Sleep Quality, Psychologic Profiles, Cardiac Activity, and Salivary Biomarkers in Young Subjects with Different Degrees of Rhythmic Masticatory Muscle Activity: A Polysomnography Study

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Aims: To investigate the objective and subjective characteristics of sleep and psychosomatic and physiologic variables in young subjects with different frequencies of rhythmic masticatory muscle activity (RMMA) during sleep. **Methods:** A total of 54 young (mean age 23.8 ± 2.1 years), healthy subjects underwent polysomnographic (PSG) recordings for 2 nights. Sleep and psychosomatic states were assessed prior to PSG using validated questionnaires, and the following PSG variables were assessed before and after sleep: subjective sleep quality, physical symptoms, anxiety level, and salivary biomarkers. Second-night sleep and oromotor variables were scored according to standard criteria as well as the quantitative autonomic activity during the night. These variables were compared among the high- (H-RMMA, n = 21, mean RMMA index: 5.7 times/hour) and low- (L-RMMA, n = 13, 2.6 times/hour) frequency RMMA and control (CTL, n = 20 subjects, 1.0 time/hour) groups. **Results:** Sleep and psychosomatic states did not differ among the three groups. No group differences were noted for nonrhythmic oromotor events. Sleep architecture did not differ among the three groups except for sleep latency being shorter (P = .008) and microarousal index being higher (P = .013) in the H-RMMA group. Mean heart rate during sleep was lower (Stage N2, P = .008; Stage N3, P = .036; Stage R, P = .045) in the H-RMMA group, but the heart rate variability did not differ among the three groups. Sleep quality and anxiety level before and after sleep did not differ among the three groups. Cortisol did not differ among the three groups, while chromogranin A in the morning was slightly lower in the L-RMMA group (median: 9.1 pmol/mg) than in the H-RMMA group (12.3 pmol/mg) (P = .049). **Conclusion:** In otherwise healthy subjects presenting normal physiologic variables, neither significant nor consistent differences in sleep architecture, psychologic states, heart rate variability, or salivary biomarkers in relation to the frequency of RMMA were found. *J Oral Facial Pain Headache 2019;33:105–113. doi: 10.11607/ofph.2231*

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Sleep bruxism (SB) is classified into sleep-related movement disorders by the International Classification of Sleep Disorders, version 3.[1] SB is defined as nonfunctional masticatory muscle activity characterized by repeated grinding during sleep with or without a grinding noise.[1–5] In clinical dentistry, SB is recognized as a risk factor for tooth attrition, tooth fracture, temporomandibular disorders (TMD), orofacial pain, occlusal trauma, and prosthetic treatment complications.[2,3]

Physiologically, SB is characterized by the frequent occurrence of rhythmic masticatory muscle activity (RMMA) with or without tooth grinding noise during sleep.[2] To make a diagnosis of SB, clinicians must assess self-reports of tooth grinding and facial pain/headache along with objective evidence of tooth wear via oral examination and the presence of RMMA on polysomnographic (PSG) recordings. PSG studies have demonstrated that RMMA is associated with transient arousal phenomenon (ie, microarousal and sleep stage shifts).[6,7] Although transient RMMA events may be present in normal subjects without complaints (ie, with a low frequency of RMMA episodes per hour of sleep), the presence of these events does not disrupt sleep

when they occur. Previous studies have shown that sleep macro- and microstructures are usually normal in young and otherwise healthy SB patients (ie, a high frequency of RMMA episodes per hour of sleep).[8,9] Descriptive and analytic PSG studies proposed that transient sleep arousals seem to facilitate the onset or genesis of RMMA during the sleep of young subjects; onset of RMMA was associated with transient arousal in most young and healthy SB patients.[6,10]

It is believed that stress/anxiety is one of the causal or exacerbating factors for SB. SB can be considered a behavior but is recognized as a sleep-related motor disorder over a certain threshold (presence of signs and symptoms and increased occurrence of RMMA).[11,12] In case-control and epidemiologic studies in which SB is assessed by self-report, stress and/or anxiety are significantly associated with SB.[13,14] In other studies using ambulatory electromyographic (EMG) recordings, however, the association between SB and stress/anxiety is more controversial.[15] In sleep medicine, psychologic conditions such as stress and anxiety are known to influence objective and subjective sleep quality. Stress and anxiety may be associated with hyperarousal, a condition frequently found in patients with insomnia that is characterized by difficulty falling asleep and maintaining sleep continuity or by misinterpretation of sleep quality.[16,17] Insomnia and poor sleep maintenance are a concomitant complaint in the general SB population.[18,19] In addition, the association between psychologic conditions and SB has often been investigated using salivary biomarkers; ie, salivary cortisol[13] and chromogranin A.[20–22] However, controversy remains as to the specificity of the findings for awake-time stress and sleep recordings. Furthermore, these findings were derived from data using single-channel EMG ambulatory recordings without assessment of sleep macro- and microstructures. Collectively, the roles of psychologic factors and stress hormones (salivary cortisol and chromogranin A) in the probability of RMMA onset/frequency per hour of sleep have not been systematically investigated. Therefore, the belief in the role of wake-time anxiety and/or stress in the pathophysiology of SB is a topic of debate.[15,23,24]

Currently, there are few studies that investigate whether inter-individual differences in the occurrence of RMMA may be explained by subjective and objective measures related to sleep and psychologic factors. Therefore, this study investigated subjective and objective variables for sleep and psychosomatic profiles in young adult subjects with different levels and frequencies of RMMA as determined by the full PSG protocol. The hypothesis was that SB would be associated with changes in sleep architecture, cardiac activity related to stress and anxiety levels assessed by self-report, and salivary measures of cortisol and chromogranin A.

Materials and Methods

Subjects

A total of 72 participants (mean age: 24.4 ± 2.6 years, 32 women and 40 men, mean BMI: 20.7 ± 1.7 kg/m^2) including university students, staff, and their acquaintances were enrolled in the study. This study was approved by the ethics committee of the Osaka University Dental Hospital and the Graduate School of Dentistry (H25-E9-5), and all participants read and signed a written consent form according to the Helsinki Declaration and understood that they were free to withdraw from the experiment at any time.

Questionnaire and Clinical Examination

Prior to the sleep evaluation with PSG, all subjects completed the self-administered questionnaires, reporting sleep quality using the Japanese version of the Pittsburgh Sleep Quality Index (PSQI),[25] gastroesophageal symptoms using the F scale,[26] and a broad range of psychologic problems using the 9-item subscale of the Symptom CheckList-90 Revised (SCL-90-R).[27]

On the day of the PSG recording, orodental examinations and an interview were conducted to assess the presence and level of tooth wear, jaw muscle hypertrophy, morning orofacial symptoms (pain or fatigue of the jaw), and the self-awareness of SB.[28] Anxiety levels during the evening before and during the morning after the PSG recording were assessed using the Japanese version of the State-Trait Anxiety Inventory (STAI).[29] The morning after waking, subjects completed a self-administered questionnaire to assess sleep quality, jaw symptoms related to discomfort, and oral dryness. Sleep quality in comparison to usual sleep was assessed using a 5-grade Likert scale (1 to 5), with 1 representing poor quality and 5 representing good quality. Jaw symptoms and oral dryness were assessed using a 100-mm visual analog scale (VAS) with anchors of not at all (0) to intolerable symptom (100).

PSG Recordings

Video-PSG recordings were carried out for 2 consecutive nights in the sleep research laboratory at Osaka University Graduate School of Dentistry. Subjects went to bed between 10:30 and 11:00 pm after electrodes setting and woke up between 6:30 and 7:00 am. The first night recording was conducted for habituation, and the data from the second night were analyzed.

PSG included the following biosignals: electroencephalograms (EEGs; C4–M1, C3–M2, O2–M1, O1–M2, F4–M1, and F3–M2); electrooculograms (EOGs); electrocardiograms (ECGs); EMGs of the chin/suprahyoid, bilateral masticatory muscles (masseter, temporalis), and bilateral anterior tibialis

muscles; snoring sounds; oronasal thermal airflow; nasal pressure; chest and abdominal movements; arterial oxygen saturation; body position; and laryngeal movements. All signals were recorded using acquisition and analysis software (Embla N7000, REMbrandtTM PSG software, Natus Medical).

Saliva Sampling

Unstimulated saliva was collected using Salivette (SARSTEDT) in the evening before sleep recording and in the morning on awakening. Saliva samples were immediately stored at −20°C. Samples were assayed for salivary cortisol using the enzyme immune assay (YK 241 Cortisol [Saliva] EIA kit, YK070 Human Chromogranin A ELA kit, Yanaihara Institute). The intra- and interassay coefficients of variation were < 6% and < 15%, respectively. Salivary cortisol (μg/dL) and chromogranin A (pmol/mg) were quantified blind to RMMA status.

Data Analysis

Sleep stages and related events were scored by a trained sleep technologist according to the American Academy of Sleep Medicine (AASM) scoring manual version 2.1.[30] Awakening was considered to be > 15 seconds arousal, and microarousal to be 3 to 15 seconds arousal. The following variables were calculated: total sleep time; sleep latency; wake after sleep onset (WASO); sleep efficiency; the percentage of time spent in each sleep stage; the latency from sleep onset to Stage R; microarousal index (per hour); awakening index (per hour); and apnea-hypopnea index (per hour).

Oromotor events were scored according to a previous report.[8] RMMA was also scored with the contraction patterns of the masseter muscle according to a previous study.[12] Masseter activities unrelated to RMMA, usually nonrhythmic, were scored as nonspecific masseter activity (NSMA).[31] The following oromotor variables were calculated: RMMA index (number of RMMA episodes per hour of sleep); NSMA index (number of NSMA episodes per hour of sleep); and RMMA + NSMA index (number of RMMA and NSMA episodes per hour of sleep). The number of RMMA episodes with teeth grinding sounds per night was also counted. Subjects were categorized into three groups: *(1)* control (CTL) group (RMMA index < 2 episodes/hour); low-frequency (L-RMMA) group (RMMA index of ≥ 2 and < 4 episodes/hour); and *(3)* a high frequency (H-RMMA) group (RMMA index ≥ 4 episodes/hour).[12] Again, all sleep and oromotor activity scoring was done blind to subject status.

Heart Rate Analysis

Heart rate analysis was performed using the complex demodulation method (CDM).[32] The spectral power analysis was done using a computer program with 200 millisecond time resolution capability (HRV LOG-Pro-DSA Analysis, Norupro Light Systems), and the following data were calculated for Stage N2, Stage N3, and Stage R during the entire night after removing the epochs with artifacts: mean heart rate (HR); the spectral power in each frequency band of low-frequency wave (LF = 0.04–0.15 Hz) and high-frequency wave (HF = 0.15–0.4 Hz); and the ratio of LF to HF (LF:HF). Calculated data were combined with each sleep stage scored using PSG.

Statistical Analyses

The variables sex, self-reports of SB, and jaw symptoms were compared among the three groups using Pearson chi-square method. Sleep and oromotor activity variables, salivary cortisol, and scores for the questionnaires were compared using Kruskal-Wallis test with post hoc Mann-Whitney *U* test. Significance was set at $\alpha = .05$. All statistical analyses were performed using SYSTAT 13 (HULINKS).

Results

Of the 72 subjects, 4 were excluded due to lack of a data set or technical errors. Of the remaining 68 subjects scored, 14 were excluded due to the presence of apnea-hypopnea events (ie, apnea-hypopnea index ≥ 5 episodes/hour). Finally, the analysis was done for 54 subjects: 20 were classified into the CTL group, 13 into the L-RMMA group, and 21 into the H-RMMA group (Fig 1).

Sex, age, and BMI did not differ among the three groups (Table 1). The incidence of self-reported tooth grinding was twice as high in the H-RMMA group, but statistical analysis revealed this trend was not significant ($P = .057$). Neither morning masticatory muscle symptoms nor the number of teeth with tooth wear differed among the three groups (Table 1). Sleep quality assessed with the PSQI and gastroesophageal symptoms assessed using the F scale did not differ among the three groups (Table 1). SCL-90-R data were obtained by subdividing into the following nine items: *(1)* somatization; *(2)* obsessive compulsive; *(3)* interpersonal sensitivity; *(4)* depression; *(5)* anxiety; *(6)* hostility; *(7)* phobic anxiety; *(8)* paranoid ideation; and *(9)* psychoticism. However, none of these items differed among the three groups.

Oromotor and Sleep Variables

RMMA index significantly increased from the CTL to the H-RMMA groups ($P < .001$), while NSMA index did not differ among the three groups (Table 2). The number of tooth grinding sounds during the night lineally increased from the CTL to the H-RMMA groups ($P < .001$) (Table 2).

Table 1 Demographic Data

Variables	CTL (a) (n = 20)	L-RMMA (b) (n = 13)	H-RMMA (c) (n = 21)	*P* value
Demographic				
Sex,[a] n	F: 12; M: 8	F: 10; M: 3	F: 8; M: 13	.085
Age,[b] y	24.0 (22.0–33.0)	24.0 (21.0–29.0)	23.0 (20.0–28.0)	.90
BMI,[b] kg/m^2	20.8 (18.7–24.6)	20.2 (18.9–23.3)	20.0 (16.0–23.7)	.67
Clinical examination				
Self-awareness,[a] n (%)	6/20 (30)	5/13 (38)	14/21 (67)	.057
Jaw symptoms,[a] n (%)	6/20 (30)	5/13 (30)	5/21 (24)	.65
Teeth with wear[a]	3.5 (0.0–12.0)	1.0 (0.0–12.0)	3.0 (0.0–14.0)	.78
PSQI score[a,c]	5.0 (3.0–9.0)	5.0 (2.0–9.0)	5.0 (1.0–9.0)	.74
F scale score[a]	3.0 (0.0–15.0)	6.0 (0.0–17.0)	3.0 (0.0–18.0)	.91
SCL-90-R scores				
Somatization[b]	49.0 (35.0–71.0)	53.0 (44.0–66.0)	49.0 (38.0–69.0)	.80
Obsessive compulsive[b]	52.0 (37.0–64.0)	58.0 (37.0–72.0)	55.5 (37.0–66.0)	.19
Interpersonal sensitivity[b]	53.0 (44.0–71.0)	47.0 (44.0–66.0)	47.0 (44.0–59.0)	1.0
Depression[b]	52.0 (38.0–66.0)	57.0 (34.0–66.0)	50.0 (34.0–65.0)	.83
Anxiety[b]	44.0 (37.0–68.0)	54.0 (37.0–59.0)	46.0 (37.0–66.0)	.24
Hostility[b]	48.0 (40.0–72.0)	49.0 (41.0–66.0)	52.0 (30.0–65.0)	.82
Phobic anxiety[b]	47.0 (44.0–65.0)	47.0 (44.0–66.0)	47.0 (44.0–59.0)	1.0
Paranoid ideation[b]	49.0 (38.0–58.0)	49.0 (41.0–70.0)	45.0 (41.0–74.0)	.57
Psychoticism[b]	53.0 (44.0–71.0)	53.0 (44.0–66.0)	53.0 (44.0–74.0)	.73

Data are presented as median (minimum–maximum) unless otherwise indicated. CTL = control group; L-RMMA = low RMMA group; H-RMMA = high RMMA group; BMI = body mass index; PSQI = Japanese version of the Pittsburgh Sleep Quality Index; SCL-90-R = Symptom CheckList-90 Revised.
[a]Pearson chi-square test and post hoc tests.
[b]Kruskal-Wallis test and post hoc Mann Whitney *U* tests.
[c]One missing data.

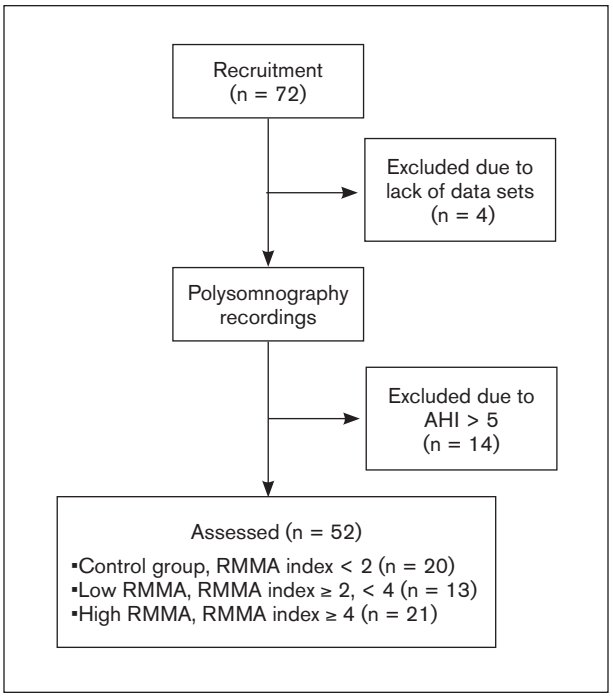

Fig 1 Schematic description. AHI = apnea hypopnea index; RMMA = rhythmic masticatory muscle activity.

Total sleep time, WASO, and sleep efficiency did not differ among the three groups. The percentage of sleep stage duration did not differ among the three groups, except for Stage N1 (*P* = .012). Post hoc Mann-Whitney *U* tests revealed that the percentage of Stage N1 was significantly higher in the H-RMMA group compared to the CTL (*P* = .037) and L-RMMA groups (*P* = .005). Sleep latency significantly decreased between the CTL and H-RMMA groups (*P* = .008). Post hoc Mann-Whitney *U* tests revealed that the H-RMMA group (*P* = .005) and the L-RMMA group (*P* = .027) showed shorter sleep latency (about 4 minutes) than the CTL group. REM latency did not differ among the three groups (Table 2).

A group difference was found in the microarousal index (*P* = .013). Post hoc Mann Whitney *U* tests revealed that the microarousal index was significantly higher in the H-RMMA group than in the CTL (*P* = .007) and the L-RMMA groups (*P* = .022). Awakening frequency during sleep did not differ among the three groups.

Autonomic Nervous System

Mean HR was significantly lower in the H-RMMA group than in the other two groups in Stages N2 (*P* = .044), N3 (*P* = .036), and R (*P* = .045) (Table 3). The analysis of heart rate variability (a proxy of autonomic activity) showed that LF (sympathetic proxy), HF (parasympathetic proxy), and LF:HF (sympathetic proxy) did not differ among the three groups (Table 3).

Subjective Sleep Quality and Anxiety

State and trait anxiety did not differ among the three groups in the evening or morning. Subjective estimation of sleep latency significantly differed among the three groups (*P* = .003). Post hoc Mann-Whitney *U* tests showed that the estimated self-reported sleep latency

Table 2 Sleep Variables

Variables	CTL (a) (n = 20)	L-RMMA (b) (n = 13)	H-RMMA (c) (n = 21)	*P* value			
					Paired comparison		
				Group	a vs b	a vs c	b vs c
Sleep architecture							
TST (min)	458.0 (372.0–486.0)	435.5 (374.0–497.0)	445.0 (412.0–503.0)	.73			
SL (min)	7.8 (2.5–29.0)	4.0 (1.0–11.0)	3.0 (0.0–17.0)	.008	.027	.005	.43
WASO (min)	24.8 (11.0–89.5)	22.0 (12.5–44.5)	19.0 (8.5–62.5)	.17			
SE (%)	94.9 (83.0–97.3)	95.2 (89.4–97.1)	96.1 (87.3–98.4)	.21			
REML (min)	99.3 (65.5–208.5)	75.5 (59.0–159.0)	77.0 (5.5–182.5)	.17			
Sleep stage (%)							
N1	9.2 (3.8–13.2)	7.6 (4.0–11.2)	11.8 (6.6–18.0)	.012	.28	.037	.005
N2	46.9 (33.9–55.6)	45.1 (36.0–56.4)	44.6 (34.9–53.7)	.65			
N3	22.1 (8.6–37.6)	26.4 (15.4–33.8)	21.4 (10.2–32.5)	.26			
R	19.0 (13.2–24.9)	19.6 (11.3–25.8)	17.5 (12.7–24.3)	.59			
W	3.4 (1.1–15.6)	2.7 (1.4–9.6)	3.1 (1.6–11.2)	.53			
Arousals							
Microarousal index (/h)	6.9 (3.8–11.8)	7.0 (4.6–9.1)	10.7 (5.2–15.3)	.013	.93	.007	.022
Awaking index (/h)	3.2 (1.2–6.7)	3.3 (1.8–6.3)	3.3 (2.0–9.8)	.63			
Respiratory events							
AHI (/h)	0.76 (0.13–4.6)	1.5 (0.14–4.6)	1.1 (0.13–4.8)	.37			
Oromotor events							
RMMA index (/h)	1.0 (0.0–1.8)	2.6 (2.1–3.9)	5.7 (4.2–12.0)	< .001	< .001	< .001	< .001
NSMA index (/h)	5.7 (2.7–10.5)	7.6 (2.4–10.6)	6.6 (2.9–13.6)	.49			
RMMA+NSMA index (/h)	7.0 (3.1–12.1)	10.4 (4.6–14.1)	13.7 (9.2–19.3)	< .001	.006	< .001	.007
Teeth grinding sound (/night)	0.0 (0.0–5.0)	1.0 (0.0–12.0)	9.0 (1.0–78.0)	< .001	.002	< .001	.007

Data are presented as median (minimum–maximum). CTL = control group; L-RMMA = low RMMA group; H-RMMA = high RMMA group; TST = total sleep time; SL = sleep latency; WASO = wake after sleep onset; SE = sleep efficiency; REML = REM latency; AHI = apnea-hypopnea index; RMMA = rhythmic masticatory masseter activity; NSMA = nonspecific masseter activity. Statistical analyses were conducted with Kruskal-Wallis and post hoc Mann Whitney *U* tests.

Table 3 Heart Rate Analysis During the Entire Night

Variables	CTL (a) (n = 20)	L-RMMA (b) (n = 13)	H-RMMA (c) (n = 21)	*P* value			
					Paired comparisons		
				Group	a vs b	a vs c	b vs c
LF (ms^2/Hz)							
Stage N2	2,225.6 (438.3–4,257.4)	2,551.0 (1,098.3–10,169.4)	2,093.7 (750.0–6,299.2)	.70			
Stage N3	2,336.5 (495.1–4,521.7)	1,810.9 (868.6–4,280.2)	2,335.6 (474.3–7,061.2)	.83			
Stage R	2,586.2 (495.1–4,521.7)	2,179.2 (941.8–11,480.3)	2,694.9 (1,069.1–7,761.4)	.32			
HF (ms^2/Hz)							
Stage N2	882.6 (293.1–2,270.6)	1,303.9 (428.3–2,210.0)	770.8 (134.5–3,695.6)	.32			
Stage N3	780.9 (179.5–2,510.0)	1,023.9 (369.8–2,434.9)	748.4 (84.4–2,263.1)	.53			
Stage R	835.8 (259.5–2,643.4)	1,103.9 (331.5–3,632.4)	759.5 (169.8–4,128.5)	.60			
LF/HF							
Stage N2	2.2 (1.3–3.6)	2.1 (1.1–4.4)	2.4 (1.2–4.0)	.17			
Stage N3	2.5 (1.1–3.7)	2.1 (1.2–3.5)	2.3 (1.3–4.4)	.17			
Stage R	2.5 (0.62–4.5)	2.2 (1.4–4.3)	2.7 (1.5–5.5)	.13			
Mean HR (bpm)							
Stage N2	57.6 (40.1–71.3)	58.3 (50.1–64.8)	52.3 (40.6–66.6)	.044	.93	.040	.029
Stage N3	60.2 (41.2–74.1)	59.4 (51.1–65.8)	53.6 (40.0–70.3)	.036	.84	.025	.035
Stage R	58.3 (41.0–71.8)	58.7 (52.0–66.8)	53.4 (41.9–65.8)	.045	.93	.040	.029

Data are presented as median (minimum–maximum). CTL = control group; L-RMMA = low RMMA group; H-RMMA = high RMMA group; LF = low-frequency wave; HF = high-frequency wave; HR = heart rate. Statistical analyses were conducted using Kruskal-Wallis and post hoc Mann-Whitney *U* tests.

of the L-RMMA ($P < .034$) and the H-RMMA groups ($P = .001$) was shorter than the CTL group (Table 4).

A group difference was noted for the frequency of awakenings ($P = .033$). Post hoc Mann-Whitney *U* tests showed that the L-RMMA group reported more frequent awakenings in the laboratory than at home in comparison to the CTL group ($P = .024$) and the H-RMMA group ($P = .029$) (Table 4). However, sleep quality in the laboratory in comparison to that at home was rated differently among the three

Table 4 Anxiety Scores and Subjective Assessments of Sleep Upon Waking in the Morning

Variables	CTL (a) (n = 20)	L-RMMA (b) (n = 13)	H-RMMA (c) (n = 21)	*P* value			
					Paired comparisons		
				Group	a vs b	a vs c	b vs c
Anxiety (STAI)							
State (evening)	38.0 (21.0–48.0)	32.5 (26.0–45.0)	38.0 (21.0–48.0)	.47			
State (morning)	36.0 (20.0–51.0)	36.0 (23.0–44.0)	36.0 (20.0–51.0)	.89			
Trait (evening)	41.0 (20.0–51.0)	43.5 (30.0–53.0)	41.0 (20.0–56.0)	.37			
Trait (morning)	41.5 (20.0–57.0)	40.0 (30.0–53.0)	41.5 (20.0–51.0)	.37			
Sleep							
Sleep latency (min)	20.0 (3.0–60.0)	10.0 (5.0–30.0)	10.0 (0.0–30.0)	.003	.034	.001	.25
Intermittent awakenings (/night)	2.0 (1.0–3.0)	3.0 (2.0–5.0)	2.0 (2.0–3.0)	.033	.024	.80	.029
Sleep quality	3.0 (2.0–4.0)	3.0 (1.0–3.0)	3.0 (1.0–5.0)	.021	.003	.72	.041
Physical condition							
Jaw symptoms	0.0 (0.0–1.5)	0.0 (0.0–1.2)	0.0 (0.9–4.1)	.70			
Oral dryness (cm)	0.8 (0.0–5.6)	3.2 (0.0–5.4)	2.1 (0.0–8.6)	.014	.020	.011	.71

Data are presented as median (minimum–maximum). CTL - control group; L-RMMA = low RMMA group; H-RMMA = high RMMA group; STAI = Japanese version of the State-Trait Anxiety Inventory. Statistical analyses were conducted using Kruskal-Wallis and post hoc Mann-Whitney *U* tests.

Table 5 Salivary Markers

Variables	CTL (a) (n = 20)	L-RMMA (b) (n = 13)	H-RMMA (c) (n = 21)	*P* value			
					Paired comparisons		
				Group	a vs b	a vs c	b vs c
Cortisol (μg/dL)							
Evening	0.078 (0.023–0.28)[a]	0.056 (0.028–0.17)	0.058 (0.017–0.18)	.29			
Morning	0.30 (0.061–1.2)	0.34 (0.086–1.4)	0.36 (0.029–1.3)	.58			
Morning/evening	4.6 (0.44–32.4)	7.6 (0.84–37.3)	5.9 (0.18–35.9)	.20			
Chromogranin A (pmol/mg)							
Evening	7.2 (2.2–31.0)	6.7 (1.1–14.1)	6.6 (0.53–19.1)	.68			
Morning	11.1 (4.6–29.7)	9.1 (4.3–12.9)	12.3 (4.8–19.3)	.049	.11	.85	.007
Morning/evening	1.6 (0.63–5.8)	1.2 (0.4–10.7)	2.2 (0.80–21.5)	.091			

Data are presented as median (minimum–maximum). CTL = control group; L-RMMA = low RMMA group; H-RMMA = high RMMA group. Statistical analyses were conducted using Kruskal-Wallis and post hoc Mann Whitney *U* tests.
[a]One missing data.

groups (P = .021). The L-RMMA group rated laboratory sleep better than the CTL group (P = .003) and the H-RMMA group (P = .039). The L-RMMA and H-RMMA groups reported a higher score for oral dryness in the morning, while other measures, such as jaw symptoms, did not differ among the three groups (Table 4).

Salivary Markers

Salivary cortisol significantly increased in the morning in comparison to the evening estimates for all three groups (P < .001) (Table 5).[33,34] However, no significant group difference was noted. The ratio of cortisol concentration between the evening and morning did not differ among the three groups (Table 5). When all participants were further classified into two groups using a cut-off at the median ratio of cortisol between evening and morning, subjects with a high ratio of cortisol showed fewer awakenings, a lower percentage of stage N1, and higher sleep efficiency than those with a low ratio.

Chromogranin A also significantly increased in the morning in comparison to the evening estimates for all three groups (P < .001) (Table 5).[35] The concentration in the morning was significantly lower in the L-RMMA group than in the H-RMMA group (P = .007). The ratio of chromogranin A between the evening and the morning did not differ among the three groups (Table 5). Sleep variables did not differ between the two groups when divided according to high and low ratios of chromogranin A.

Discussion

The present PSG study demonstrated that sleep macrostructure did not differ among young subjects exhibiting different degrees of RMMA. Significant differences were found for sleep latency, the percentage of Stage N1, and microarousal index, although all sleep variables were within the normal range. Sympathetic tone did not differ among the

three groups, although mean heart rate was significantly lower in the H-RMMA group than the other two groups. Subjective anxiety level and salivary markers did not differ among the three groups, although salivary chromogranin A was statistically higher in the H-RMMA group than the L-RMMA group in the morning. These results suggest that the inter-individual differences in the occurrence of RMMA cannot be simply explained by the sleep architecture or by the presence of anxiety and stress in young subjects, although minor differences were found for these variables.

In this study, subjects were divided into three groups according to the RMMA index.[12] However, nonrhythmic oromotor activity (NSMA index) did not differ among the three groups. Although the number of RMMA episodes with tooth grinding noise was significantly higher in the H-RMMA group than the CTL group, the frequency of self-reported tooth grinding did not show a significant difference among the three groups. Since self-awareness of tooth grinding noise is less precise unless witnessed,[28] the high percentage (92.6%) in this sample who slept alone may affect this result. Neither morning jaw symptoms nor tooth wear differed among the three groups. In addition, no difference was noted for the PSQI, SCL-90-R, or F scale scores. Therefore, the three groups in this study were characterized by the different degrees of RMMA occurrence during sleep without other confounding factors, such as major clinical symptoms.

In general, sleep architecture was within a normal range in this study population, as reported in previous studies.[12,18,31] Although sleep macrostructure did not show major differences among the three groups, some variables associated with sleep-disrupting influences, such as the percentage of Stage N1 and the frequency of microarousals, were significantly higher in the H-RMMA group than in the other groups. However, the frequent occurrence of RMMA in the H-RMMA group did not increase Stage W. The above results support previous findings. RMMA occurs in association with microarousals and with a higher level of arousal fluctuations, such as a cyclic alternating pattern, and was usually followed by the sleep stage shift to stage N1 rather than awakening.[7,36,37] In addition, sleep latency was significantly shorter in the H-RMMA and the L-RMMA groups than the CTL group. Interestingly, there were minor differences in cardiac variables, showing a lower heart rate in NREM and REM sleep stages in the H-RMMA group than the other two groups. These results suggest two possibilities. In comparison to the CTL group, the H-RMMA group may have higher sleep pressure or more recuperative function to maintain normal sleep processes over the frequent occurrence of RMMA. Alternatively, the arousals and sleep stage shifts related to RMMA are neither frequent nor intense enough to alter sleep macrostructure in young subjects in the H-RMMA group, since RMMA occurs as frequently as respiratory events at a mild level of OSA (eg, 5–10 per hour of sleep). Therefore, these results suggest that factors other than sleep fragmentation may underlie the exaggerated occurrence of RMMA during sleep in young subjects.

All young participants in this study had normal sleep variables without any signs of sleep disturbance. In addition, the results from the psychologic assessments with the PSQI and SCL-90-R did not differ among the three groups. State and trait anxiety scores were normal and did not differ among the three groups in the evening or in the morning. Subjective reports of sleep latency during the PSG recordings were consistent with objective sleep latency; sleep latency was significantly longer in the CTL group than in the L-RMMA and the H-RMMA groups. Therefore, these results suggest that young subjects with frequent RMMA do not have a symptomatic level of hyperarousal and anxiety/stress conditions that can lead to sleep disturbance such as difficulty in falling asleep and maintaining sleep continuity.[16,17] Interestingly, however, the L-RMMA group rated the night of PSG recordings differently in terms of sleep quality, nocturnal awakening, and oral symptoms such as oral dryness in comparison to the CTL and H-RMMA groups. This possibly supports the previous finding that SB subjects with a low frequency of RMMA differently reported somatic symptoms.[12] Whether such a subtle difference in subjective reports in sleep quality and oral symptoms may be related to a different coping style to the laboratory sleep environment in this population requires further investigation.[38,39]

Previous studies attempted to assess the association between psychological factors and SB. For example, many cross-sectional studies demonstrated the positive association between psychologic measures (ie, stress and anxiety) and self-reported SB (ie, tooth grinding or self-awareness).[13,14,23] Subjective assessment of SB does not always reflect the increase of oromotor activity since SB awareness may be biased by the sleep environment[28] and accompanying somatic conditions.[40] In fact, these associations were controversial when objective assessments for oromotor activity during sleep were performed.[13,41–44] The results of the present PSG study support a recent finding that psychologic condition is not correlated with the number of masseter EMG events based on a more reliable SB diagnosis using a portable EMG/ECG device.[24]

The association between psychologic factors and SB was studied by assessing several neuroendocrine substances. Either nocturnal masseter EMG activity

level or self-report of SB was positively correlated with urinary catecholamine levels[45,46] and with morning salivary cortisol.[13] Other studies showed daytime salivary chromogranin A was positively correlated with self-reported SB[21] but negatively with nocturnal masseter EMG level.[20] Similar to the sleep and psychological assessment results in this study, no significant correlation between the occurrence of RMMA and salivary markers was found. Salivary cortisol did not differ among the three groups in the evening or morning, while chromogranin A was higher in the H-RMMA group than the L-RMMA group in the morning only. Cortisol is released from the adrenal cortex in relation to hypothalamic-pituitary-adrenal (HPA) axis activity,[34] while chromogranin A is released with catecholamine from the adrenal medulla, sympathetic nerve endings, and the submandibular gland.[47] The concentration of salivary cortisol and chromogranin A showed distinct diurnal variations[33–35]: two markers showed high concentration at waking in the morning, but after awakening, cortisol further increased while chromogranin A rapidly decreased. Cortisol secretion was found to be negatively correlated with the amount of Stage N1 and nocturnal awakenings during sleep.[48] Collectively, group differences of sleep architecture found in this study are not enough to differentiate neuroendocrine responses assessed by salivary markers. The discrepancy between the present and previous findings on salivary markers in SB research may be partly due to a lack of control for the daily circadian phase prior to data collection. Since the occurrence of RMMA during the night is also related to the circadian cycle,[7] the inter-individual variability of the circadian phase may be a confounding factor in investigating the association between the neuroendocrine system and RMMA in future studies.

Conclusions

The present cross-sectional PSG study showed that young subjects with different degrees of RMMA showed similar objective and subjective sleep characteristics, psychosomatic profiles, cardiac activity, and salivary biomarkers, except for minor differences. Therefore, these variables cannot differentiate the different degrees in the occurrence of RMMA in young subjects. The physiologic variables assessed in this study were within normal range; however, the present study cannot exclude the possibility of intra-individual variations (ie, daily variation) for RMMA occurrence[49–51] in association with psychologic factors. Therefore, future studies are needed to demonstrate the physiologic roles of the balance between sleep disruption and protective influences on the inter- and intraindividual variations in RMMA occurrence.

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