1) | 10 (71.4) | 0.165 | 18.4 ± 7.5 | 24.7 ± 9.8 | < 0.001 |
| Phasic mentalis | 8 (57.1) | 10 (71.4) | 0.165 | 16.7 ± 7.7 | 22.5 ± 10.1 | < 0.001 |
| Tonic mentalis | 0 (0) | 1 (7.1) | n.a. | 2.9 ± 1.5 | 5.0 ± 3.7 | 0.086 |
| FDS | n.a. | 6 (46.2) | n.a. | n.a. | 16.4 ± 8.7 | n.a. |
| AT | 0 (0) | 5 (35.7) | n.a. | 13.7 ± 7.6 | 31.1 ± 21.1 | 0.002 |
| “Any” mentalis + AT bilateral | 1 (7.1) | 9 (64.3) | 0.001 | 32.5 ± 9.4 | 52.2 ± 16.6 | 0.005 |

Significant p values after Bonferroni correction for multiple comparisons are represented by bold text. AT, anterior tibialis muscle; EMG, electromyography; FDS, flexor digitorum superficialis muscle; n.a., not applicable; RWA, rapid eye movement sleep without atonia; SD, standard deviation; SINBAR, Sleep Innsbruck Barcelona group.

Clinical Visit

At follow-up, RBD developed in 1 of 14 isolated RWA subjects (7.1%) with an RBD suggestive history, a positive Innsbruck RBD-Inventory score of 0.6, and observed violent movements during REM sleep in the v-PSG. None of the isolated RWA subjects developed manifest parkinsonism. The mean UPDRS III score was 0.6 ± 0.9 . None of the risk factors for RBD (head injury, smoking, farming, welding, pesticide exposure) were frequent among the subjects with isolated RWA.

Biomarkers of Neurodegeneration

Neurodegenerative biomarkers results are provided in **Table 2**. Ten subjects with isolated RWA (71.4%) were positive for at least one biomarker. The subject who developed RBD had hyposmia.

With respect to the individual biomarkers, four of 14 cases of the isolated RWA group (28.6%) had mild cognitive dysfunction, and four of 12 cases (33.3%) had SN hyperechogenicity. The eight cases in the isolated RWA group who exceeded the SINBAR cutoff values for RBD at baseline showed comparable results (see **Table S1**, supplemental material).

Quantitative Assessment of EMG Activity

Table 3 and **Figure 1** provide the baseline and follow-up EMG activity measures as well as the number of subjects exceeding SINBAR cutoff values for RBD.⁷

Except for tonic EMG activity, all measured types of EMG activity in the mentalis and both AT muscles increased over the observational period. Eight of the subjects with isolated

RWA exceeded the SINBAR cutoff values for RBD at baseline. Overall, the number of subjects exceeding the cutoff values also increased over the observational period. This comparison reached statistical significance for the combination of “any” mentalis and phasic in both AT muscles EMG activity ($p = 0.004$), but not for EMG activities in the single muscles (all $p > 0.05$). Similar results were observed when regarding only the eight subjects exceeding the SINBAR cutoff values for RBD at baseline (see **Table S2**, supplemental material). The subject in whom RBD developed was over the SINBAR cutoff for phasic and “any” in mentalis muscle at baseline, and for all indices except tonic EMG activity in the mentalis muscle at follow-up.

DISCUSSION

This is the first long-term follow-up investigation in subjects with an isolated finding of RWA, including assessment of biomarkers for synuclein-mediated neurodegenerative disease and v-PSG. Major findings are that neurodegenerative biomarkers are positive in a substantial number of subjects with isolated RWA, and that REM-related EMG activity measures increase with time in subjects with isolated RWA.

RWA as Precursor of RBD

In this study 1 of 14 subjects with isolated RWA (7.1%) met criteria for RBD at follow-up. This percentage would be higher than the reported RBD prevalence of 0.38%–2.1% in the general population,^{34,35} and would support that RWA is a precursor of RBD. Nevertheless, the small sample size in this study

precludes firm conclusions. Interestingly, in one additional patient of the isolated RWA cohort, RBD developed after the follow-up visit. This patient also complained about memory impairment.

Neurodegenerative Biomarkers are Positive in a Substantial Number of Subjects with Isolated RWA

We found a positivity in at least one neurodegenerative biomarker in most of the subjects with isolated RWA, although none of the neurodegenerative biomarkers alone seemed to predict a higher risk of conversion to RBD. This could be explained by the protean manifestation of synuclein-mediated neurodegenerative disorders, with different structures involved in different patients and over time. Therefore, a varied association with neurodegenerative biomarkers should be expected.

Concerning individual biomarkers, the most frequently positive were mild cognitive impairment and SN hyperechogenicity. Cognitive impairment has been demonstrated in patients with iRBD, including impairment in attention, verbal memory, and executive functions.²¹ In our cohort, we found scores below the cutoff on the MoCA, which has been validated in patients with RBD.²²

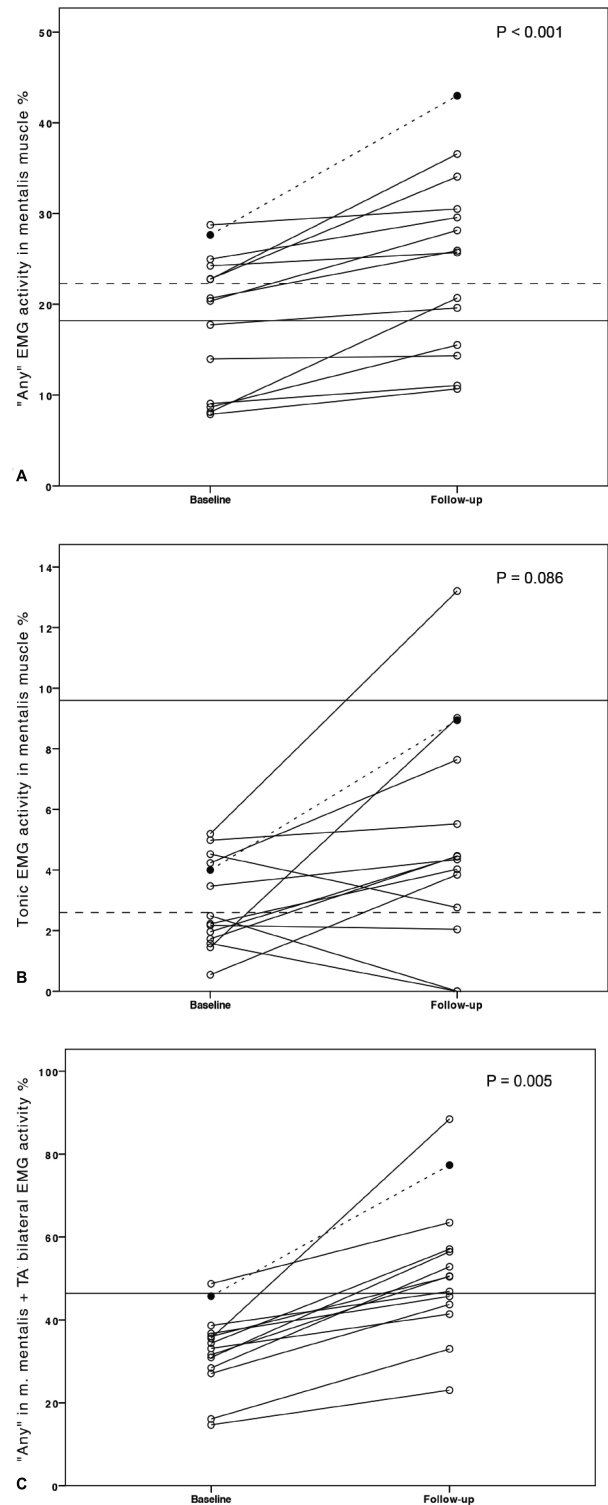
Abnormal hyperechogenicity of the SN pars compacta is present in 80% to 90% of patients with PD.³⁶ It is present early in the disease, and shows no progression with time.³⁷ We found SN hyperechogenicity in 33.3% of subjects with isolated RWA. This number is increased compared to that of healthy normals.³⁶

The subject in whom RBD developed at follow-up had hyposmia. Olfactory dysfunction has been demonstrated in 40% to 60% of patients with RBD.^{13,14} A significant difference in olfactory performance has been reported between patients with iRBD in whom parkinsonism developed compared to those in whom parkinsonism did not develop in a 5-year follow-up.¹⁶ In the current study, we did not find an increased prevalence of olfactory dysfunction among subjects with isolated RWA. Further studies investigating a combination of biomarkers in larger groups of RWA subjects are needed.

Loss of REM Sleep Atonia Progresses Over Time

We found a significant increase in all REM sleep EMG activity measures except for tonic chin EMG activity over the observational period. An increase in both tonic and phasic EMG activity with time was already described in patients with iRBD¹⁰ and an increase in tonic but not phasic chin EMG activity was reported in patients with RBD in whom a neurodegenerative disease later developed.¹¹ Different pathophysiological mechanisms may underlie the presence of excessive tonic and phasic EMG activity during REM sleep. Tonic EMG activity seems to reflect degeneration of the sublaterodorsal nucleus in the brainstem, with subsequent disinhibition of spinal motoneurons eventually resulting in increased EMG activity during REM sleep.³⁸ Phasic EMG activity seems to depend on activation of brainstem locomotor generators during REM sleep and on an alteration of pathways in the intermediate ventromedial medulla.³⁹ Degeneration of the sublaterodorsal nucleus, resulting in increased tonic EMG activity during REM sleep, could be more closely linked with an increased risk of PD.¹¹ In our

Figure 1



Individual "any" (A) and tonic (B) mentalis EMG activity indices and EMG activity indices for the combination of "any" in mentalis muscle and both anterior tibialis muscles (C) in the isolated RWA subjects at baseline and follow-up. Circles represent individuals. The subject in whom RBD developed is presented as a filled black circle, with dotted line. Horizontal solid lines: SINBAR RBD cutoff-values;⁷ dotted lines 90th percentile of normative values.⁸ AT, anterior tibialis muscle; EMG, electromyography; SINBAR, Sleep Innsbruck Barcelona group; RBD, rapid eye movement sleep behavior disorder; RWA, rapid eye movement sleep without atonia.

study, the patient in whom RBD developed showed a marked increase in tonic chin EMG activity over time (from 4% to 8.9%). However, also other subjects in whom RBD or parkinsonism did not develop showed a marked increase of tonic chin EMG activity. Further investigations with a higher number of subjects and longer follow-up periods are needed to clarify this question. We do not think that this increase in REM-related EMG activity is related to the reduction of REM sleep as observed in our study; indeed the latter is most likely explained by the known physiological decrease of REM sleep with age.⁴⁰

Potential Limitations

The investigated sample size of 14 subjects is small, particularly considering that our baseline cohort included 50 patients. The high attrition rate is a limitation of the current study. It was only in part due to loss to follow-up; more important factors were denial of study participation and the presence of exclusion criteria at follow-up. The neurodegenerative biomarkers were assessed only at follow-up. Prospective studies investigating their association with isolated RWA over time could be more informative. The duration of the follow-up period was less than 10 y. Based on the results of the current study one might expect that longer follow-up periods of 15–20 y, for example, would have been preferable. Almost all RWA subjects have mild SRBD, or had SRBD under nCPAP treatment, which might have had an influence on RWA itself. Nevertheless, AHI was stable, whereas RWA worsened over the observational period. Moreover, a recent study by McCarter et al.⁴¹ showed that nCPAP treatment does not affect RWA.⁴¹ Confirmation of our findings in healthy normal sleepers with an isolated finding of RWA is a necessary next step.

CONCLUSION

This is the first long-term follow-up study in subjects with an isolated finding of RWA. The relevance of RWA as early sign of synuclein-mediated neurodegeneration needs to be further investigated; however, we demonstrated in RWA subjects a significant progression of EMG activity during REM sleep with time, probably reflecting ongoing degeneration of structures that control REM sleep atonia. The possible role of RWA as an early marker of neurodegeneration is further supported by the high frequency of neurodegenerative biomarkers in subjects with isolated RWA. If these findings will be replicated in larger studies with longer observational periods, the finding of isolated RWA will have implications for defining at-risk cohorts for PD.

ABBREVIATIONS

AASM, American Academy of Sleep Medicine
 AHI, apnea-hypopnea index
 AT, anterior tibialis
 EMG, electromyography
 FDS, flexor digitorum superficialis
 FM-100, Farnsworth-Munsell 100
 iRBD, idiopathic RBD
 MoCA, Montreal Cognitive Assessment Scale
 nCPAP, nasal continuous positive airway pressure

PD, Parkinson disease
 PLMS, periodic leg movements in sleep
 PSG, polysomnography
 RBD, REM sleep behavior disorder
 RWA, REM sleep without atonia
 SINBAR, Sleep Innsbruck Barcelona group
 SN, substantia nigra
 SRBD, sleep-related breathing disorders
 UPDRS III, Unified Parkinson's disease rating scale motor score
 UPSIT, University of Pennsylvania smell identification test
 v-PSG, video-PSG

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SUPPLEMENTAL MATERIAL

Table S1—Assessment of neurodegenerative biomarkers in subjects with rapid eye movement sleep without atonia above SINBAR cutoffs at baseline polysomnography.

| Neurodegenerative Biomarker | N Assessed | N Positive (%) | Mean ± SD |
|-----------------------------|------------|----------------|-------------|
| MoCA score | 8 | 1 (12.5) | 27.5 ± 2.6 |
| Purdue Pegboard | 8 | n.a. | 12.3 ± 2.4 |
| Color vision error score | 7 | 0 (0) | 24.6 ± 10.9 |
| UPSIT | 8 | 4 (50) | 29.5 ± 5.7 |
| Orthostatic hypotension | 8 | 0 (0) | n.a. |
| SN echogenic area | 8 | 2 (25) | 0.16 ± 0.07 |

MoCA, Montreal Cognitive Assessment; SD, standard deviation; SINBAR, Sleep Innsbruck-Barcelona group; SN, substantia nigra; UPSIT, University of Pennsylvania smell identification test.

Table S2—REM-related EMG activity measures for the subjects with rapid eye movement sleep without atonia over SINBAR cutoffs at baseline polysomnography, comparing baseline and follow-up.

| EMG Activity % | N Over SINBAR Cutoff (%) | | | Mean ± SD | | |
|-------------------------------|--------------------------|-----------|-------|------------|-------------|-------------------|
| | Baseline | Follow-Up | p | Baseline | Follow-Up | p |
| “Any” mentalis | 8 (100) | 8 (100) | n.a. | 23.9 ± 3.0 | 31.7 ± 5.9 | 0.004 |
| Phasic mentalis | 8 (100) | 8 (100) | n.a. | 22.6 ± 3.1 | 29.7 ± 6.1 | 0.004 |
| Tonic mentalis | 0 (0) | 1 (12.5) | n.a. | 3.6 ± 1.3 | 6.0 ± 3.9 | 0.066 |
| FDS | n.a. | 2 (25) | n.a. | n.a. | 14.0 ± 6.8 | n.a. |
| AT | 0 (0) | 2 (25) | n.a. | 13.2 ± 8.1 | 27.9 ± 14.6 | 0.006 |
| “Any” mentalis + AT bilateral | 1 (12.5) | 6 (75) | 0.041 | 36.4 ± 7.8 | 53.9 ± 11.3 | < 0.001 |

Significant p values after Bonferroni correction for multiple comparisons are represented by bold text. AT, anterior tibialis muscle; EMG, electromyography; FDS, flexor digitorum superficialis muscle; n.a., not applicable; PSG, polysomnography; SD, standard deviation; SINBAR, Sleep Innsbruck Barcelona group.