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ORIGINAL ARTICLE

Normative and isolated rapid eye movement sleep without atonia in adults without REM sleep behavior disorder

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Abstract

Study Objectives: Values for normative REM sleep without atonia (RSWA) remain unclear. Older age and male sex are associated with greater RSWA, and isolated elevated RSWA has been reported. We aimed to describe normative RSWA and characterize isolated RSWA frequency in adults without REM sleep behavior disorder (RBD).

Methods: We visually quantified phasic, "any," and tonic RSWA in the submentalis (SM) and anterior tibialis (AT) muscles, and the automated Ferri REM Atonia Index during polysomnography in adults without RBD aged 21–88. We calculated RSWA percentiles across age and sex deciles and compared RSWA in older (≥ 65) versus younger (<65) men and women. Isolated RSWA (exceeding diagnostic RBD cutoffs, or >95th percentile) frequency was also determined.

Results: Overall, 95th percentile RSWA percentages were SM phasic, any, tonic = 8.6%, 9.1%, 0.99%; AT phasic and "any" = 17.0%; combined SM/AT phasic, "any" = 22.3%, 25.5%; and RAI = 0.85. Most phasic RSWA burst durations were ≤1.0 s (85th percentiles: SM = 1.07, AT = 0.86 seconds). Older men had significantly higher AT RSWA than older women and younger patients (all $p < 0.04$). Twenty-nine (25%, 18 men) had RSWA exceeding the cohort 95th percentile, while 17 (14%, 12 men) fulfilled diagnostic cutoffs for phasic or automated RBD RSWA thresholds.

Conclusions: RSWA levels are highest in older men, mirroring the demographic characteristics of RBD, suggesting that older men frequently have altered REM sleep atonia control. These data establish normative adult RSWA values and thresholds for determination of isolated RSWA elevation, potentially aiding RBD diagnosis and discussions concerning incidental RSWA in clinical sleep medicine practice.

Statement of Significance

We quantified polysomnographic REM sleep without atonia (RSWA) in patients without REM sleep behavior disorder to determine normative values, and to clarify the frequency of patients with isolated excessive RSWA. Isolated RSWA elevations were present in 25% of patients, while 14% of patients without dream enactment had sufficient RSWA elevation to fulfill previously established diagnostic cutoffs for RBD. Older men had higher RSWA than older women or younger patients, mirroring the biology of RBD. These data establish normative adult RSWA values and thresholds for isolated RSWA elevation, potentially aiding RBD diagnosis and discussions concerning incidental RSWA in clinical sleep medicine practice. Future prospective cohort studies will be necessary to determine the potential clinical significance of isolated RSWA.

Key words: isolated REM sleep without atonia; REM sleep behavior disorder; polysomnogram; normative values

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Introduction

Rapid eye movement (REM) sleep muscle activity, also known as REM sleep without atonia (RSWA), is the neurophysiologic substrate of REM sleep behavior disorder (RBD) [1–4]. Several visual manual and automated methods for RSWA scoring and diagnostic cutoffs for RBD diagnosis have been established [5–15]. Adults without RBD have also been shown to have measurable RSWA, especially those receiving antidepressant medications [16].

However, there have been a very limited number of previous studies of RSWA normative values. A previous pivotal study of normative motor activity during polysomnography in 100 community-dwelling adults in Innsbruck, Austria, demonstrated similar levels of RSWA across age deciles, and in this study community controls without sleep disturbance complaints or dream enactment behavior history displayed measurable RSWA using standard SINBAR scoring criteria for RSWA diagnosis and research applications in RBD [17]. However, compared with patients with RBD, phasic muscle activity in those without RBD was quite low, and tonic muscle activity even lower, confirming findings of several previous systematic studies of RBD involving controls without dream enactment [5, 6, 12, 17]. Previous studies of automated RSWA utilizing the Ferri REM Atonia Index (RAI) in adults without RBD found that older adults had higher RSWA levels [18, 19]. We also previously studied RSWA in patients without clinical dream enactment behaviors, finding varying RSWA amounts through the lifespan. Interestingly, similar to the findings of Ferri *et al.* [18, 19], greater RSWA was seen most often in older adults, especially men, paralleling the demographic characteristics of RBD [20].

The Innsbruck group also analyzed patients without dream enactment who had been described qualitatively as having incidental/isolated RSWA during polysomnography, noting that a subset had sufficient RSWA to meet diagnostic RBD cutoffs [21]. An important pilot follow-up study of 14 patients from the Innsbruck isolated RSWA cohort found the presence of covert neurodegenerative markers in 71.4%, supporting isolated RSWA as a marker for synucleinopathy even without clinical dream enactment symptoms of RBD [22].

Establishing normative values for RSWA in patients without dream enactment remains an important priority for clinical sleep medicine practice and a research frontier to establish whether isolated RSWA is also a prodromal marker of synucleinopathy. Establishing normative RSWA values can further aid the diagnostic discrimination of neurologically normal sleepers from RBD, since some RBD patients with polysomnographically verified RBD have insufficient RSWA to fulfill established diagnostic cutoffs [7]. Normative RSWA levels may also help to distinguish patients with idiopathic/isolated RBD from mimickers such as "pseudo-RBD" presentations resulting from sleep apnea [23]. Normative RSWA values may also aid clinicians in counseling patients found to have RSWA as an incidental finding during clinical polysomnography done for common indications other than parasomnias, such as sleep apnea.

Clearer understanding of the distribution of normative RSWA in adults without RBD also lays the foundation for understanding patients considered to have isolated RSWA (i.e. patients without reported dream enactment but with RSWA levels considerably higher than age-sex similar peers). Patients with isolated RSWA may be at risk for RBD or other overt forms of synucleinopathy. Determining neurophysiologic standards for isolated RSWA is crucial to enable natural history studies of these patients for possible covert isolated Lewy body disease and to determine phenoconversion risk for overt dream enactment, subsequent idiopathic/isolated RBD diagnosis, or for evolving other forms of overt synucleinopathy. Determining the clinical significance of isolated RSWA as a synucleinopathy biomarker could aid efforts to "turn back the clock" for future neuroprotective trials, enabling treatment of milder prodromal synucleinopathies earlier in the disease course before the development of more devastating consequences of overt motor, cognitive, and autonomic impairments.

We aimed to quantify RSWA throughout the adult lifespan and describe normative amounts of RSWA in patients without RBD encountered in routine clinical sleep medicine practice. We also aimed to identify patients with isolated RSWA, characterizing the frequency of patients whose RSWA amounts exceeded previously determined diagnostic thresholds for RBD or who exceeded the cohort 95th percentile of muscle activity thresholds or those for their respective age decile.

Methods

The Mayo Clinic Institutional Review Board provided human subjects research approval for our study.

Subjects

A total of 118 adult patients, seen between 2008 and 2015 for indications other than reported dream enactment behavior, parasomnia, or sleep-related movement disorders such as restless legs syndrome or periodic limb movement disorder at the Mayo Clinic Center for Sleep Medicine, were selected for retrospective RSWA analysis from our polysomnogram (PSG) database. We included adult patients without reported dream enactment symptoms, aged 21 years and older. No patient included in this study had documented endorsement of dream enactment symptoms on a screening intake sleep questionnaire answered by all patients in our sleep medicine practice, which included a negative answer to the question, "Have you ever been told you scream, shout or make unusual movements such as swinging arms about, acting out dreams, etc., during sleep?", as well as an in-person sleep medicine consultation with review of the questionnaire and a complete history and examination. Additionally, we reviewed electronic medical records to ensure exclusion of a documented history of dream enactment symptoms and clinical RBD or parasomnia diagnosis before or within 2 years following polysomnography, and to exclude any patients who received antidepressant medications.

We selected ~10 men and ~10 women for each age decile with an apnea hypopnea index (AHI) <15/h and periodic limb movement index (PLMI) <15/h to include a relatively broad, representative sample that would yield normative levels of RSWA through the adult lifespan for each of 7 age deciles: 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and 80–89 years. All included patients had either a normal PSG or diagnoses of primary snoring or mild sleep-disordered breathing, and the PLM threshold for all subjects was <15/h (except above age 70, in which too few patients were available to fulfill this criteria, and inclusion PLMI was therefore liberalized to <50/h).

Polysomnogram recordings

Video PSG recordings were conducted using a 16-channel Nicolet NicVue digital system with sensitivity at 5–7 μV/mm. Electroencephalogram (EEG) was bandpass filtered from 0.3 to 70 Hz (Cardinal Health Corporation, Madison, WI) and digitized at a sampling rate of 500 Hz. EEG recordings were performed according to the International 10–20 system electrode placements (Fp1, Fp2, Fpz, Fz, Cz, C3, C4, O1, O2, Oz) including electrooculography (left and right outer canthus, LOC and ROC placements), submentalis (SM) and bipolar linked anterior tibialis (AT) electromyography (EMG), and an electrocardiogram. Extensor digitorum communis (EDC) EMG was not routinely recorded but was analyzed where available, since EDC recording was generally utilized only for studies of patients with suspected parasomnia. Respirations were analyzed using an oronasal thermistor and nasal pressure sensor for airflow monitoring, with thoracoabdominal impedance plethysmography to monitor effort. Oxyhemoglobin saturation was evaluated by pulse oximetry. Thirty-second epochs of PSG were used to score sleep in accordance with standard criteria [16]. We used the occurrence of the first REM in the electrooculographic channel to determine the onset of the REM sleep period [11]. The end of the REM sleep period was determined by absence of REMs in three consecutive minutes or upon observance of awakening, K complex, or spindles. SM and AT EMG channels were digitized at a sampling rate of 500 Hz and amplified at 5 μV/mm with low- and high-frequency filters set at 10 and 70 Hz, respectively.

Analysis of REM sleep muscle activity

The reference background EMG amplitude during REM sleep varied from 0.5 to 2.0 μV in all subjects. All visual, manual, and automated quantitative analysis of EMG activity was performed utilizing HypnoLab sleep-scoring software (ATES Medica Labs, Verona, Italy). Overall tonic, phasic, and "any" (either tonic, phasic, or both forms of muscle activity occurring within the same mini-epoch) percent muscle activity were visually and manually scored by previously published methods [6, 13]. Phasic and "any" percent muscle activity were also calculated separately for SM and AT muscles. In addition, the duration of each phasic muscle burst during REM sleep was measured directly, and bursts fulfilling scoring standards [6, 13] were individually recorded for each muscle, resulting in an overall average phasic muscle activity burst duration. We excluded any 3-s mini-epoch containing a breathing-related, snoring-related, or spontaneous arousal from final analysis [6, 13].

Phasic muscle activity was defined as lasting between 0.1 and 14.9 s with an amplitude >4 times the lowest background muscle activity voltage. The end of a phasic burst was defined as return of muscle activity to background for ≥200 ms. We calculated percent of phasic muscle activity by dividing the number of 3-s mini-epochs containing phasic muscle activity by the total number of 3-s mini-epochs during REM sleep. Similarly, the percentage of "any" muscle activity was calculated as the number of 3-s mini-epochs containing either phasic and/or tonic muscle activity, divided by the total number of REM 3-s mini-epochs (i.e. "any" 3-s mini-epochs containing both phasic- and tonic muscle activity was only counted once, to avoid artificially inflated muscle activity percentages) [6].

We used 30-s epochs to score tonic muscle activity in the SM muscle. An epoch was considered positive for tonic activity if >50% of the epoch (i.e. ≥15 s in duration), had muscle activity continuously greater than double the background EMG voltage, or ≥10 μV [6, 7, 9, 11, 13]. Tonic muscle activity percentage was calculated as the total number of positive 30-s epochs divided by the total number of analyzable 30-s REM sleep epochs. "Phasic-on-tonic" muscle activity (i.e. concurrent phasic and tonic muscle activity occurring within the same 3-s mini-epoch) was scored positively in addition to underlying tonic activity only if the overlying phasic burst was greater than twice the background tonic EMG activity within that same 3-s mini-epoch [6, 8].

The automated Ferri REM Atonia Index (RAI) for the SM muscle was also calculated using the automated RAI implemented within HypnoLab sleep-scoring software [5, 19]. Before RAI analysis, 30-s epochs containing a breathing-related artifact, snoring, or arousal were excluded, and the SM signal was notch filtered at 60 Hz and rectified [5, 6]. Last, a further complementary method of RSWA assessment was also analyzed according to American Academy of Sleep Medicine (AASM) visual manual scoring criteria for excessive phasic muscle activity for 30-s epochs, defined as a 30-s epoch containing ≥5 3-s mini-epochs containing phasic muscle activity within that epoch [10]. This definition was used to generate AASM phasic muscle activity percentages for the SM and AT muscles individually and combined.

A total of four scorers of RSWA were blinded to patient group and had high inter-rater reliability with a κ coefficient of 0.889. κ coefficients were calculated according to previously published methods [6, 12, 13].

We defined subjects with isolated RSWA by one of four definitions, including: (1) patients who exceeded the 95th percentile for the overall cohort; (2) patients who exceeded the 95th percentile for their age-sex decile; (3) patients who exceeded the 95th percentile for their age decile; or (4) patients with RSWA levels exceeding our previously defined RSWA cutoffs for RBD in the SM, AT, or both muscles [13].

Statistical analyses

Clinical, demographic, and PSG data are presented as means, standard deviations, and frequencies. For our primary study aims, descriptive statistics with means, standard deviations, and determination of the 10th, 25th, 50th, 75th, 90th, and 95th percentile RSWA levels were calculated for each age-sex decile and the overall cohort. A logarithmic transformation was applied for centile smoothing across ages due to population distribution of SM/AT phasic and SM/AT "any" muscle activity. For comparison of RSWA metrics, patients were analyzed as seven different age deciles. A histogram depicting the duration of each individual scored phasic muscle activity burst across all subjects was constructed. We also used a kernel density plot analysis, which is useful to estimate the function of a curve created by the histogram distribution, to represent the average SM and AT phasic muscle activity burst durations at the individual subject level.

As secondary analyses, we explored subgroup comparisons for RSWA metrics, demographics, clinical characteristics, and PSG metrics similar to a prior study of normative RSWA [17].

Patients were analyzed as four different quartiles of those aged 20–39, 40–59, 60–74, and ≥75 years. Group comparisons were performed using nonparametric tests (Wilcoxon rank sum test in case of two groups, Kruskal–Wallis test in case of more than two groups) using R statistical software. Given the exploratory, pilot nature of our secondary analyses, significance was set at p <0.05 for all comparisons without correction for multiple comparisons, as per previously published methods analyzing normative RSWA data [17]. Relationships between clinical independent variables; dependent tonic, phasic, and "any" muscle activity; and phasic muscle activity burst durations were analyzed utilizing multivariable linear or logistic regression. To normalize residual distributions and respect assumptions for linear and logistic regression analysis, we utilized logarithmic transformations on several variables when indicated.

Results

Clinical and demographic data

Of the 118 patients, all were Caucasian, and 62 (53%) were women with an average age of 54.0 (range 21–88) years. Comparisons across the age quartiles of those aged 20–39, 40–59, 60–74, and ≥75 years are shown in Table 1 (with all age deciles shown in Supplementary Table 1). The most frequent diagnoses for PSG were primary snoring (67%) and mild obstructive sleep apnea (19%), with the other 14% having insomnia or various sleep disturbances without a specific clinical diagnosis. Twenty-four patients (20%) used beta-blockers at the time of PSG. Epworth Sleepiness Scale (ESS) scores were not significantly different across age groups. The AHI was significantly higher (p < 0.001), while REM and total sleep times and sleep efficiency (all p < 0.01) were significantly lower in older than younger age quartiles. None of the subjects had any neurologic diagnoses.

Normative RSWA data in adults

A total of 149,670 total REM mini-epochs were analyzed, with 5,598 removed due to artifact. An average of 1,221 mini-epochs were scored per subject (*SD* = 598) and an average of 47 mini-epochs of rejected artifact were removed per subject (*SD* = 44). We directly measured a total of 13,937 phasic muscle activity bursts across all subjects (8,811 AT, 5,126 SM), averaging 73.8 AT and 42.8 SM phasic bursts per subject (SM [Median, *SD*] = 30, 46.6; AT [Median, *SD*] = 35.5, 165.3). A histogram plot of all phasic muscle burst measurements in aggregate across all subjects is shown in Figure 1A and B, demonstrating that most phasic burst durations were shorter than 1.0 s (AT = 7,696 bursts, 87% SM = 4,293 bursts, 84%). The highest percentile phasic burst durations across all subjects were 95th percentile: SM = 2.24, AT = 2.06 s; 90th percentile: SM = 1.45, AT = 1.22 s; and at the 85th percentile: SM = 1.07, AT = 0.86 s. A kernel density plot, representing each patient's averaged SM and AT phasic muscle activity burst durations, is shown in Figure 1C and D. The highest percentile-averaged phasic burst durations for each subject in the SM and AT muscles were 95th percentile: SM = 1.09, AT = 1.17 s; 90th percentile: SM = 0.97, AT = 1.00 s; and at the 85th percentile: SM = 0.89, AT = 0.88 s (Figure 1C and D).

Normative RSWA metric percentiles, also calculated across ages and sexes, are shown in Figure 2 and Supplementary Figures 1 and 2. The 95th percentiles of all RSWA metrics by age decile, collapsing men and women together, were also calculated and

Table 1. Demographic and polysomnography data for 118 adult subjects without parasomnias

Variable	20–39 years[A]	40–59 years[B]	60–74 years[C]	≥75 years[D]	Group differences
Age (year)	29.0 (4.4)	48.7 (5.5)	67.2 (4.8)	81.6 (3.8)	—
Sex (M/F)	16/16	18/17	13/16	10/12	—
BMI	28.7 (9.3)	30.3 (6.9)	28.7 (7.1)	27.6 (3.0)	—
ESS	9.8 (5.3)	9.1 (5.1)	7.8 (5.2)	9.9 (7.5)	—
β-blocker (*n*)	6	6	6	6	—
AHI	1.3 (1.2)	1.1 (0.7)	4.4 (6.9)	6.9 (6.1)	C > A, B D > A, B
PLMI	3.2 (4.1)	3.8 (6.7)	5.9 (10.1)	7.4 (7.1)	—
PLMI (M/F)	3.1 (3.5)/ 3.2 (4.7)	4.1 (8.7)/ 3.5 (4.3)	9.1 (13.6)/ 3.2 (5.2)	7.9 (8.4)/ 7.1 (6.2)	
TST (min)	389.3 (93.2)	372.7 (70.4)	299.6 (99.4)	222.5 (10.0)	A > C,D B > C,D C > D
REM time (min)	81.9 (32.3)	74.5 (30.6)	54.3 (32.6)	40.8 (3.2)	A > C, D B > C, D
SE	84.7 (13.1)	79.6 (16.5)	72.4 (13.4)	67.2 (1.8)	A > C, D B > D
ISL	19.5 (17.9)	17.2 (21.5)	20.1 (16.9)	23.6 (2.0)	—
IRL	100.1 (58.9)	93.7 (54.6)	104.9 (84.0)	131.23 (1.1)	—
N1 (%)	8.9 (4.5)	9.5 (7.3)	10.4 (8.8)	9.9 (5.7)	—
N2 (%)	52.0 (6.6)	56.1 (22.6)	47.1 (12.2)	49.5 (1.1)	—
N3 (%)	18.48 (7.9)	19.9 (8.9)	24.4 (13.4)	24.1 (1.4)	—
REM (%)	20.6 (4.9)	19.2 (6.1)	18.1 (7.2)	16.5 (7.6)	—
Arousal Index	16.8 (7.7)	16.8 (7.0)	16.6 (9.3)	20.1 (8.2)	—

Means (standard deviations) are shown for each major clinical and polysomnographic finding, with statistical group comparisons between age quartiles. Group differences indicate p <0.05.

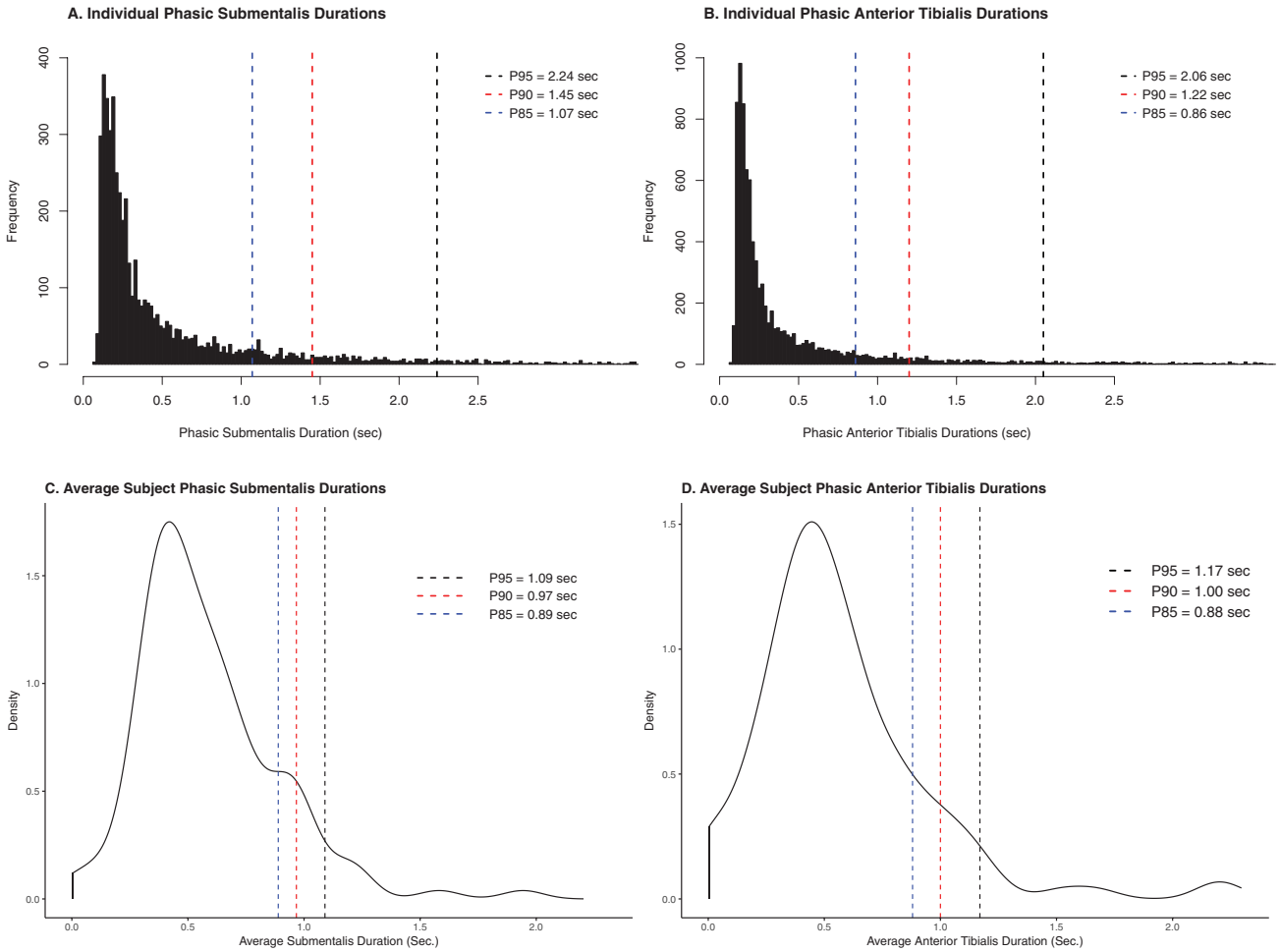

Figure 1. Frequency histograms of phasic RSWA durations. Panels A and B depict the frequency of each individual phasic burst duration measured across all subjects within the entire cohort for both the submentalis (A) and the anterior tibialis (B). Panels C and D depict a kernel density plot of each subject's average phasic burst duration for both the submentalis (C) and the anterior tibialis (D). The blue dashed line represents the 85th percentile, the red dashed line the 90th percentile, and the black line as the 95th percentile.

are given in Table 2. The 95th percentile RSWA muscle activity percentages for the entire cohort were SM phasic 8.6%, SM "any" 9.1%, SM tonic 0.99%, AT phasic 17.0%, AT "any" 17.0%, combined SM/AT 22.3%, combined SM/AT "any" 25.5%, and RAI 0.85.

Older subjects (≥65) had significantly higher AT phasic, AT "any," SM/AT phasic and SM/AT "any" densities than younger subjects (<65 years, all $p < 0.03$; Figure 3; Supplementary Figure 3). Older men had significantly higher AT phasic and AT "any" than older women, and also than all younger subjects (all $p < 0.04$). Detailed comparisons between age deciles are shown in the Supplementary Table 2 and Supplementary Figure 4. In a multivariate linear model, men had significantly higher AT phasic and AT "any" after controlling for age and PLMI (both $p < 0.004$). SM duration and SM phasic density were negatively associated with age after controlling for sex (both $p < 0.01$, Supplementary Table 3).

Frequency of isolated RSWA

Overall, 68 (58%) unique patients (mean age = 53.4, 35 men, 33 women) met one or more of our definitions for isolated RSWA, including 29 (25%) patients who exceeded the 95th percentile for the overall cohort, 34 (29%) patients who exceeded the 95th percentile for their age-sex decile, 38 (32%) who exceeded the 95th percentile for their age decile, and 17 (14%) patients that exceeded previously determined RBD diagnostic RSWA thresholds [13]. Some patients overlapped between these categories. Mean ages (sexes) of patients in these categories were 57.1 years (18 men, 11 women) who were above cohort 95th percentile RSWA levels (Table 2), 57.8 years (18 men, 16 women) who exceeded their respective age-sex decile 95th percentile RSWA levels (Table 3), and 56.7 years (22 men, 16 women) who exceeded the 95th percentile for their age decile for one or more RSWA metrics (Table 2).

Seventeen (14%) unique patients (mean age = 57.7; 12 men, 5 women) exceeded our previously established RSWA diagnostic cutoffs for RBD for the 3-s mini-epoch phasic muscle activity scoring, 30-s tonic muscle activity scoring, or automated RAI, with five patients meeting multiple cutoffs [13]. Details concerning these patients are presented in the Supplementary Data section.

Using the AASM scoring method for 30-s epochs, 20 (17%) patients (mean age = 48.7; 7 men, 13 women) exceeded SM phasic, and 24 (20%) exceeded SM phasic or tonic RSWA

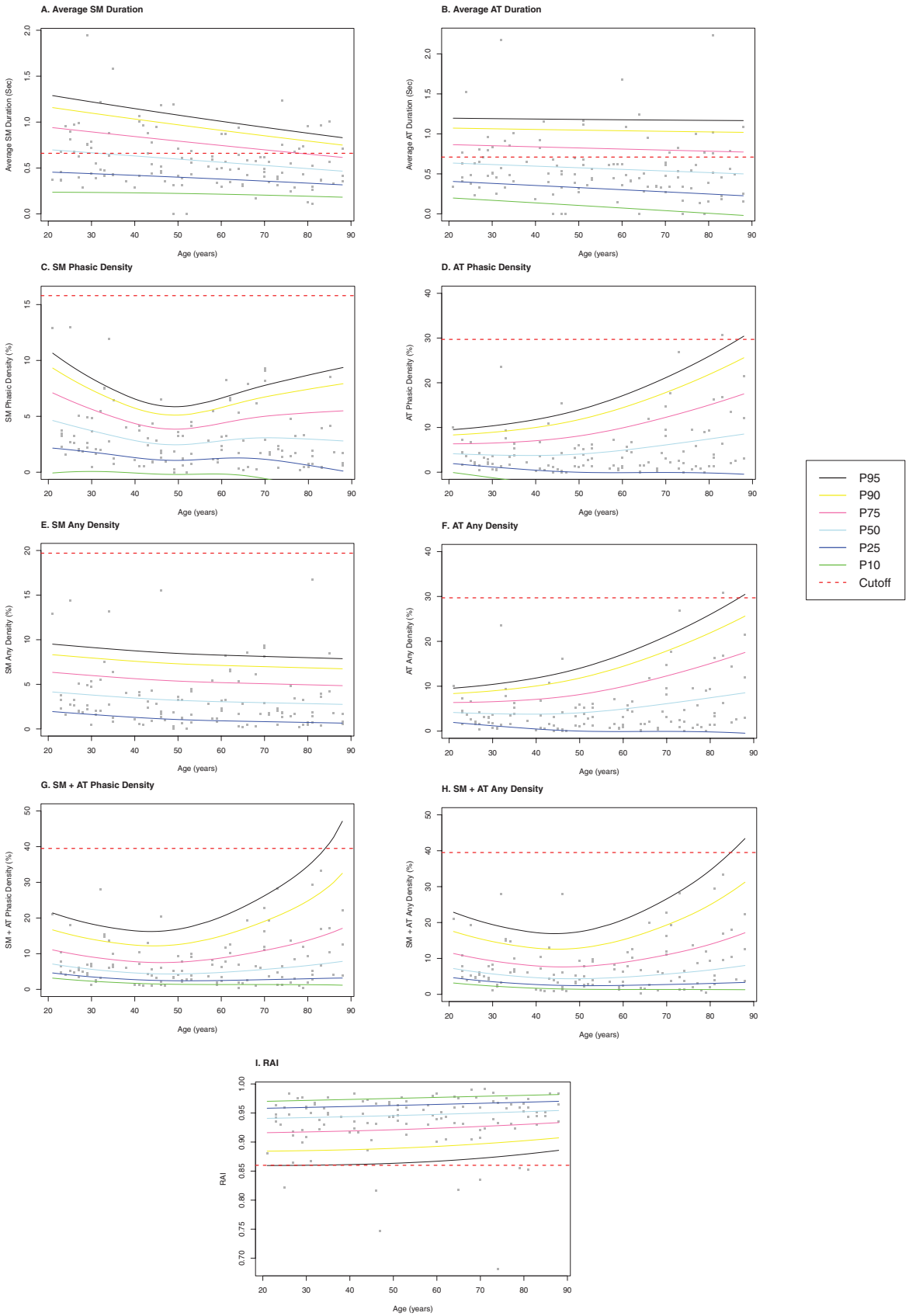

Figure 2. Normative RSWA metrics and percentiles for adults aged 20–88 years. Shown are the percentile curves across the age deciles for (A) SM and (B) AT durations; (C) SM phasic, (D) AT phasic, (E) SM "any," (F) AT "any," (G) SM/AT phasic, and (H) SM/AT "any" muscle activities, and (I) the automated Ferri REM atonia index (RAI). The black, yellow, pink, light blue, dark blue, and green lines indicate the 95th, 90th, 75th, 50th, 25th, and 10th percentiles, respectively. The dashed red line indicates the McCarter et al. [13] RBD RSWA cutoff value for reference. Chin/leg phasic and "any" were logarithmically transformed to accurately calculate the percentile curves [24].

Table 2. Normative RSWA 95th percentile values for 118 adults without parasomnias

Variable	Total (n = 118)	20s (n = 17)	30s (n = 15)	40s (n = 19)	50s (n = 16)	60s (n = 17)	70s (n = 19)	80s (n = 15)
Average SM duration (s)	1.1	1.2	1.3	1.2	0.7	0.9	0.8	1.0
Average AT duration (s)	1.2	1.1	1.4	1.0	1.1	1.3	0.8	1.4
SM phasic density (%)	8.6	12.9	8.9	4.6	4.7	8.0	9.1	11.0
AT phasic density (%)	17.0	7.8	13.6	11.3	6.4	21.9	18.6	24.2
SM "any" density (%)	9.1	13.2	9.2	5.5	4.7	8.3	9.1	11.0
AT "any" density (%)	17.0	7.8	13.5	11.4	6.4	21.9	18.6	24.2
SM + AT phasic density (%)	22.3	18.7	19.2	13.7	9.5	28.0	23.3	30.6
SM + AT any density (%)	25.5	19.6	19.2	14.4	9.5	28.5	23.3	30.6
Tonic (%)	1.0	1.3	1.8	1.2	0	0.2	0.1	1.2
RAI	0.849	0.856	0.896	0.809	0.925	0.884	0.820	0.907

95th percentile for each age decile is shown, with number of subjects per age decile indicated in each column.

diagnostic cutoffs for RBD (mean age = 51.8; 10 men, 14 women) [13]. Details concerning these patients are presented in the Supplementary Data section. Patients who exceeded our phasic burst duration diagnostic cutoffs were more numerous than patients who met phasic or tonic diagnostic cutoffs based on 3-s mini-epoch scoring (Supplementary Table 4, details in the Supplementary Data section). There were no associations between RSWA elevations exceeding the 95th percentile levels or RBD diagnostic thresholds with sex or age >65 ($p > 0.05$).

Discussion

We established normative ranges of RSWA throughout the adult lifespan for patients without parasomnias seen in clinical sleep medicine practice, and confirmed that there are significantly greater amounts of RSWA in older adults (particularly older men) than in younger adults without dream enactment behavior. These data may further aid diagnosis in rare RBD patients whose RSWA levels do not fulfill previously established diagnostic thresholds, but who have dream enactment behaviors highly suspicious for RBD [8]. Further, these normative RSWA data demonstrate that isolated RSWA elevations in the absence of dream enactment symptoms are not uncommon, and may guide discussions concerning incidental findings of RSWA during clinical polysomnography practice.

We defined isolated RSWA as RSWA levels exceeding previously established RBD diagnostic cutoffs [13],the 95th percentile for the overall cohort, the 95th percentile for each patient's respective age decile, or the 95th percentile for each patient's respective age-sex decile. We found evidence for isolated RSWA by these admittedly *prima facie* definitions in 14%–32% of patients overall (those exceeding previously established RBD diagnostic cutoffs, overall cohort 95th percentile, age decile 95th percentile, and age-sex 95th percentile RSWA values). The three 95th percentile definitions we propose identified subjects with isolated RSWA in our cohort at a similar frequency to that reported in the previous normative RSWA study in the Innsbruck community (i.e. 25%–32% of our subjects fulfilled our definitions of isolated RSWA, compared with 25% of subjects who met the SINBAR submentalis phasic muscle activity diagnostic cutoff) [17]. Recently, isolated RSWA patients were shown to have higher reports of sleep-related motor and behavioral symptoms on REM sleep behavior disorder screening instruments, and patients with Parkinson's disease have been shown to manifest REM sleep motor behaviors during polysomnography without yet reporting clinically overt dream enactment behaviors, suggesting that a spectrum of sleep-related motor behaviors may occur in association with RSWA in the absence of clinically overt RBD [25, 26]. There may be a spectrum between isolated RSWA, and evolving REM sleep motor behaviors that represent a *forme fruste* of prodromal RBD [3]. Further prospective cohort research studies will be necessary to characterize any concurrent or future clinically significant associated sleep behaviors or neurologic prognostic consequences in patients with isolated RSWA, and to determine if this interesting group is also at risk for underlying synucleinopathy similar to patients with clinically overt RBD.

We also again noted significant differences in RSWA across ages and sexes similar to our previous findings in adults without RBD, confirming that elderly men have higher amounts of AT phasic muscle activity during REM sleep [20], in contrast to findings of previous cohorts that focused on the mentalis and flexor digitorum superificialis muscles and did not analyze the anterior tibialis muscle for RSWA [17, 18]. While RBD primarily affects older men, the reason for the RSWA difference between the sexes is unknown. AT RSWA was associated with PLMI, although there were no significant PLMI differences between men or women in any age strata and no difference in the association between PLMI and male or female sex in regression modeling. While PLMI is associated with higher amounts of RSWA, PLMs do not appear to be the primary driver for higher levels of RSWA in older adults, especially in older men. One possible explanation is that REM sleep muscle atonia control has different specific somatotopic control regions within the ponto-medullary region that govern atonia in the upper- and lower body segments, and that the degree of regional REM sleep atonia control varies between the sexes. Further preclinical animal research and additional prospective human studies are needed to determine differential somatotopic control mechanisms for REM sleep muscle atonia, and whether such mechanisms explain differences in RSWA between older men and women.

Our data also revealed some interesting and somewhat surprising trends in RSWA in younger patients, including relatively higher amounts of tonic muscle activity in those aged 20–40 years, comparable to and even exceeding those seen in the over 80-year-old subgroup. A similar "bell or U-shaped" bimodal distribution pattern of higher RSWA in both younger and older adults, with lower amounts of RSWA in middle adult life was noted previously by Ferri using the automated RAI [18]. The possibility of a bimodally maximal distribution for RSWA was also suggested in our data by findings of decreased RAI (consistent with lower atonia, and higher RSWA) in those in the

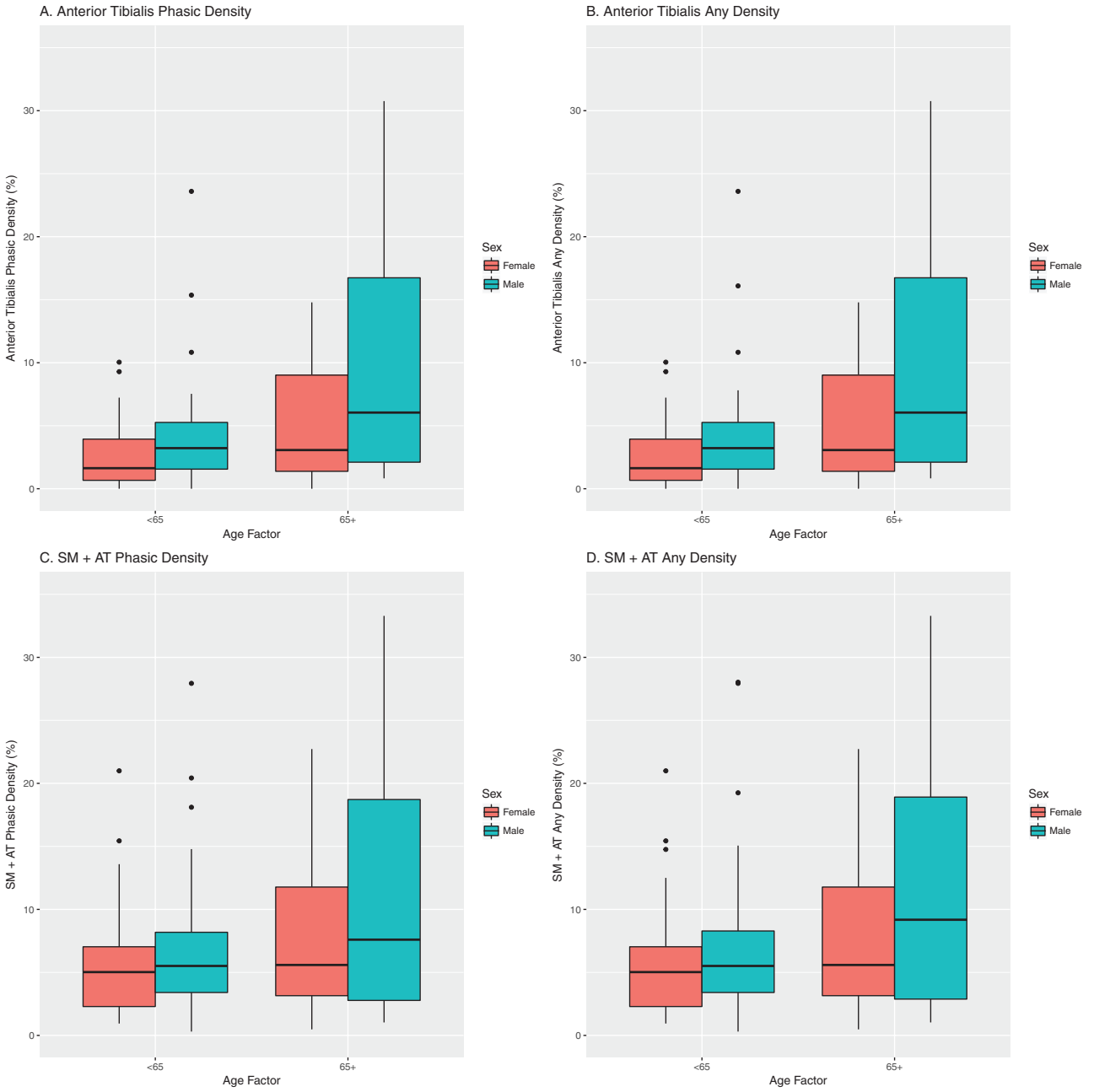

Figure 3. RSWA comparison for men and women who are older or younger than 65 years of age. Shown above are the RSWA levels for submentalis (SM) and anterior tibialis (AT) muscles, for the: (A) AT phasic, (B), AT "any," (C) SM/AT phasic, and (D) SM/AT "any" muscle activities. RSWA was significantly higher in older men in the AT but not the SM, with AT also driving the significant difference for the SM/AT combination metric for men.

70-year-old age decile, and older patients had more variation in RSWA levels than younger patients, suggesting that age-related differences in RSWA are driven by a subset of elderly who have altered REM atonia control. Further clarification of RSWA levels through the lifespan by additional large-scale normative RSWA cohort studies, and determination of possible mechanisms for altered age-related REM atonia control (i.e. altered neurotransmission or neurodegeneration in atonia control networks) is needed.

Interestingly, the 95th percentile for average phasic durations for the entire cohort of both SM and AT phasic activity were each ~1 s (Figure 1C and D), which is longer than our previously published RBD diagnostic cutoff of 0.66 s for the SM and 0.71 s for the AT muscles [6, 16]. Additionally, the 95th percentile for the total individually measured SM and AT phasic bursts (Figure 1 A and B) were >2.0 s, demonstrating that some patients without RBD have significantly longer phasic burst durations than previously recognized. This difference is likely explained by a larger, more heterogeneous sample of patients without RBD throughout the adult lifespan in the current study, including patients with more variable, longer duration phasic RSWA bursts than in our previous case–control studies. These findings imply that future studies using a minimum phasic duration of ≥1.0 s for RSWA scoring, rather than 100 msec, may be more specific in

Table 3. Number (percentage) of patients with isolated RSWA who exceeded 95th percentile by age-sex deciles

Variable	20s	30s	40s	50s	60s	70s	80s
Average SM duration (s)	1 (6%)	1 (7%)	2 (11%)	0	2 (12%)	1 (5%)	1 (7%)
Average AT duration (s)	1 (6%)	1 (7%)	1 (6%)	0	2 (12%)	0	2 (13%)
SM phasic density (%)	2 (12%)	2 (13%)	1 (6%)	0	3 (18%)	3 (16%)	1 (7%)
AT phasic density (%)	0	2 (13%)	0	0	2 (12%)	1 (5%)	0
SM "any" density (%)	2 (12%)	1 (7%)	1 (6%)	0	1 (6%)	3 (16%)	2 (13%)
AT "any" density (%)	1 (6%)	2 (13%)	0	0	2 (12%)	1 (5%)	0
SM + AT phasic density (%)	1 (6%)	1 (7%)	1 (6%)	0	1 (6%)	1 (5%)	0
SM + AT any density (%)	2 (12%)	1 (7%)	1 (6%)	0	2 (12%)	1 (5%)	0
Tonic (%)	1 (6%)	1 (7%)	1 (6%)	0	1 (6%)	1 (5%)	1 (7%)
RAI	2 (12%)	0	1 (6%)	0	1 (6%)	3 (16%)	1 (7%)
Total unique subjects exceeding 95th percentile	4 (24%)	5 (33%)	4 (22%)	0	8 (47%)	7 (37%)	6 (40%)

The number of patients (percentage) in each decile is shown.

distinguishing between RBD and normal values without RBD or RSWA elevation. Longer phasic burst duration (≥1.0 s) may also represent a frontier for identifying abnormal REM sleep atonia control, signifying the presence of isolated RSWA and/or a state that Hogl, Stefani, and Videnovic have recently described as prodromal RBD [3].

Prodromal RBD is an evolving concept without a well-established consensus definition. It may involve notable visual and/or quantitative abnormalities in REM sleep atonia with variable clinical features of video-polysomnography–recorded REM sleep behavioral or motor events not fulfilling diagnostic RBD criteria of clear-cut complex vocal or motor behaviors thought to represent actual dream enactment behaviors [3, 26, 27]. Determining whether isolated RSWA ≥1.0 s in duration is a plausible biomarker for "prodromal RBD" will require prospective cohort studies analyzing development of clinical dream enactment and measurement of other degenerative markers for synucleinopathy, such as hyposmia, constipation, and covert cognitive, autonomic, and motor impairments.

The 95th percentile RSWA level of ≥1.0 s identified in this study also provides new direct evidence for the upper limit biological duration of measured RSWA for use in future studies of isolated RSWA in patients without dream enactment, which must also be considered in light of patient's medical histories, comorbidities, and medications. Given that REM atonia loss on the automated Ferri REM Atonia Index below a threshold of 0.8 was recently shown to be associated with clinical symptoms of probable RBD, further analyses of automated isolated RSWA thresholds is also a promising future direction for prospective cohort studies [25].

For the current study, we adapted a practical, purposely broad, inclusive exploratory definition for isolated RSWA as quantitative RSWA levels that either exceeded the 95th percentile for the cohort or subjects' sex and age decile, or that either met or exceeded our previously determined idiopathic/isolated RBD diagnostic cutoffs [13]. The theoretical proposed construct of isolated RSWA will require additional validation in future large-scale prospective cohort studies to determine whether isolated RSWA has potential clinical significance for evolution of idiopathic/isolated RBD or other forms of overt synucleinopathy. For purposes of illustration, Figure 4 demonstrates actual submentalis and anterior tibialis phasic muscle activity percentages from the current study in adults without RBD, with superimposition of our overall cohort 95th percentile threshold and our previously established idiopathic/isolated RBD diagnostic RSWA threshold. Patients should be followed clinically and analyzed prospectively if they exceed our defined normative 95th percentile RSWA levels, or if they exceed iRBD diagnostic cutoffs on well-defined standard polysomnogram EMG channels such as submentalis, flexor digitorum superficialis, other arm muscles, or anterior tibialis. Prospective cohort studies of patients with varying levels of isolated RSWA could help define and determine the natural history of this patient group with excessive RSWA, and develop quantitative bounds for discriminating subclinical isolated RSWA without clinical accompaniments, from "prodromal RBD" (i.e., having only subtle vocal or motor behavioral events recording during polysomnography without more elaborate recorded complex motor behaviors, and lacking a clinical history of dream enactment behavior), from isolated/idiopathic RBD.

Several potential explanations for the significantly higher AT phasic activity in the age 80–89 decile can be entertained. These findings could relate to concurrent lumbosacral or peripheral neuropathic pathologies leading to nonspecific but quantifiable motor activity during REM sleep (e.g. fragmentary myoclonus, fasciculation potentials, or possibly the impact of comorbidities or comedications). However, and more provocatively, the presence of covert incidental Lewy body disease (iLBD) is also possible. Previously published autopsy series found iLBD to be common among the elderly in the general population [24,28,29]. iLBD refers to Lewy bodies and Lewy neurites present at autopsy in the nervous systems of older individuals with no ante mortem features of cognitive impairment, Parkinsonism, or autonomic dysfunction [30–32]. Evidence suggests an iLBD frequency of approximately 20%–25% at autopsy, which, interestingly, closely parallels the 25% frequency of isolated RSWA in both our current study and the previous Innsbruck study [28, 29]. Like isolated RSWA and clinical RBD, iLBD is more common in men than women [31]. Interestingly, previous pathologic evidence suggests that the frequency of isolated incidental Lewy body pathology at autopsy in the pons, including evidence for direct involvement of the locus ceruleus/subceruleus region [24] (which is the main locus of REM sleep atonia control), is also found in about 20%–25% of cases, highly similar to the frequency of isolated RSWA in our patients (14%–32% of the cohort) and the previously published Innsbruck (25%) normative RSWA study [12]. Indeed, in one large neuropathological series, most patients with iLBD had Stage IIa (brainstem predominant) disease, and 50% had alpha-synucleinopathy pathology in the pons with a density score paralleling those found in Parkinson disease or dementia with Lewy bodies [29]. One of the extremely rare autopsied cases of

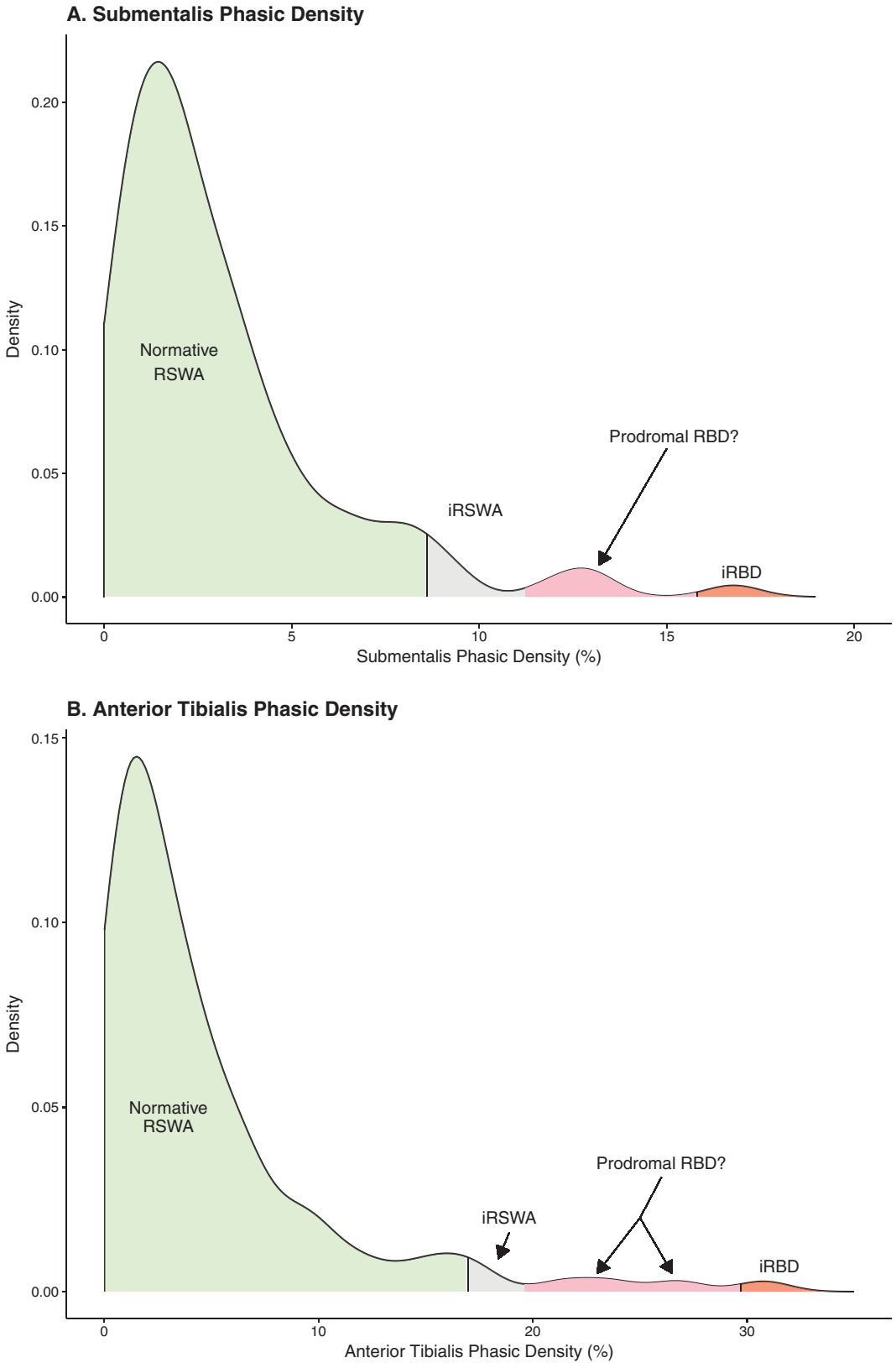

Figure 4. Kernel density plot of actual submentalis phasic muscle activity percentages, with superimposed shading indicating theoretical proposed bounds of isolated RSWA and idiopathic/isolated RBD. A kernel density plot shows (A) submentalis (SM) phasic muscle activity density of patients from this study with the green-shaded area indicating patients with normal RSWA levels, the gray-shaded area representing patients having sufficient RSWA to exceed the 95th percentile level (i.e. meeting a threshold for proposed "isolated RSWA"), transitioning to a theoretical pink-shaded area indicating elevated levels of RSWA associated with overt vocal or motor REM behavioral events captured during polysomnography, merging with the red shaded area which indicates sufficiently elevated levels of RSWA to meet previously established diagnostic cutoff levels for isolated/idiopathic RBD, together with clinically manifest dream enactment behavior [13]. (B) Similar illustration of theoretical bounds of normative, isolated RSWA, prodromal RBD, and idiopathic/isolated RBD for the anterior tibialis (AT) muscle.

idiopathic/isolated RBD also found evidence for brainstem predominant LBD, although another more recent report demonstrates that more widespread peripheral autonomic and central nervous system pathology can be present concomitant with clinically isolated/incidental RBD [33, 34]. Ante mortem studies of patients with idiopathic/isolated RBD using neuromelanin sensitive MRI of the pontine locus ceruleus/subceruleus region also found an association between neuromelanin signal loss in the dorsolateral pontine region and higher RSWA levels, implying a direct correlation between dysfunction in this region (presumably mediated by alpha-synuclein pathology) and levels of RSWA [35]. However, future clinicopathological studies are needed to determine if RSWA above normative thresholds during life is associated with the pathological substrate of Lewy body pathology in the subceruleus region of the brainstem at autopsy. Future longitudinal prospective cohort studies of older adults will be necessary to determine whether isolated RSWA could possibly be related to covert Lewy body pathology.

This study has several limitations. Due to its retrospective nature, we were unable to control for confounding biases such as sleep complaints or medical comorbidities. We excluded patients with known neurologic diseases, parasomnia, or dream enactment symptoms as well as those receiving centrally active medications such as antidepressants known to impact REM sleep muscle atonia. Additionally, due to the exploratory nature of the secondary aims of this study, statistical adjustments were not made for multiple comparisons, consistent with previously published methods analyzing normative RSWA [17]. If a more conservative correction for multiple analyses for the two muscles analyzed had been applied using Bonferroni's method, the alpha level would have been 0.025 rather than 0.05, and the difference in RSWA between patients older and younger than age 65 years would no longer have been significant following correction. However, multiple regression adjusting for covariates found a highly significant association between age and RSWA, a superior method for demonstrating this association since linear regression uses the whole data to analyze this relationship. Using the smallest and largest effect sizes and variability for group differences in various RSWA metrics between younger and older patients found in our study, we determined that sample sizes ranging from 94 to 140 patients would be necessary to prove these group RSWA differences for the two muscles analyzed at a more stringent alpha level of 0.025. Additional future large-scale prospective studies should recruit healthy adult men and women without sleep complaints or medication use to determine whether age and sex cause differences in REM sleep muscle activity, to establish normative quantitative RSWA metrics throughout the lifespan, and to identify a subset of patients with defined isolated RSWA to be recruited to longitudinal cohort outcome studies for development of clinical dream enactment or other symptoms or signs of covert underlying synucleinopathy. Additionally, utilization of time intensive, expert visual/manual approaches to RSWA quantification are not pragmatic or feasible in most busy clinical sleep medicine practices, so additional future studies validating automated RSWA quantification approaches such as RAI, automated SINBAR [36], the automated Mayo method (as developed by Jeppson *et al.*, an automated implementation of our method as used herein), and others [14, 36–42] are needed to encourage further widespread adaption and utilization of RSWA quantification applications in daily clinical practice for the accurate and timely identification of isolated RSWA. Such studies correlating "gold standard" visual/manual approaches with automated methods are also needed to further delineate and understand differences in estimated RSWA amounts. While in general the RSWA estimates provided by automated atonia estimates and visual/manual RSWA methods parallel one another, these can yield different results even for the same patient. Possible reasons for different results between automated and visual/manual methods include their differing measurement methodologies, as well as differential artifact rejection approaches necessitated by each method. Human error in over/under scoring of visual/manual RSWA estimates is certainly possible, and the automated Ferri RAI and other automated methods may be detecting very small phasic muscle activity bursts that do not meet visual scoring criteria. Variable artifact rejection requirements between the two techniques could also serve to explain different findings, since for visual scoring, we and others delete respiratory and arousal related muscle artifacts on a miniepoch (3 s) basis, whereas for the Ferri RAI, one must exclude an entire 30 s epoch to avoid including erroneous muscle activity in the RAI calculation, leading to differential artifact rejection which could alter the RSWA estimates between the two methods.

Last, we submit that these data may be useful in clinical sleep neurology practice. Which factors might explain the discrepancy between the lower 95th percentile RSWA upper bound thresholds determined in this study, and relatively higher, diagnostic thresholds for RBD diagnosis [6, 7, 13]? Moreover, which values should be used when seeing a patient with clinically suspected isolated/idiopathic RBD? We propose that both normative value and diagnostic cutoff approaches have merit in different clinical scenarios. Well-established RSWA diagnostic thresholds for RBD are clearly preferred for most cases encountered in sleep clinics. However, normative RSWA data are useful adjuncts for determining excessive isolated RSWA in patients without dream enactment, and also toward diagnosing difficult RBD cases with a high clinical index of suspicion (e.g. recorded dream enactment behaviors or highly suspect clinical history) who do not yet fulfill previously determined RSWA cutoffs. RSWA diagnostic thresholds were derived from studies typically involving clear-cut clinical cases of RBD with a relatively long duration of dream enactment symptoms (varying from 5.7 to 10 years) [6, 9, 12, 13], and RSWA amounts have shown progressive increases over time in available longitudinal studies [22, 43]. As such, RBD patients with milder clinical phenotypes and/or earlier disease onset may not have yet developed sufficient RSWA to fulfill established RSWA diagnostic thresholds established by case control studies that included well established and more advanced RBD cases versus controls. Normative 95th percentile RSWA thresholds provide an alternative objective reference for abnormal REM sleep atonia control to support diagnosis in RBD patients, who may also merit close longitudinal follow-up with repeat PSG if the diagnosis remains unclear.

In summary, REM sleep muscle activity appears to be higher in older men, specifically in the AT muscle. While periodic limb movements correlate with higher AT muscle activity, they do not seem to be driving sex differences. These findings could suggest either a biological predisposition to altered REM sleep muscle atonia control in men or possibly, and even more provocatively, the presence of an underlying neurodegenerative disorder such as covert incidental/isolated Lewy body pathology. We also established normative RSWA values for men and women across

the adult lifespan, which potentially aid findings of incidental RSWA in clinical sleep practice. In practice, isolated RSWA should be recognized in patients exhibiting phasic muscle activity densities or durations exceeding the 95th percentile for their sex and age. The distribution percentiles for average phasic muscle activity burst durations suggest that >1.0-s phasic bursts of RSWA are rare. This finding suggests a new, potentially more convenient threshold for phasic burst duration measurement that could aid design of a more rapid, evidence-based RBD RSWA diagnostic threshold determination. Further prospective basic and clinical research of quantitative REM sleep muscle activity and the brainstem centers involved in REM atonia control are necessary to confirm these findings and to elucidate mechanisms for differences in RSWA due to age and sex.

Supplementary material

Supplementary data are available at *SLEEP* online.

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