Neuroscience 588 (2025) 100–111

Contents lists available at ScienceDirect

Neuroscience

journal homepage: www.elsevier.com/locate/nsc

Research Article

Diurnal variations and intermittent arousals modulate jaw-opening and -closing muscle activity level during sleep in rats

Yiwen Zhu [a], Masaharu Yamada [a], Noriko Minota [a,b], Ayano Katagiri [a], Takafumi Kato [a,*]

[a] Department of Oral Physiology, Graduate School of Dentistry, The University of Osaka, Suita, Osaka, Japan
[b] Department of Oral and Maxillofacial Surgery, Graduate School of Dentistry, The University of Osaka, Suita, Osaka, Japan

ARTICLE INFO

Keywords:
Jaw muscle activity
Sleep-wake cycle
Diurnal variation
Intermittent arousal

ABSTRACT

Increased jaw muscle activity during sleep, which is associated with conditions such as sleep bruxism and obstructive sleep apnea, is a significant clinical concern in dentistry. The present study aimed to investigate the factor influencing natural variations on jaw muscle activity during sleep throughout 24 h. In ten male SD rats, electrophysiological recording was conducted to monitor electroencephalography (EEG), electromyographic (EMG) activity of neck, jaw-closing (masseter), and jaw-opening (digastric) muscles while freely behaving throughout 24 h. Jaw muscle activity level during sleep exhibit a diurnal variation, with lower level in the light phase than in the early dark phase. The jaw muscle activity level was significantly higher during intermittent arousals than during quiet sleep period. Jaw muscle activity levels during intermittent arousals were significantly higher in dark phase within arousals during NREM sleep. Within intermittent arousals, jaw muscle activity level was negatively correlated with EEG delta power in NREM sleep or EEG theta power in REM sleep. Clustering analysis further revealed that multiple muscles including neck and jaw muscles are often activated within intermittent arousals. These findings revealed the occurrence of intermittent arousals under diurnal influences underlie the variations of jaw muscle activity during sleep in 24 h.

Introduction

Jaw-closing muscles play significant roles on oral motor functions such as mastication, by exerting the appropriate force to break down the food (Kato et al., 2011). During sleep, jaw-closing muscles were found to be activated in humans. However, excessive jaw-closing muscle activities during sleep are often associated with the undesirable orodental problems such as tooth destruction, breakage of dental restoration and prostheses and orofacial pain/headaches (Kato et al., 2013b). These conditions have been reported in patients with sleep disorders such as sleep bruxism and sleep apnea. Patients with sleep bruxism have been reported to show an increased activity of jaw-closing muscles during sleep (Toyota et al., 2022). Patients with obstructive sleep apnea can exhibit jaw-closing muscle activities after respiratory events (Kato et al., 2013a; Li et al., 2023).

Interestingly, the activation of jaw-closing muscles was found to occur in association with stage-dependent changes in sleep such as non-rapid eye movement (NREM) and rapid eye movement (REM) sleep as jaw-closing muscle activities occurred more frequently during NREM sleep compared to REM sleep in patients with sleep bruxism and obstructive sleep apnea (Kato et al., 2013a; Toyota et al., 2022). Moreover, in addition to state dependent changes of sleep, jaw muscle activation is time-related to periodic or transient arousals (i.e., cortical activation) in these patients (Kato et al., 2001; Kato et al., 2013a; Imai et al., 2021). Therefore, the fluctuations of jaw-closing muscle activities related to intermittent arousals over the changes in vigilance states can represent physiological indicators for investigating the pathophysiology of the conditions with excessive jaw-closing muscle activities during sleep.

In experimental animals, similar phenomena have been observed that jaw-closing muscle activities were influenced by the natural variations such as diurnal changes, sleep-wake states, and arousal events. Research on rabbits (Langenbach et al., 2004; Grünheid et al., 2005) and rats (Kawai et al., 2007) has demonstrated diurnal changes in jaw-closing muscle activity over 24-hour periods. These studies highlight the effects of time-of day on jaw-closing muscle activity patterns. Investigations into jaw-closing muscle activity across sleep-wake states have been conducted in rats, mice, and guinea pigs (Burgess et al., 2008; Katayama et al., 2015; Kato et al., 2018). These studies have shown that jaw-closing muscle activity varies significantly among wakefulness,

* Corresponding author at: Department of Oral Physiology, Graduate School of Dentistry, The University of Osaka, Japan.
E-mail address: kato.takafumi.dent@osaka-u.ac.jp (T. Kato).

https://doi.org/10.1016/j.neuroscience.2025.10.002
Received 3 May 2025; Accepted 4 October 2025
Available online 10 October 2025

NREM, and REM sleep. Furthermore, some research has explored jaw-closing muscle activities in association with arousals during sleep in guinea pigs (Kato et al., 2010; Kato et al., 2018), which suggests that cortical arousal can involve in the elevation of jaw-closing muscle activity during sleep. However, the comprehensive impact of these natural variance on jaw-opening and −closing muscle activities have not been fully understood in experimental animals. In order to build an animal model of sleep-related oromotor disorders including sleep bruxism, it is essential to grasp the characteristics of jaw muscle activity in natural sleep of rats.

Therefore, the aim of the present study was to examine the integrated effects of diurnal changes, vigilance states and intermittent arousals on the activity of jaw muscles over a 24-hour period in rats.

Materials and methods

Animals and surgical preparation

Ten male Sprague-Dawley rats were used in this study. The animals were individually housed in a light-controlled environment and climate (temperature: 23 ± 0.5 °C) with free access to food and water under a 12 h light/dark cycle (lights on at 8:00). The Animal Experimentation Committee approved the protocol of the present animal study, which was conducted at Graduate School of Dentistry, The University of Osaka (approval no. R04-010). This study adheres to the ARRIVE 2.0 guidelines as far as applicable.

Before surgery, a custom-made multiple pin socket was soldered for electrophysiological recording. The socket contained five channels, each with two pins. Two pairs of silicone-coated multistranded stainless wires (F = 0.3 mm) attached by stainless-steel screws (F = 1.4 mm) and three pairs of polyurethane-coated stainless wires (F = 0.08 mm) were soldered to the pins of socket as recording electrodes.

Surgery was performed in 5-week-old rats (body weight: 137.2 ± 4.7 g) under anesthesia with combination of Medetomidine hydrochloride (0.375 mg/kg), Midazolam (2 mg/kg), and Butorphanol (2.5 mg/kg) (M/M/B; i.p.). Body temperature was maintained around 37 °C with a heating pad. An incision was made in the skin on the midline of the head to expose the skull after local anesthesia of 2 % lidocaine. Two holes were drilled onto the frontal skull and one pair of screws were fixed for electroencephalography (EEG) recording. Another two holes were drilled onto the occipital bone for reference electrodes fixation. For electromyogram (EMG) recording of the dorsal neck muscle, left side of masseter muscle (Mas, jaw-closing muscle) and anterior digastric muscle (Dig, jaw-opening muscle), the skin of the dorsal neck areas and submandibular was incised after local anesthesia. Three pairs of wires were tunneled subcutaneously and inserted to the exposed muscles. Then, all the incisions were sutured, and the socket was fixed onto skull with dental resin. Buprenorphine hydrochloride (0.02 mg/kg) and Benzylpenicillin potassium (2000 unit/rat) were intraperitoneally injected after surgery to avoid infection and release pain. All the rats were allowed to recover for 2 weeks.

Electrophysiological recording

During the recording session, each animal was placed in a noise-attenuated recording chamber with free access to food and water. A lightweight cable was connected to the multi-pin socket implanted on the rat's head, while the opposite end was attached to a multichannel slip-ring, allowing signal collection during free movement. Recordings began at 20:00 and continued for 24 h. Throughout the session, electrophysiological signals were collected using VitalRecorder® software (Kissei Comtec Ltd., Matsumoto, Japan) with optimal bandwidths (EEG: 0.3–100 Hz; EMG: 100–1000 Hz; 60-Hz hum filters for all).

Sleep scoring

Vigilance states—wakefulness (W), non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep—along with EEG and EMG activity, were scored in 10-second epochs using Sleepsign® software (Kissei Comtec Ltd.). The signals were synchronously digitized with a sampling rate of 512 Hz for all channels. EEG and EMG were amplified 1000-fold and filtered at 0.5 - 20 Hz and 20 - 100 Hz respectively. Vigilance states were manually classified based on EEG and neck muscle EMG activity by two well-trained raters. Specifically, wakefulness (W) was characterized by low-amplitude, high-frequency EEG activity and elevated neck muscle tone; NREM sleep was identified by high-amplitude, synchronized EEG activity with a predominance of delta waves and reduced neck muscle tone; and REM sleep was marked by desynchronized EEG activity with a predominance of theta waves and neck muscle atonia. A wakefulness or sleep episode was defined as four or more consecutive epochs. The percentage of time spent in each state, the number of episodes, and the duration of each episode were calculated. Intermittent arousals were visually identified based on the disappearance of slow waves and the appearance of fast waves during NREM sleep (Cardis et al., 2021; Schott et al., 2023) and the disappearance of theta waves during REM sleep (Cardis et al., 2021; Bueno-Junior et al., 2023). No more than three consecutive epochs of W were classified as intermittent arousals during NREM sleep (ANR) or during REM sleep (AR). The frequency of ANR and AR was also determined.

EEG power spectrum analysis and quantification of EMG activity

Delta power during NREM sleep and theta power during REM sleep are indicators of sleep depth in rodents (Vertes, 1981; Opp et al., 1997). To assess the power densities of the EEG spectrum during NREM and REM sleep, fast Fourier transformation (FFT) was applied to each 10-s epoch. The EEG signal was filtered using a band-pass filter with a range of 0.5 - 20 Hz. Spectral power was calculated from 1024-point windows that were multiplied by the hanning window function, yielding a spectral resolution of 0.50 Hz. To minimize the influence of artifacts, outliers—defined as values below [Q1(the 25th percentile) - 1.5 * IQR (interquartile Range: Q3 (the 75th percentile) - Q1)] or higher than [Q3 + 1.5 * IQR]—were excluded from the analysis (Park and Chung, 2019). Then, the power of delta waves (0.5 - 4 Hz) during NREM sleep without arousals was compared to that within arousals (ANR), and the power of theta waves (4 - 9 Hz) during REM sleep without arousals was compared to that within arousals (AR).

EMG activities were rectified and integrated for every 10-s epoch, with the minimum value subtracted to eliminate baseline noise (Lu et al., 2005; Kato et al., 2007; Kato et al., 2015). The maximum EMG activity of the masseter muscle was extracted from each chewing episode, along with the corresponding EMG activities of the neck and digastric muscles within the same epoch. The mean values of these muscle activities were calculated and was set as 100 % to normalize muscle activity for each epoch.

Correlation analysis of EEG power and EMG activity during NREM and REM sleep

To further relate the EMG activities of neck and jaw muscles during sleep to cortical activity level, Huber regression was chosen for modeling the correlation between EEG and EMG activity during NREM and REM sleep in each subject due to its robustness in handling non-normal distributions and outliers (Huber, 1992; Owen, 2007). Unlike simple linear regression, which assumes normally distributed residuals and is sensitive to skewness, Huber regression could minimize the impact of extreme values by using a combination of squared loss for small residuals and absolute loss for large ones (Huber, 1992; Owen, 2007). Huber regression modelling was conducted using the Robust

Linear Model (RLM) from the statsmodels library with Python (version 3.11.4). The threshold value (δ) for the Huber loss function in statsmodels is $\delta = 1.345$. This value is commonly used based on the 95 % confidence interval of a normal distribution, meaning that approximately 95 % of residuals are treated as normal, while larger residuals (outliers) are subjected to linear loss. Regression coefficient (β) was calculated to estimate the effect of EEG activity on muscle activity. *t*-values were calculated as the ratio of the regression coefficient to its robust standard error.

To account for the potential confounding effect of arousal duration on the relationship between EEG power and EMG activity, we further segmented each arousal episode by its duration and grouped them into 1-epoch, 2-epoch, or 3-epoch segments. For each arousal segment, the minimum delta/theta power across the segment was used to represent EEG activity, while the maximum EMG value was used to represent muscle activity. This ensured that the EEG-EMG relationship was not biased by arousal length or within-segment variability.

To synthesize the regression results across the 10 subjects, a *meta*-analysis model (Hooijmans et al., 2014) was employed with Python to estimate the weighted averaged (WA) of β and *t*-value between EEG and EMG activity during NREM and REM sleep.

Clustering analysis of EMG activity during NREM and REM sleep

Based on observations in present study, among neck, Mas and Dig muscles, two or three muscles could be activated within the same 10-s epoch of intermittent arousal during NERM and REM sleep. To further analyze the multiple activation of neck and jaw muscles within intermittent arousals, clustering analysis was combined to distinguish muscle contractions and muscle tone during NREM and REM sleep.

Fig. 1 - A illustrates an example of the histogram of EMG activity of Mas during NREM sleep throughout 24 h. The logarithmic transformation (log EMG values) of the EMG data was applied to neck, Mas and Dig during NREM and REM sleep throughout 24 h (Fig. 1 - B). The log-transformation can convert the skewed dataset into normality distribution. A cluster analysis using Gaussian Mixture Models (GMMs) was then performed on the log-transformed EMG values (Fig. 1 - C). This analysis was conducted using the Gaussian mixture package in Scikit-learn (version 1.3.0) with Python (version 3.11.4). The GMM method employs the expectation–maximization algorithm to identify overlapping Gaussian distributions within the dataset. The Bayesian Information Criterion (BIC) was used to determine the optimal number of components in the mixture model. Results from the BIC suggested that the log EMG values consisted of at least two components. Consistent with bimodal distribution of jaw muscle activity observed in human and mice during sleep-wake cycle (Katayama et al., 2015; Toyota et al., 2022). Given that sleep can include periods with or without active muscle contractions, the two components were defined as cluster 1, representing estimated muscle contraction, and cluster 2, representing estimated muscle tone. The probability density function of these clusters was calculated using the following formula: $f(x) = \mathscr{W} \cdot \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$ (Toyota et al., 2022). The intersection of two clusters were also detected by code. To assess the reproducibility of the GMM-based cluster assignments, silhouette scores (Saranya and Poonguzhali, 2024) were calculated using Python code. We found consistently high silhouette scores of three muscles across animals during NREM (Neck: 0.65 ± 0.016; Mas: 0.62 ± 0.023; Dig: 0.66 ± 0.018) and REM sleep (Neck: 0.67 ± 0.034; Mas: 0.66 ± 0.037; Dig: 0.61 ± 0.017), suggesting the consistency of the classification of two clusters.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9.0 after assessing the normality of data distribution by the Shapiro-Wilk Test. Time-course changes of percentage in arousal frequency and EMG activities were compared by One-way repeated measures ANOVA with Dunnett's multiple comparisons test or Friedman test with Dunn's multiple comparisons test. EMG activities and arousal frequency during transition were compared by Bonferroni's or Dunn's post hoc. Paired *t* test or Wilcoxon matched-pairs signed rank test was conducted to compare sleep variables in each state between light phase and dark phase. The differences of EEG and EMG between NREM/REM sleep and arousals were also compared by Paired *t* test or Wilcoxon matched-pairs signed rank test. One-way repeated measures ANOVA with Tukey's multiple comparisons test or Friedman test with Dunn's multiple comparisons test was used to compare EMG activities among vigilance states in the dark and light phase and the differences among the ratio of muscle activation. The significance of differences was set at $p < 0.05$. A post-hoc power analysis (G*Power, Version 3.1.9.6) for a representative comparison yielded an effect size of dz = -1.18228 (n = 10) and a power of 0.913. As other comparisons showed similar effect sizes, a sample size of 10 rats was considered sufficient.

Results

Sleep architecture throughout 24 h

Fig. 2 - A shows the example of the physiological recording for 10 min. Typically, rats fall in NREM sleep, then enter into REM sleep, and finally return to wakefulness. NREM sleep was characterized as high amplitude and low frequency EEG activity while REM sleep by low

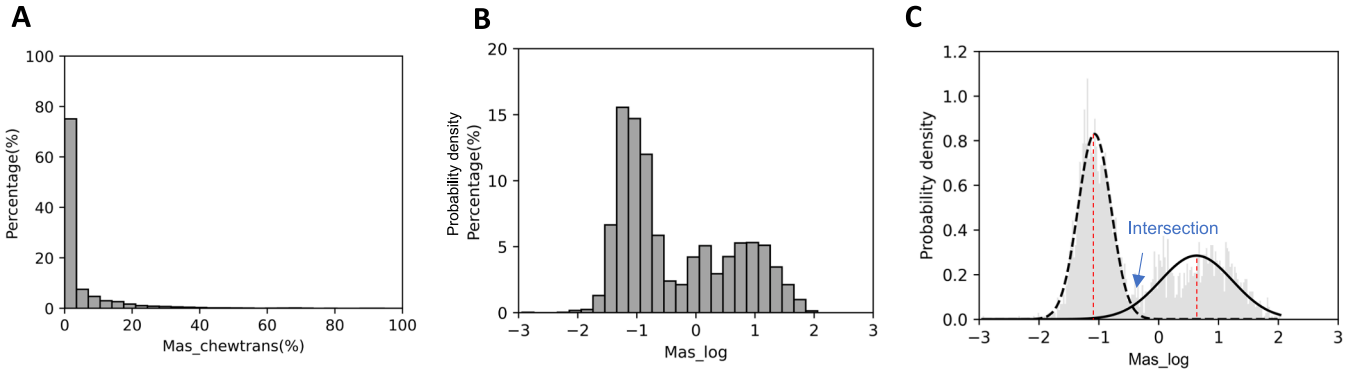

Fig. 1. Clustering analysis of EMG activities A: An example of the histogram of normalized EMG activity of Mas during NREM sleep throughout 24 h. B: An example of the histogram of logarithmic transformed value of Mas throughout 24 h. C: An example of the distribution of the clusters estimated. The red dashed lines show the mean value of each cluster, and the blue arrow points the intersection of two clusters. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

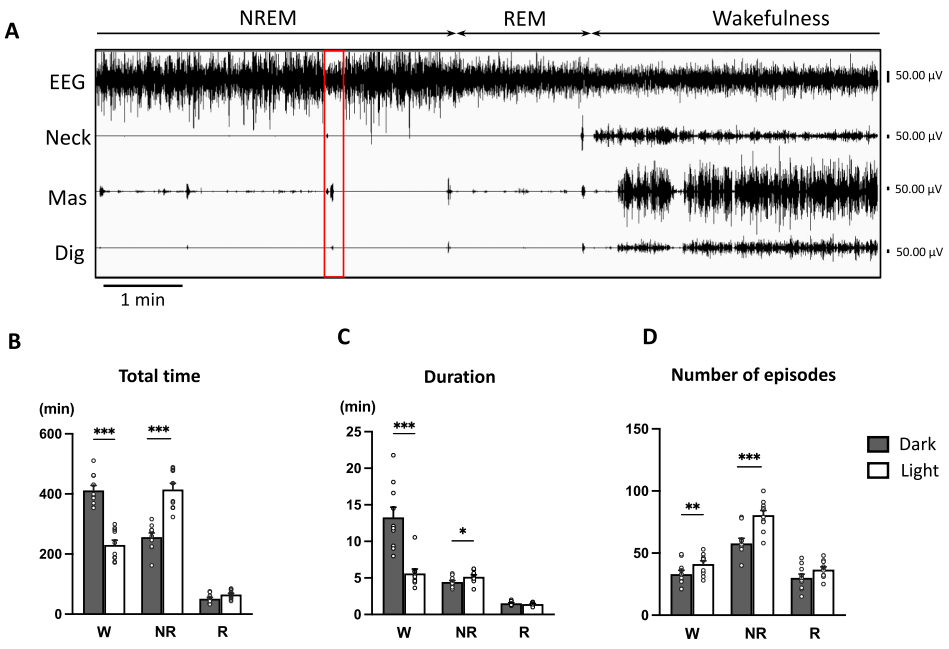

Fig. 2. Sleep architecture throughout 24 h recording. A: The raw waveform of the physiological recording, including EEG, EMG of neck, Mas and Dig muscle. The vigilance states of NREM sleep, REM sleep and wakefulness were manually identified. Red frame highlights an intermittent arousal during NREM sleep. B-D: The comparison of total time in each vigilance state, mean duration and the number of episodes in each vigilance state between dark phase and light phase. N = 10, Mean ± SEM, Paired *t* test, *p < 0.05, **p < 0.005, ***p < 0.001. W: wakefulness; NR: NREM sleep; R: REM sleep. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

amplitude and high frequency EEG activity; during wakefulness, the muscle tone was higher than that during sleep.

The sleep architecture differed between dark and light phases. The total time spent in wakefulness predominated during dark phase ($t(9) = 7.395$, $p < 0.001$). The mean duration of wakefulness episode was significantly longer during dark phase than during light phase ($t(9) = 5.928$, $p < 0.001$), though there were fewer wakefulness episodes in dark phase ($t(9) = 3.354$, $p = 0.008$). Rats spent significantly more time in NREM sleep during light phase than that during dark phase ($t(9) = 7.255$, $p < 0.001$) since the mean duration of each NREM sleep episode during light phase was significant longer than that during dark phase ($t(9) = 2.322$, $p = 0.05$). The number of NREM sleep episodes was significant higher during light phase than during dark phase ($t(9) = 4.857$, $p < 0.001$). However, under this experimental condition, there was no difference of the time spent for REM sleep between light and dark phase ($t(9) = 1.903$, $p = 0.09$) (Fig. 2 - B, C, D).

Time-course change of EMG activities in sleep-wake cycle

The average EMG activities of neck, Mas, and Dig muscle for 4-hour periods were analyzed across 24 h (Fig. 3), with comparisons to 20:00-23:00. Neck EMG activities did not show no time-course changes. Mas EMG activity during wakefulness ($\chi^2(5) = 19.20$, $p = 0.002$) was significantly higher in the initial period of dark phase (20:00-23:00) than in the initial (8:00-11:00: Dunn's post hoc, $p = 0.006$) and intermediate 4-hour period (12:00-15:00: Dunn's post hoc, $p = 0.003$) of light phase. Besides, the Mas EMG activity during wakefulness dropped within the transition period from dark phase (4:00-7:00) to light phase (8:00-11:00) (Dunn's post hoc, $p = 0.01$). The Mas EMG activity during NREM sleep ($\chi^2(5) = 12.06$, $p = 0.03$) was significantly higher in the initial period of dark phase than in the initial (Dunn's post hoc, $p = 0.01$) and intermediate 4-hour period (Dunn's post hoc, $p = 0.02$) of light phase. However, there was no significant difference during phase transition. The Dig EMG activity during wakefulness and NREM sleep ($F(5,45) = 2.701$, $p = 0.03$) was significantly higher in the initial period of dark phase than in the initial (Dunnett's post hoc, $p = 0.02$) and intermediate 4-hour period (Dunnett's post hoc, $p = 0.02$) of light phase. The Dig EMG activities dropped within the transition period from dark phase (Bonferroni's post hoc, $p = 0.03$) to light phase. The Dig EMG activity during NREM sleep ($F(5,45) = 3.256$, $p = 0.01$) was significantly higher in the initial period of dark phase than in the initial (Dunnett's post hoc, $p = 0.005$) and intermediate 4-hour period (Dunnett's post hoc, $p = 0.005$) of light phase. However, there was no significant difference during phase transition. In REM sleep, there was no significant difference in time-course manner for three muscles. In general, the EMG activities of three muscles in wakefulness were significantly 4 to 7 times higher than that in NREM and REM sleep, but there was no significant difference in EMG activities of three muscles between NREM and REM sleep (Fig. 3).

Arousals frequency, EEG and EMG activations within arousals

Compared to 20:00-23:00 ($F(5,45) = 6.270$, $p < 0.001$), the frequency was significantly higher in 8:00-11:00 (Dunnett's post hoc, $p = 0.045$) and 12:00-15:00 (Dunnett's post hoc, $p = 0.005$) (Fig. 4 - A). Besides, it also increased significantly during the transition period from dark phase to light phase (Bonferroni's post hoc, $p = 0.028$) (Fig. 4 - B). Therefore, the average frequency of ANR during light phase was significantly higher than that during dark phase (Wilcoxon test, $p = 0.002$). However, the frequency of AR did not differ over 24 h (Fig. 4 - A, B). Considering the diurnal variation in sleep amount, the variation in arousal frequency reflects differences in sleep time rather than a direct time-of-day effect.

The delta power was significantly lower within ANR than without ANR (dark: $t(9) = 7.060$, $p < 0.001$; light: $t(9) = 6.498$, $p < 0.001$). Similarly, the theta power was significantly lower within AR than without AR (Wilcoxon test, dark: $p = 0.002$; light: $p = 0.002$) (Fig. 4 - C, D). EMG activities of three muscles were significantly elevated within ANR or AR in both dark and light phase (Fig. 4 - E, F). More importantly, the EMG activity of Mas and Dig muscles within ANR were significantly higher in dark phase than that in light phase (Mas: $t(9) = 3.379$, $p = 0.005$; Dig: $t(9) = 3.991$, $p = 0.003$). However, there was no difference

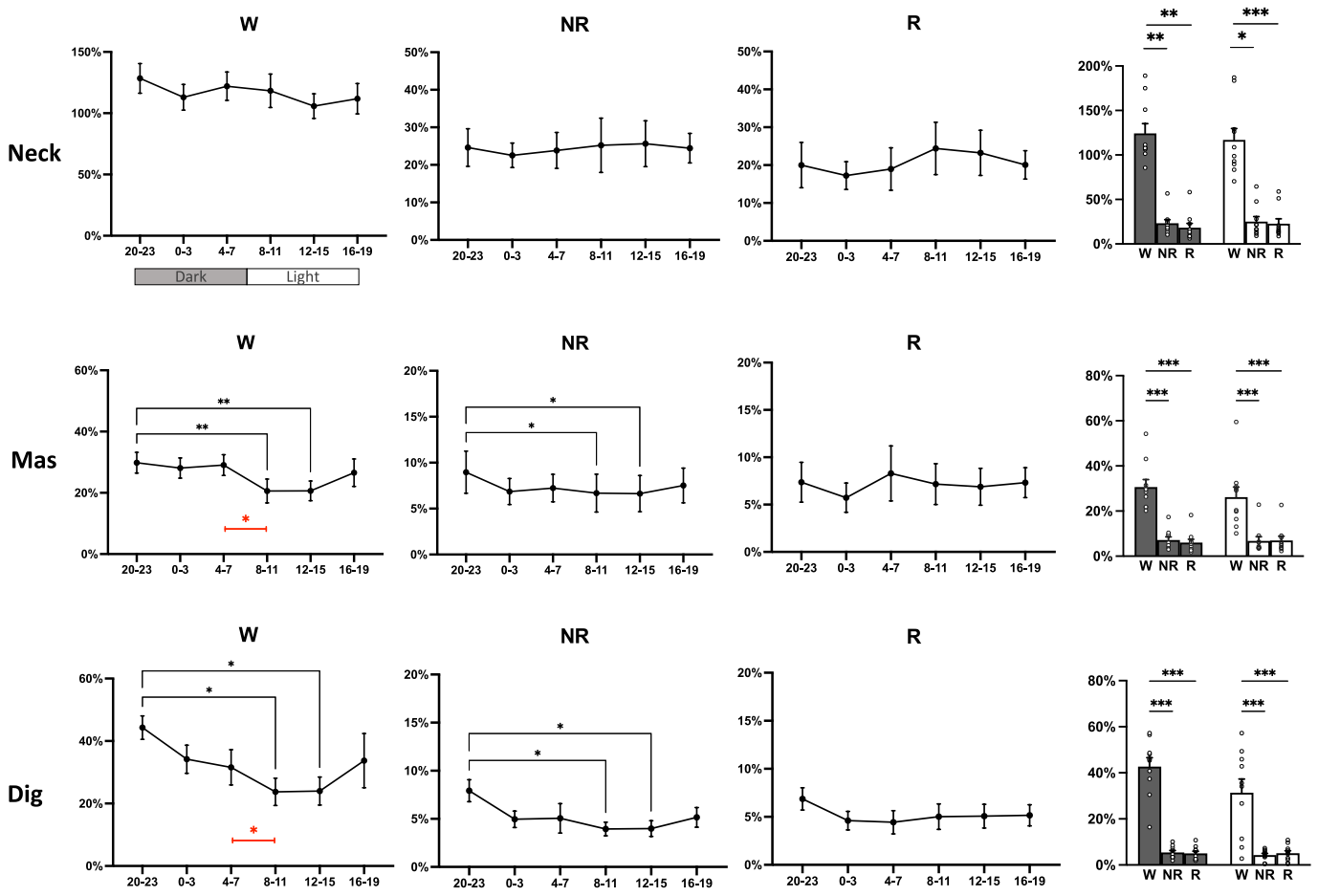

Fig. 3. Time-course change of EMG activities in sleep-wake cycle. Line charts show the time-course change of EMG activities of neck, Mas and Dig muscle in three vigilance states throughout 24 h. One-way repeated measures ANOVA with Dunnett's post hoc or Friedman test with Dunn's post hoc. The bar charts show the mean values of EMG activities of neck, Mas and Dig muscle in each state during dark (black bars) and light (white bars) phase. One-way repeated measures ANOVA with Tukey's post hoc or Friedman test with Dunn's post hoc. N = 10, Mean ± SEM; *p < 0.05, **p < 0.005, ***p < 0.001. W: wakefulness; NR: NREM sleep; R: REM sleep.

of muscle activations within AR between dark and light phase.

The relationship between arousal duration and delta power and EMG activity level

Intermittent arousals during NREM sleep were categorized with duration: 1 epoch (10-sec); 2 epochs (20-sec) and 3 epochs (30-sec). Fig. 5 shows the delta power and EMG activity level of neck, masseter and digastric muscle in different arousal duration during NREM sleep. Delta power was significantly lower in the longer arousal in the dark phase ($F(2, 18) = 56.55$, $p < 0.001$; Tukey's post hoc: $p < 0.001$) and light phase ($F(2, 18) = 38.32$, $p < 0.001$; Tukey's post hoc: $p < 0.001$). Neck EMG activity was significantly higher in the longer arousal in the dark phase ($F(2, 18) = 27.69$, $p < 0.001$; Tukey's post hoc: 1 vs. 2: $p = 0.03$; 1 vs. 3: $p < 0.001$; 2 vs. 3: $p = 0.006$) and light phase ($F(2, 18) = 28.09$, $p < 0.001$; Tukey's post hoc: 1 vs. 2: $p = 0.004$; 1 vs. 3: $p < 0.001$; 2 vs. 3: $p = 0.004$). Masseter EMG activity was significantly higher in the longer arousal in the dark phase ($F(2, 18) = 19.04$, $p < 0.001$; Tukey's post hoc: 1 vs. 2: $p = 0.003$; 1 vs. 3: $p < 0.001$; 2 vs. 3: $p = 0.07$) and light phase ($F(2, 18) = 15.10$, $p < 0.001$; Tukey's post hoc: 1 vs. 2: $p = 0.003$; 1 vs. 3: $p < 0.001$). Digastric EMG activity was significantly higher in the longer arousal in the dark phase ($F(2, 18) = 9.548$, $p = 0.001$; Tukey's post hoc: 1 vs. 2: $p = 0.03$; 1 vs. 3: $p = 0.001$) and light phase ($F(2, 18) = 11.63$, $p < 0.001$; Tukey's post hoc: 1 vs. 2: $p = 0.05$; 1 vs. 3: $p < 0.001$). Besides, in the same 2-epoch or 3-epoch arousal, masseter EMG activity level was significantly higher in dark phase than that in light phase (20-sec: $t(9) = 2.977$, $p = 0.02$; 30-sec: $t(9) = 3.152$, $p = 0.01$).

In REM sleep, the similar phenomenon was observed too, however, the statistical analysis was difficult to conduct due to the lack of enough data of 3-epoch arousal.

Correlation between cortical activity and EMG activity during NREM and REM sleep

An example of a scatter plot from one rat that related the EMG activity of the neck, Mas, and Dig muscles to the delta power during NREM sleep and the theta power during REM sleep is shown in Figs. 6 and 7, upper panels. Majority of epochs with arousals tend to cluster at lower EEG power values and higher EMG activity values while the majority of epochs without arousals were associated with higher EEG power and lower EMG activity. In addition, arousals of different durations are distinguished. It revealed that longer arousals are generally associated with lower delta power and higher EMG activity, consistent with the results in Fig. 5. According to Huber regression modeling, EMG activities of three muscles were in inverse proportion to EEG power during sleep in all rats with a statistically significant linear correlation (Table 1 and 2), Besides, the results of *meta*-analysis showed that the averaged regression coefficient (β) was also statistically significant. During NREM sleep, EMG activity of three muscles were significantly correlation to delta wave power (Table 1; Fig. 6 lower panel). During REM sleep, three muscles were significantly correlation to theta wave power (Table 2; Fig. 7 lower panel).

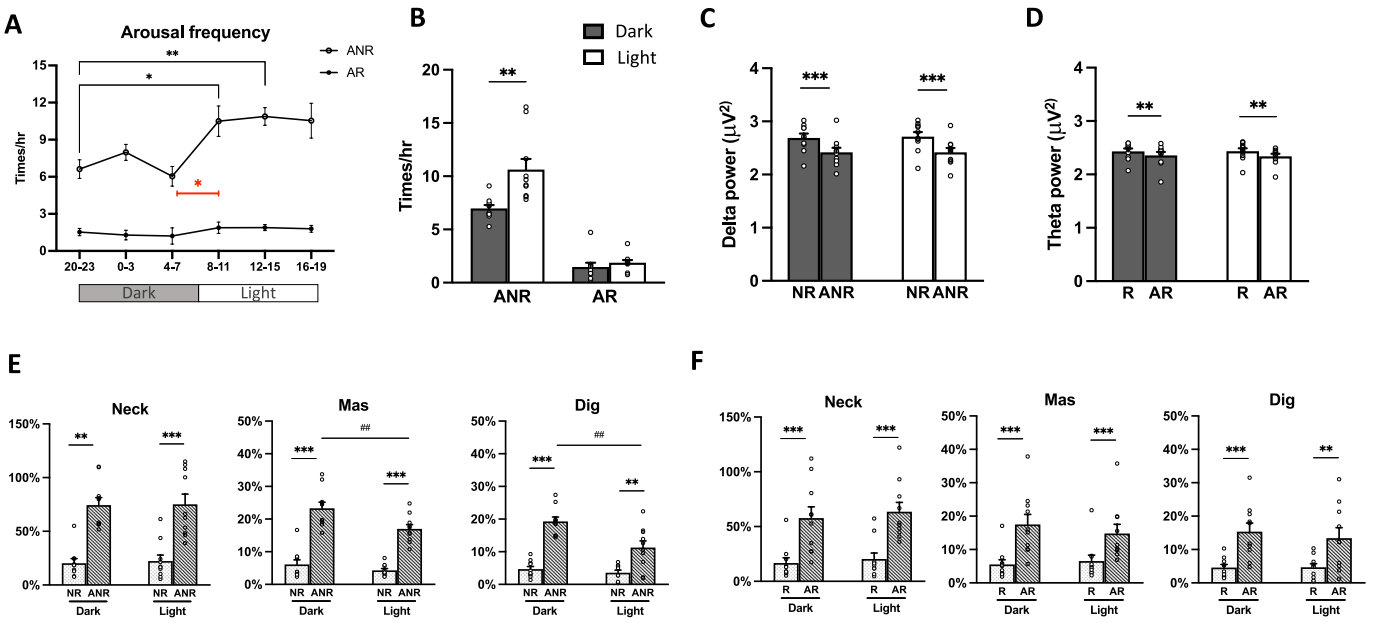

Fig. 4. Arousals frequency, EEG and EMG activations within arousals. A: The time-course change of arousal frequency (times per hour) throughout 24 h. One-way repeated measures ANOVA with Bonferroni's or Dunnett's post hoc or Friedman test with Dunn's post hoc. B: The frequency of ANR and AR in dark and light phase. C: The mean value of delta power during NREM sleep and ANR in dark and light phase. D: The mean value of theta power during REM sleep and AR in dark and light. E: The comparisons of EMG activities of neck, Mas and Dig muscle between ANR and NR during dark and light phase. F: The comparisons of EMG activities of neck, Mas and Dig between AR and R during dark and light phase. B-F: Mean ± SEM, Paired *t* test or Wilcoxon matched-pairs signed rank test, *p < 0.05, **p < 0.005, ***p < 0.001; ##p < 0.005. N = 10. NR: NREM sleep; R: REM sleep; ANR: arousals within NREM sleep; AR: arousals within REM sleep.

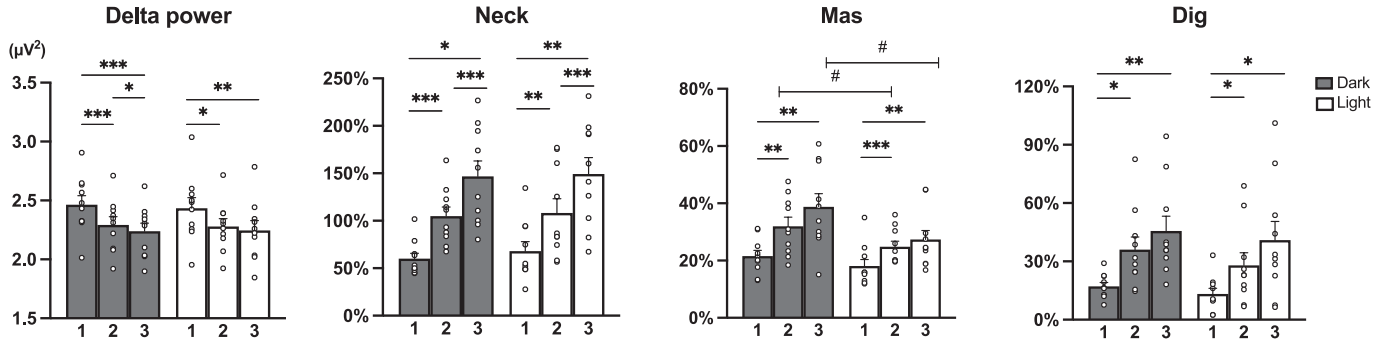

Fig. 5. The relationship between arousal duration and delta power and EMG activity level. Comparison of delta power and EMG activity level between different arousal duration in the dark and light phase. The numbers under horizontal axis represent the length of arousal duration; 1: one-epoch; 2: two-epoch; 3: three-epoch. N = 10, Mean ± SEM. One-way repeated measures ANOVA with Tukey's post hoc, *p < 0.05, **p < 0.005, ***p < 0.001. Diurnal differences were compared by Paired *t* test, #p < 0.05.

Activations of multiple muscles within intermittent arousals during NREM and REM sleep

Within arousals in NREM sleep (Fig. 8), the activations of multiple muscles were significantly more frequent than single or no activation for three muscle pairs. For neck-Mas pair, the ratio in area 1 was significantly higher than those in other areas ($\chi^2(5) = 16.82$, $p < 0.001$; Dunn's post hoc: area 1 vs. area 2: $p = 0.04$; area 3: $p = 0.002$; area 4: $p = 0.002$); for neck-Dig pair, the ratio in area 1 was significantly higher than those in other areas ($\chi^2(5) = 19.08$, $p < 0.001$; Dunn's post hoc: area 1 vs. area 2: $p = 0.02$; area 3: $p = 0.002$; area 4: $p < 0.001$); and for Mas-Dig pair, the ratio in area 1 was significantly higher than those in other areas ($\chi^2(5) = 20.52$, $p < 0.001$; Dunn's post hoc: area 1 vs. area 2: $p < 0.001$; area 3: $p = 0.003$; area 4: $p = 0.03$).

Similar results were also observed in REM sleep (Fig. 9). For neck-Mas pair, the ratio in area 1 was significantly higher than those in other areas ($\chi^2(5) = 18.34$, $p < 0.001$; Dunn's post hoc: area 1 vs. area 2: $p = 0.04$; area 3: $p = 0.01$; area 4: $p < 0.001$); for neck-Dig pair, the ratio in area 1 was significantly higher than those in other areas ($F(3, 27) = 31.22$, $p < 0.001$; Tukey's post hoc: area 1 vs. area 2: $p = 0.004$; area 3: $p < 0.001$; area 4: $p < 0.001$); and for Mas-Dig pair, the ratio in area 1 was significantly higher than those in other areas ($F(3, 27) = 41.81$, $p < 0.001$; Tukey's post hoc: area 1 vs. area 2: $p = 0.003$; area 3: $p < 0.001$; area 4: $p < 0.001$).

On the contrary, during quiet sleep without arousals, the ratio of no activation was significantly higher than that of single or multiple activation. In NREM sleep (Fig. 8), for neck-Mas pair, the ratio in area 4 was significantly higher than those in other areas ($F(3, 27) = 22.81$, $p < 0.001$; Tukey's post hoc: area 4 vs. area 1: $p < 0.001$; area 2: $p = 0.002$; area 3: $p = 0.002$); for neck-Dig pair, the ratio in area 4 was significantly higher than those in other ($F(3, 27) = 24.97$, $p < 0.001$; Tukey's post hoc: area 4 vs. area 1: $p < 0.001$; area 2: $p < 0.001$; area 3: $p < 0.001$).; and for Mas-Dig pair, the ratio in area 4 was significantly higher than those in other areas ($\chi^2(5) = 17.40$, $p < 0.001$; Dunn's post hoc: area 4 vs. area 1: $p < 0.001$; area 2: $p = 0.02$; area 3: $p = 0.02$).

In REM sleep (Fig. 9), for neck-Mas pair, the ratio in area 4 was

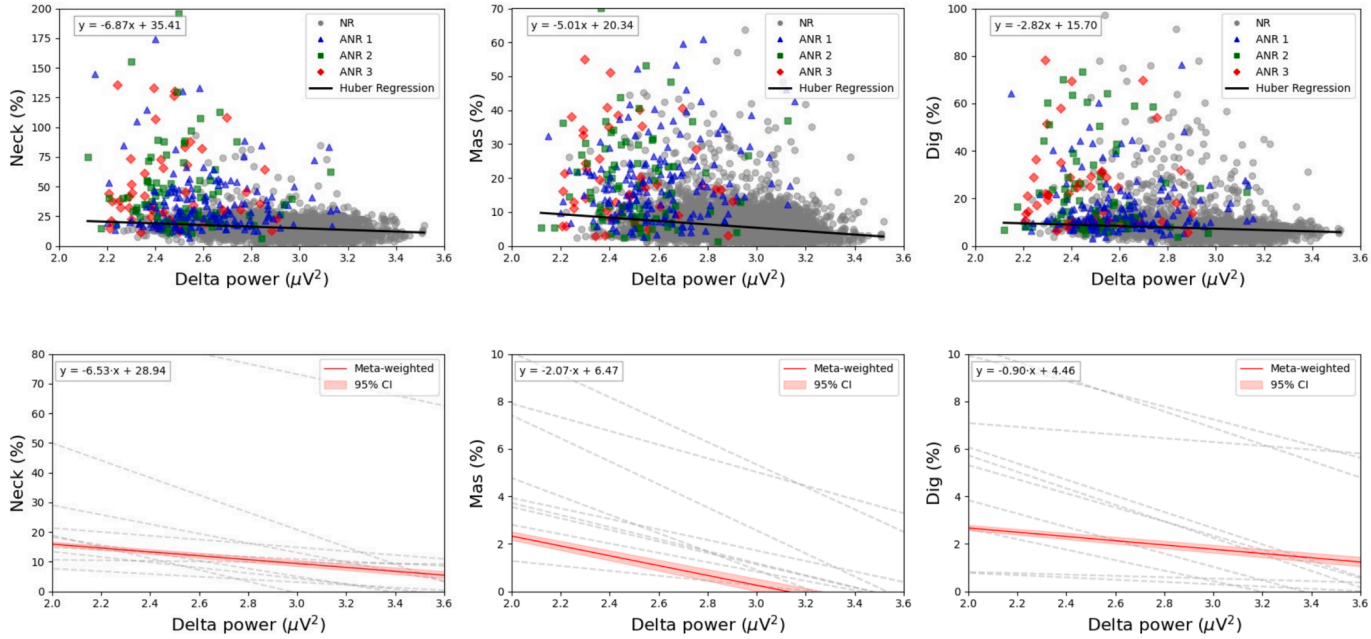

Fig. 6. Correlation analysis of delta power and muscle activity during NREM sleep. Upper panel: The example of the scatter plot of the EMG activities of neck, Mas, and Dig muscles relative to the delta power during NREM sleep. Lower panel: Single grey dot lines represent an individual correlation liner analyzed by Huber regression model. The red line shows the weighted average of ten subjects by *meta*-analysis with 95 % confidence interval showed as red shadow (N = 10). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

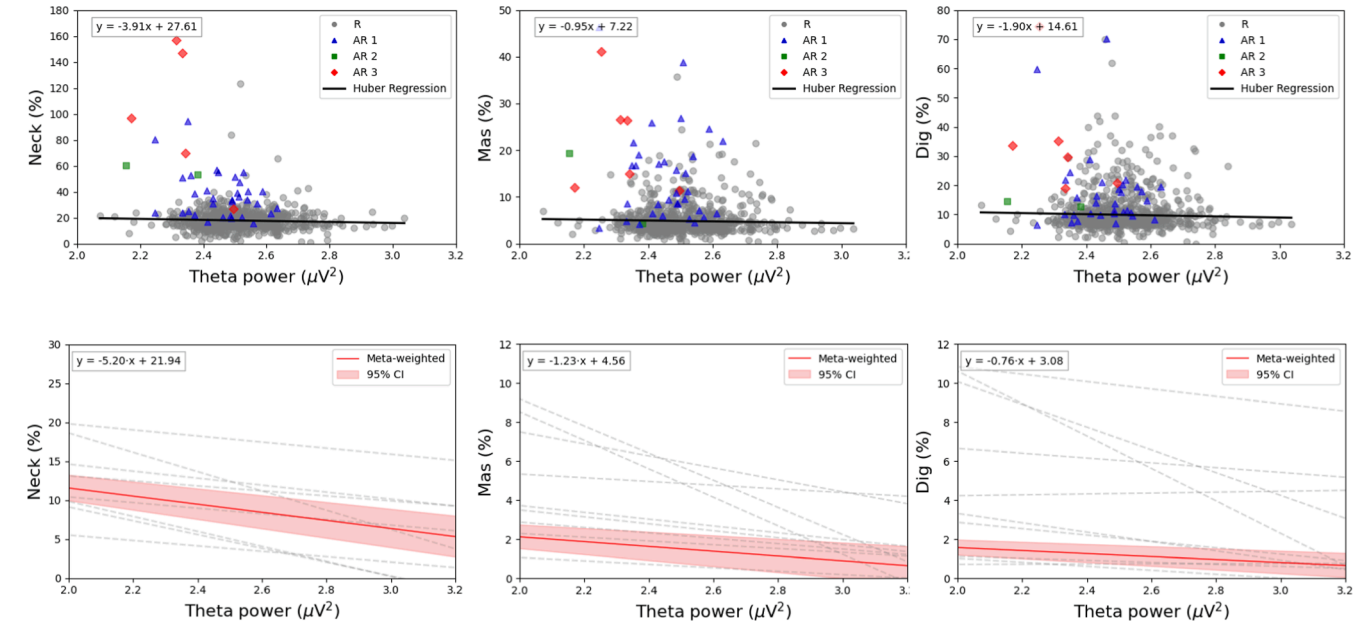

Fig. 7. Correlation analysis of theta power and muscle activity during REM sleep. Upper panel: The example of the scatter plot of the EMG activities of neck, Mas, and Dig muscles relative to the theta power during REM sleep. Lower panel: Single grey dot lines represent an individual correlation liner analyzed by Huber regression model. The red line shows the weighted average of ten subjects by *meta*-analysis with 95 % confidence interval showed as red shadow (N = 10). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly higher than those in other areas ($F(3, 27) = 26.95$, $p < 0.001$; Tukey's post hoc: area 4 vs. area 1: $p < 0.001$; area 2: $p = 0.003$; area 3: $p < 0.001$); for neck-Dig pair, the ratio in area 4 was significantly higher than those in other areas ($F(3, 27) = 18.41$, $p < 0.001$; Tukey's post hoc: area 4 vs. area 1: $p = 0.003$; area 2: $p = 0.002$; area 3: $p < 0.001$); and for Mas-Dig pair, the ratio in area 4 was significantly higher than those in other areas ($\chi^2(5) = 19.92$, $p < 0.001$; Dunn's post hoc: area 4 vs. area 1: $p < 0.001$; area 2: $p = 0.004$; area 3: $p < 0.001$).

Discussion

In this study, we measured jaw muscle activity level during sleep over 24 h and analyzed factors influencing its variability. The results showed that jaw muscle activity during NREM sleep was lower in the light phase than in the early dark phase. Jaw muscle activity within intermittent arousals was several times higher than during quiet sleep. Additionally, in NREM sleep, jaw muscle activity during intermittent arousals was higher in the dark phase than in the light phase. Jaw

Table 1
The results of Huber regression between neck and jaw muscle activity and delta wave power during NREM sleep among 10 subjects and the weighted average estimated by *meta*-analysis model. *β [CI]*: regression coefficient with 95% confidence interval; WA: weighted average.

	Neck			Mas			Dig		
	β [CI]	*t*	*p*	*β [CI]*	*t*	*p*	β [CI]	*t*	*p*
#1	-9.67 [-10.49, -8.85]	-23.25	<0.001	-4.23 [-4.60, -3.87]	-22.80	<0.001	-3.81 [-4.38, -3.24]	-13.19	<0.001
#2	-4.42 [-5.81, -3.04]	-6.27	<0.001	-2.62 [-3.03, -2.20]	-12.34	<0.001	-0.31 [-0.39, -0.22]	-7.10	<0.001
#3	-6.87 [-7.73, -6.02]	-15.79	<0.001	-5.01 [-5.54, -4.47]	-18.21	<0.001	-2.82 [-3.15, -2.50]	-16.91	<0.001
#4	-4.97 [-5.66, -4.28]	-14.07	<0.001	-3.23 [-3.80, -2.67]	-11.18	<0.001	-0.53 [-0.65, -0.42]	-9.32	<0.001
#5	-30.46 [-33.12, -27.80]	-22.46	<0.001	-7.88 [-8.73, -7.03]	-18.13	<0.001	-2.36 [-2.59, -2.12]	-19.33	<0.001
#6	-17.10 [-18.99, -15.22]	-17.79	<0.001	-5.28 [-5.76, -4.79]	-21.37	<0.001	-0.88 [-1.02, -0.75]	-12.90	<0.001
#7	-1.36 [-2.01, -0.70]	-4.06	<0.001	-2.50 [-2.88, -2.12]	-12.84	<0.001	-3.96 [-4.64, -3.28]	-11.39	<0.001
#8	-20.66 [-24.30, -17.01]	-11.12	<0.001	-2.78 [-3.01, -2.55]	-23.78	<0.001	-3.81 [-4.11, -3.50]	-24.49	<0.001
#9	-20.85 [-23.35, -18.36]	-16.40	<0.001	-1.13 [-1.27, -0.99]	-15.97	<0.001	-2.99 [-3.28, -2.70]	-20.16	<0.001
#10	-13.91 [-14.96, -12.87]	-26.14	<0.001	-2.08 [-2.27, -1.89]	-21.72	<0.001	-3.12 [-3.44, -2.81]	-19.26	<0.001
WA	**-7.09 [-7.42, -6.77]**	**-42.62**	**<0.001**	**-2.24 [-2.33, -2.16]**	**-50.48**	**<0.001**	**-0.98 [-1.03, -0.92]**	**-35.17**	**<0.001**

Table 2
The results of Huber regression between neck and jaw muscles activity and theta wave power during REM sleep among 10 subjects and the weighted average estimated by *meta*-analysis model. *β [CI]*: regression coefficient with 95% confidence interval; WA: weighted average.

	Neck			Mas			Dig		
	β [CI]	*t*	*p*	*β [CI]*	*t*	*p*	β [CI]	*t*	*p*
#1	-3.46 [-4.98, -1.94]	-4.47	<0.001	-1.39 [-3.25, 0.48]	-1.46	0.14	-1.63 [-2.82, -0.44]	-2.69	0.007
#2	-3.61 [-6.15, -1.08]	-2.79	0.005	-0.97 [-1.92, -0.01]	-1.98	0.04	0.04 [-0.28, 0.36]	0.26	0.79
#3	-3.91 [-7.83, -0.01]	-1.95	0.05	-0.95 [-2.35, 0.44]	-1.34	0.18	-1.90 [-4.42, 0.62]	-1.48	0.14
#4	-8.84 [-10.96, -6.72]	-8.16	<0.001	-3.07 [-4.52, -1.61]	-4.13	<0.001	-1.02 [-1.39, -0.65]	-5.43	<0.001
#5	0.85 [-6.68, -8.39]	0.22	0.82	2.71 [0.61, 4.81]	2.53	0.01	-0.51 [-0.92, -0.11]	-2.48	0.01
#6	-12.40 [-15.52, -9.27]	-7.78	<0.001	-7.30 [-9.24, -5.36]	-7.38	<0.001	-5.83 [-6.91, -4.76]	-10.63	<0.001
#7	-3.21 [-4.85, -1.57]	-3.84	<0.001	-1.78 [-3.18, -0.39]	-2.50	0.01	-1.23 [-3.01, 0.55]	-1.35	0.17
#8	-24.60 [-35.40, -13.80]	-4.46	<0.001	-6.95 [-9.96, -3.94]	-4.52	<0.001	-8.31 [-11.39, -5.23]	-5.29	<0.001
#9	-9.62 [-13.80, -5.43]	-4.50	<0.001	-0.88 [-1.26, -0.49]	-4.46	<0.001	-2.40 [-3.37, -1.42]	-4.83	<0.001
#10	-4.49 [-9.07, 0.09]	-1.92	0.05	-1.75 [-3.12, -0.38]	-2.50	0.01	0.23 [-1.42, 1.88]	0.27	0.79
WA	**-5.1989 [-6.02, -4.38]**	**-12.40**	**<0.001**	**-1.23 [-1.53, -0.93]**	**-7.91**	**<0.001**	**-0.76 [-0.96, -0.57]**	**-7.74**	**<0.001**

muscle activity in NREM sleep showed a negative correlation with EEG delta power, while in REM sleep, it was negatively correlated with EEG theta power. Multiple muscles, including cervical and jaw-opening/closing muscles, exhibited high activity levels during intermittent arousals. These findings suggest that jaw muscle activity during sleep is largely influenced by intermittent arousals under diurnal variations.

Diurnal variation of jaw muscle activity across sleep-wake states

The present results showed the diurnal variations of jaw muscle activity during wakefulness and NREM sleep, with a decline in the light phase from the early dark phase. The results are somewhat similar to those in the previous study in mice in which jaw muscle activity level during wakefulness and NREM sleep were significantly decreased during dark-light phase transition period (Katayama et al., 2015). The temporal pattern of the changes in jaw muscle activity level during wakefulness can be related to the functional oral movements from dark to light phases including feeding (Strubbe et al., 1986), drinking (Stephan and Zucker, 1972; Ryabinina et al., 2024) and grooming (Bolles, 1960; Li et al., 2024) while these oromotor activities less frequently occur during light phase (Witting et al., 1993; Raymond et al., 2023). As has been reported in the previous study (Eastman et al., 1984; van Betteray et al., 1991; Stephenson et al., 2012; Fisk et al., 2018), time spent in sleep was significantly shorter during dark phase than that during light phase. These diurnal changes reflect the lower sleep pressure and higher arousal level during dark phase than light phase (Deboer, 2018; Hasan et al., 2018).

During NREM sleep, jaw muscle activity was one-fourth of that

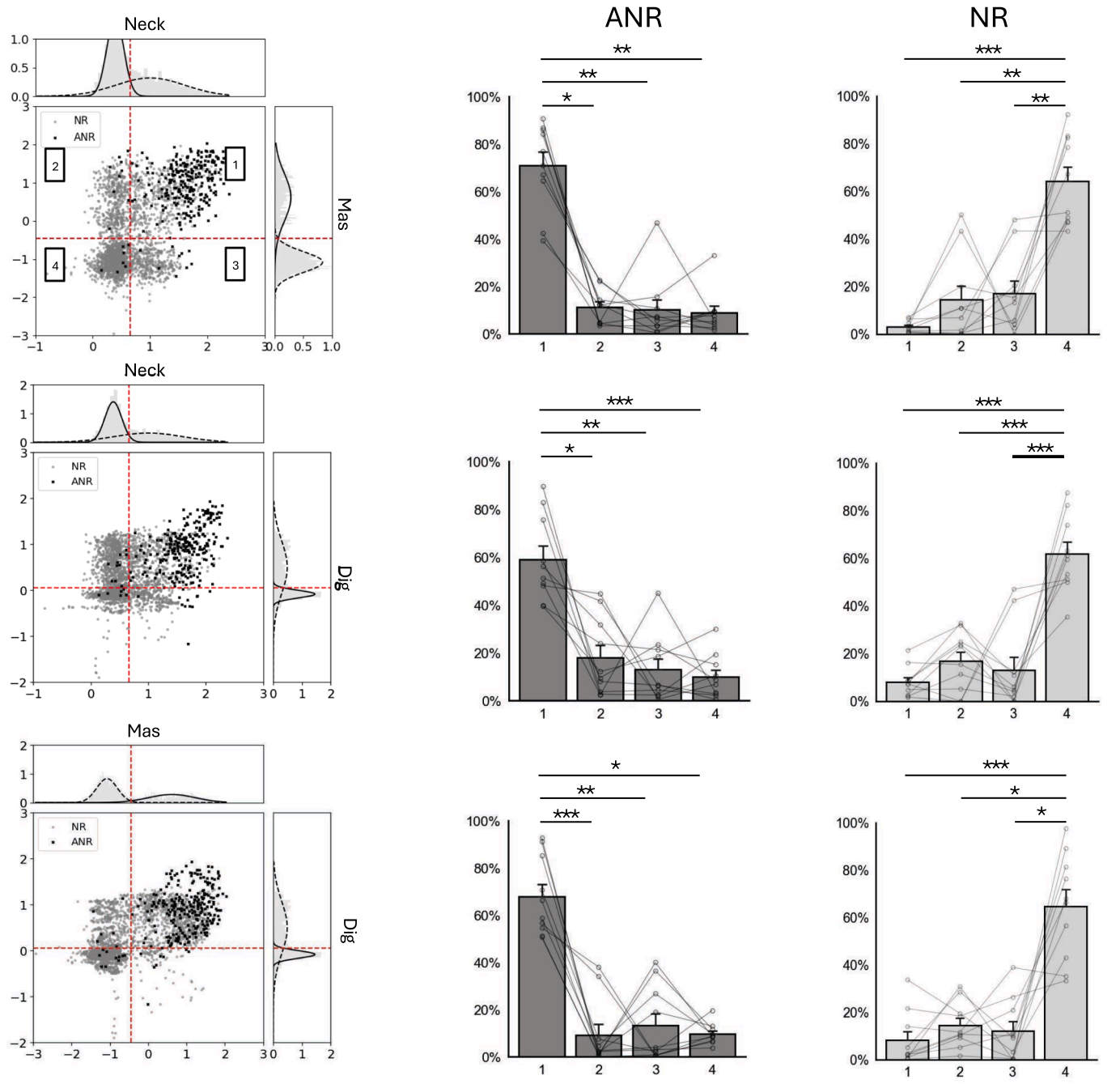

Fig. 8. Activations of multiple muscles within intermittent arousals during NREM sleep. Left panel shows the examples of the scatter plots and clustering of muscle activations during NREM sleep among neck, masseter (Mas) and digastric (Dig). In the scatter plots, the grey dots represent EMG activities during quiet NREM sleep and the black dots represent that during ANR. In the clustering plot near axis, the red dot line shows the intersection of two clusters and divided the scatter plot into four areas. Middle panel shows the ratio that the number of black dots in each area divided by the total number of black dots in all areas. Right panel shows the ratio that the number of grey dots in each area divided by the total number of grey dots in all areas. N = 10, Mean ± SEM, One-way repeated measures ANOVA with Tukey's post hoc or Friedman test with Dunnett's post hoc. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during wakefulness as functional oromotor activity disappeared (Kato et al., 2010). However, there were diurnal variations of jaw muscle activity level during sleep from dark phase to light phase. Previous studies suggested diurnal changes of sleep pressure and arousal levels during NREM sleep. For example, EEG delta power during NREM sleep was low during dark phase and increase towards the light phase with a peak in the early light phase (Steinfels et al., 1980; Yasenkov and Deboer, 2010; Zhang et al., 2017; Masuda et al., 2023). Diurnal changes of EEG delta power was inversely correlated with the autonomic activity during NREM sleep over 24 h (Jurysta et al., 2006; Thomas et al., 2014; Rothenberger et al., 2015), i.e., the autonomic activity was higher in the early dark phase and decrease towards the light phase (Sei et al., 1997; Hashimoto et al., 1999; Sheward et al., 2010; Barazi et al., 2021). Therefore, sleep pressure and arousal level can underlie the diurnal variation of jaw muscle activity levels during NREM sleep. However, there was no difference in jaw muscle activity level during REM sleep between dark and light phases. It suggests that REM sleep regulation is less affected by diurnal variation (Yasenkov and Deboer, 2010;

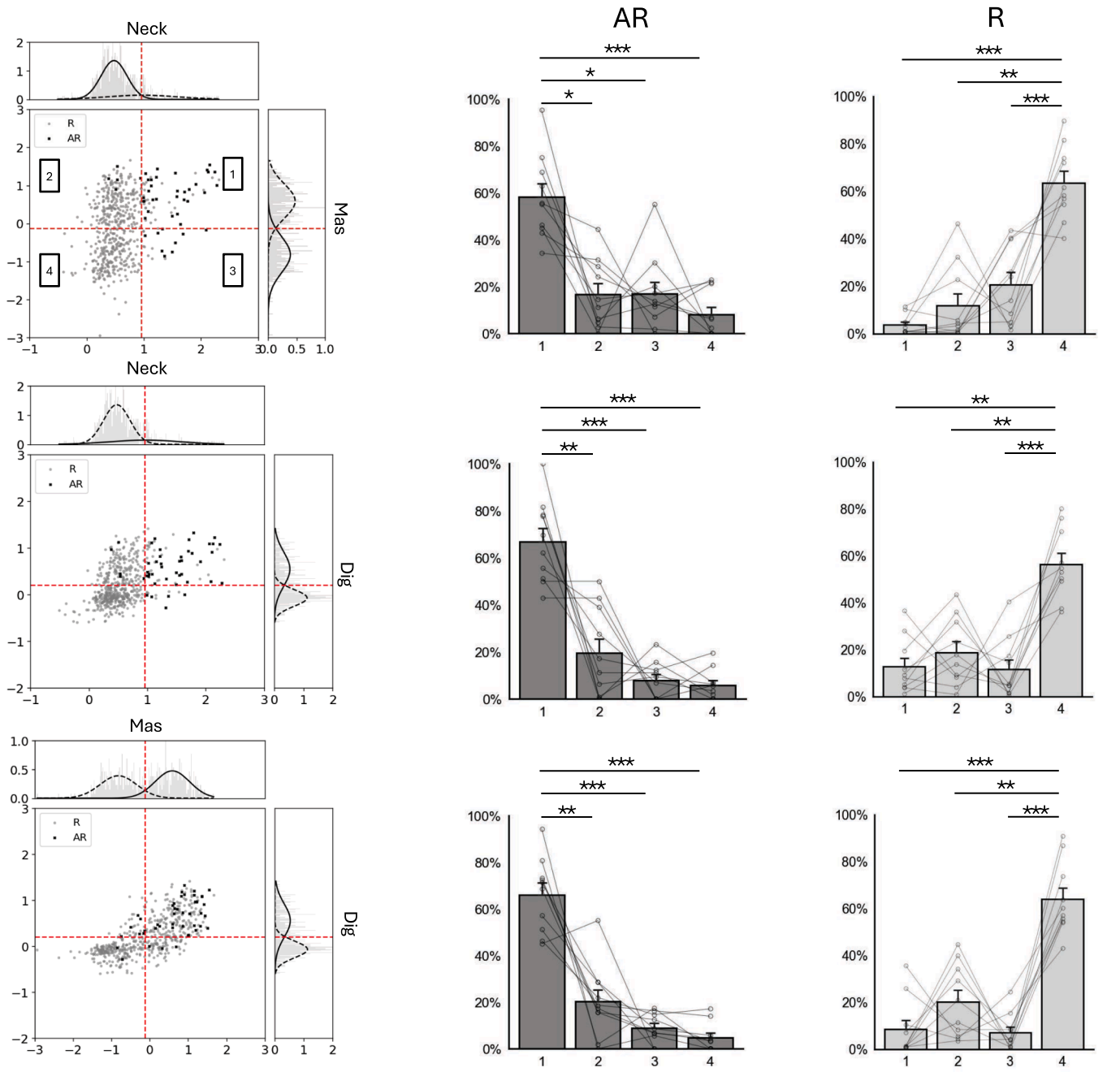

Fig. 9. Activations of multiple muscles within intermittent arousals during REM sleep. Left panel shows the examples of the scatter plots and clustering of muscle activations during REM sleep among neck, masseter (Mas) and digastric (Dig). In the scatter plots, the grey dots represent EMG activities during quiet REM sleep and the black dots represent that during AR. In the clustering plot near, the red dot line shows the intersection of two clusters and divided the scatter plot into four areas. Middle panel shows the ratio that the number of black dots in each area divided by the total number of black dots in all areas. Right panel shows the ratio that the number of grey dots in each area divided by the total number of grey dots in all areas. N = 10, Mean ± SEM, One-way repeated measures ANOVA with Tukey's post hoc or Friedman test with Dunnett's post hoc. *p < 0.05, **p < 0.005, ***p < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Amatoury et al., 2016; Park and Weber, 2020), resulting in less fluctuation in jaw muscle activity throughout a day.

Intermittent arousals contribute to jaw muscle activations during sleep as a hierarchical window

This study demonstrated that intermittent arousals significantly contribute to variability in jaw muscle activity during sleep, with activity levels 3–6 times higher than quiet sleep in NREM and REM. Longer arousal durations were associated with decreased EEG power and increased muscle activity, and that EEG power was negatively correlated with muscle activation levels. These findings suggest that EEG activity linked to arousal duration may thus serve as indicator of arousal intensity eliciting muscle activation. Besides, intermittent arousals exhibit hierarchical intensity levels, with stronger arousal level associated with increased muscle activity (Kato et al., 2010; Kato et al., 2013a). These findings are supported by the previous proposal that intermittent arousal was proved as a physiological window of a hierarchical activation from autonomic changes to EEG alterations and motor manifestations of neck and jaw muscles (Kato et al., 2001; Kato et al., 2004; Kato

et al., 2010).

Notably, jaw muscle activation during intermittent arousals in NREM sleep was significantly higher in dark phase than in light phase, suggesting that the diurnal variation in hierarchical levels of intermittent arousals, especially in relative longer arousals. The high jaw muscle activation during intermittent arousals in dark phase can be related to the increased arousal intensity, which is largely influenced by the diurnal changes of sleep and arousal pressures.

The hypothesized physiological meaning of multiple muscle activation within intermittent arousals during sleep

When muscle activity level was classified into high and low level using the Gaussian Mixture Model (GMM) classification of muscle activity levels, approximately 60 % of intermittent arousal epochs exhibited higher level of multiple muscle activity while 60 % of quiet sleep epochs showed low activity levels (Figs. 8 and 9). This finding suggests that intermittent arousals may facilitate a motor response involving multiple muscles, rather than isolated activation (Kato et al., 2004; Kato et al., 2010; Kato et al., 2015; Gouw et al., 2020).

From a functional perspective, this multi-muscle activation reflects a form of preparatory motor behavior (Benarroch, 2018). Arousal-related co-activation of jaw and neck muscles may represent a potential motor readiness, potentially serving to facilitate behavioral responses to internal or external stimuli without full awakening (Kato et al., 2004; Lavigne et al., 2004). Furthermore, this activation may serve protective roles, such as maintaining airway patency or oral motor behavior during sleep (Thie et al., 2002; Ma et al., 2013) which could have implications for understanding conditions like sleep bruxism, OSA, or sleep-related orofacial pain.

Limitations

A key limitation of this study is the use of manual scoring of vigilance states based on 10-second epochs. Although trained raters performed the scoring and inter-rater reliability was checked (Cohen's Kappa score: 0.915) (Wendt et al., 2015; Katz et al., 2025), the process is still subjective and may miss subtle changes in EEG or EMG signals. The 10-second epoch, while commonly used in rodent sleep research, may be too long to capture short events like microarousals or twitches in REM sleep (Anaclet et al., 2010; Fraigne and Orem, 2011; Kato et al., 2013b). Jaw muscle twitches during REM sleep could not be clearly separated from GMM-defined muscle tone clusters. These issues suggest that both the scoring method and the time resolution could under-estimated the arousal events and the correlation between EEG and EMG activity.

Another limitation is that the study only included healthy male rats. This was done to reduce variability and align with earlier studies, but it limits how broadly the results can be applied. Without data from female animals or disease models, it's difficult to generalize the findings to human conditions like sleep bruxism or sleep apnea.

Finally, our use of Gaussian Mixture Models (GMM) offers a way to separate EMG signals into "muscle tone" and "muscle contraction" components. The bimodal distribution is consistent with prior findings in both humans and mice (Katayama et al., 2015; Toyota et al., 2022), which supports our interpretation. However, this method is based on statistical patterns rather than direct biological confirmation.

Future studies should aim to enhance accuracy and translational relevance by employing shorter scoring epochs, validated automated methods, inclusion of both sexes and disease models, and clustering of EMG with behavioral data to validate physiological interpretations.

Conclusions

The present study highlights that arousal level can shape the variability of jaw muscle activity during sleep and that intermittent arousals act as a hierarchical window linking cortical and motor responses under diurnal changes. These findings provide insights into the natural variations of oromotor system across sleep-wake cycles and the underlying mechanism of sleep-related oromotor movements.

CRediT authorship contribution statement

Yiwen Zhu: Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis, Data curation. **Masaharu Yamada:** Writing – review & editing, Methodology, Investigation. **Noriko Minota:** Writing – review & editing, Methodology, Investigation. **Ayano Katagiri:** Writing – review & editing. **Takafumi Kato:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We sincerely thank Dr. Okamoto for her technical support. This research was supported by the Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research (B) [24K02631] and Grant-in-Aid for JSPS Fellows [25KJ1801], with partial support from the Grant-in-Aid for Challenging Research (Exploratory) [21K19594] and JST-Mirai Program [JP20348555].

References

Amatoury, J., Azarbarzin, A., Younes, M., Jordan, A.S., Wellman, A., Eckert, D.J., 2016. Arousal Intensity is a Distinct Pathophysiological Trait in Obstructive sleep Apnea. Sleep 39, 2091–2100.

Barazi, N., Polidovitch, N., Debi, R., Yakobov, S., Lakin, R., Backx, P.H., 2021. Dissecting the Roles of the Autonomic nervous System and Physical activity on Circadian Heart Rate Fluctuations in mice. Front. Physiol. 12, 692247.

Benarroch, E.E., 2018. Brainstem integration of arousal, sleep, cardiovascular, and respiratory control. Neurology 91, 958–966.

Bolles, R.C., 1960. Grooming behavior in the rat. J. Comp. Physiol. Psychol. 53, 306–310.

Bueno-Junior, L.S., Ruckstuhl, M.S., Lim, M.M., Watson, B.O., 2023. The temporal structure of REM sleep shows minute-scale fluctuations across brain and body in mice and humans. Proc. Natl. Acad. Sci. USA 120, e2213438120.

Burgess, C., Lai, D., Siegel, J., Peever, J., 2008. An endogenous glutamatergic drive onto somatic motoneurons contributes to the stereotypical pattern of muscle tone across the sleep-wake cycle. J. Neurosci. 28, 4649–4660.

Cardis, R., Lecci, S., Fernandez, L.M.J., Osorio-Forero, A., Chu Sin Chung, P., Fulda, S., Decosterd, I.Lüthi, A., 2021. Cortico-autonomic local arousals and heightened somatosensory arousability during NREMS of mice in neuropathic pain. eLife, 10, e65835.

Deboer, T., 2018. Sleep homeostasis and the circadian clock: do the circadian pacemaker and the sleep homeostat influence each other's functioning? Neurobiol Sleep Circadian Rhythms 5, 68–77.

Eastman, C.I., Mistlberger, R.E., Rechtschaffen, A., 1984. Suprachiasmatic nuclei lesions eliminate circadian temperature and sleep rhythms in the rat. Physiol. Behav. 32, 357–368.

Fisk, A.S., Tam, S.K.E., Brown, L.A., Vyazovskiy, V.V., Bannerman, D.M., Peirson, S.N., 2018. Light and Cognition: Roles for Circadian Rhythms, sleep, and Arousal. Front. Neurol. 9, 56.

Gouw, S., Frowein, A., Braem, C., De Wijer, A., Creugers, N.H.J., Pasman, J.W., Doorduin, J., Kalaykova, S.I., 2020. Coherence of jaw and neck muscle activity during sleep bruxism. J. Oral Rehabil. 47, 432–440.

Grünheid, T., Langenbach, G.E.J., Zentner, A.V., Eijden, T.M.G.J., 2005. Circadian variation and intermuscular correlation of rabbit jaw muscle activity. Brain Res. 1062, 151–160.

Hasan, S., Foster, R.G., Vyazovskiy, V.V., Peirson, S.N., 2018. Effects of circadian misalignment on sleep in mice. Sci. Rep. 8, 15343.

Hashimoto, M., Kuwahara, M., Tsubone, H., Sugano, S., 1999. Diurnal variation of autonomic nervous activity in the rat: investigation by power spectral analysis of heart rate variability. J. Electrocardiol. 32, 167–171.

Hooijmans, C.R., Inthout, J., Ritskes-Hoitinga, M., Rovers, M.M., 2014. Meta-analyses of animal studies: an introduction of a valuable instrument to further improve healthcare. ILAR J. 55, 418–426.

Huber, P.J., 1992. Robust Estimation of a Location Parameter. In: Kotz, S., Johnson, N.L. (Eds.), Breakthroughs in Statistics: Methodology and Distribution. New York, NY: Springer New York.
Imai, H., Haraki, S., Tsujisaka, A., Okura, M., Adachi, H., Ohno, Y., Yatani, H., Kato, T., 2021. A lack of specific motor patterns between rhythmic/non-rhythmic masticatory muscle activity and bodily movements in sleep bruxism. J. Prosthodont. Res. 65, 415–420.
Jurysta, F., Lanquart, J.P., Van De Borne, P., Migeotte, P.F., Dumont, M., Degaute, J.P., Linkowski, P., 2006. The link between cardiac autonomic activity and sleep delta power is altered in men with sleep apnea-hypopnea syndrome. Am. J. Physiol. Regul. Integr. Comp. Physiol. 291, R1165–R1171.
Katayama, K., Mochizuki, A., Kato, T., Ikeda, M., Ikawa, Y., Nakamura, S., Nakayama, K., Wakabayashi, N., Baba, K., Inoue, T., 2015. Dark/light transition and vigilance states modulate jaw-closing muscle activity level in mice. Neurosci. Res. 101, 24–31.
Kato, T., Rompré, P., Montplaisir, J.Y., Sessle, B.J., Lavigne, G.J., 2001. Sleep bruxism: an oromotor activity secondary to micro-arousal. J. Dent. Res. 80, 1940–1944.
Kato, T., Montplaisir, J.Y., Lavigne, G.J., 2004. Experimentally induced arousals during sleep: a cross-modality matching paradigm. J. Sleep Res. 13, 229–238.
Kato, T., Masuda, Y., Kanayama, H., Morimoto, T., 2007. Muscle activities are differently modulated between masseter and neck muscle during sleep-wake cycles in guinea pigs. Neurosci. Res. 58, 265–271.
Kato, T., Masuda, Y., Kanayama, H., Nakamura, N., Yoshida, A., Morimoto, T., 2010. Heterogeneous activity level of jaw-closing and -opening muscles and its association with arousal levels during sleep in the guinea pig. Am. J. Physiol. Regul. Integr. Comp. Physiol. 298, R34–R42.
Kato, T., Masuda, Y., Yoshida, A., Morimoto, T., 2011. Masseter EMG activity during sleep and sleep bruxism. Arch. Ital. Biol. 149, 478–491.
Kato, T., Katase, T., Yamashita, S., Sugita, H., Muraki, H., Mikami, A., Okura, M., Ohi, M., Masuda, Y., Taniguchi, M., 2013a. Responsiveness of jaw motor activation to arousals during sleep in patients with obstructive sleep apnea syndrome. J. Clin. Sleep Med. 9, 759–765.
Kato, T., Yamaguchi, T., Okura, K., Abe, S., Lavigne, G.J., 2013b. Sleep less and bite more: sleep disorders associated with occlusal loads during sleep. J. Prosthodont. Res. 57, 69–81.
Kato, T., Masuda, Y., Miyano, K., Higashiyama, M., Yano, H., Haque, T., Sato, F., Yoshida, A., 2015. Distinct association between the antagonistic jaw muscle activity levels and cardiac activity during chewing and NREM sleep in the freely moving guinea pigs. Neurosci. Lett. 592, 59–63.
Kato, T., Toyota, R., Haraki, S., Yano, H., Higashiyama, M., Ueno, Y., Yano, H., Sato, F., Yatani, H., Yoshida, A., 2018. Comparison of rhythmic masticatory muscle activity during non-rapid eye movement sleep in guinea pigs and humans. J. Sleep Res. 27, e12608.
Katz, S.L., Blinder, H., Dussah, N., Bijelić, V., Barrowman, N., Cox, G., Leitman, A.R., Hamutcu Ersu, R., 2025. Inter-rater reliability of video clips to assess for obstructive sleep apnea in children. Sleep Med. 131, 106483.
Kawai, N., Tanaka, E., Langenbach, G.E.J., Van Wessel, T., Brugman, P., Sano, R., Van Eijden, T.M.G.J., Tanne, K., 2007. Daily jaw muscle activity in freely moving rats measured with radio-telemetry. Eur. J. Oral Sci. 115, 15–20.
Langenbach, G.E.J., Van Wessel, T., Brugman, P.V., Eijden, T.M.G.J., 2004. Variation in daily masticatory muscle activity in the rabbit. J. Dent. Res. 83, 55–59.
Lavigne, G., Brousseau, M., Kato, T., Mayer, P., Manzini, C., Guitard, F., Monplaisir, J., 2004. Experimental pain perception remains equally active over all sleep stages. Pain 110, 646–655.
Li, D., Kuang, B., Lobbezoo, F., De Vries, N., Hilgevoord, A., Aarab, G., 2023. Sleep bruxism is highly prevalent in adults with obstructive sleep apnea: a large-scale polysomnographic study. J. Clin. Sleep Med. 19, 443–451.
Li, G., Lu, C., Yin, M., Wang, P., Zhang, P., Wu, J., Wang, W., Wang, D., Wang, M., Liu, J., Lin, X., Zhang, J.X., Wang, Z., Yu, Y., Zhang, Y.F., 2024. Neural substrates for regulating self-grooming behavior in rodents. J. Zhejiang Univ. Sci. B 25, 841–856.
Lu, J.W., Mann, G.L., Ross, R.J., Morrison, A.R., Kubin, L., 2005. Differential effect of sleep-wake states on lingual and dorsal neck muscle activity in rats. Respir. Physiol. Neurobiol. 147, 191–203.
Ma, S.Y., Whittle, T., Descallar, J., Murray, G.M., Darendeliler, M.A., Cistulli, P., Dalci, O., 2013. Association between resting jaw muscle electromyographic activity and mandibular advancement splint outcome in patients with obstructive sleep apnea. Am. J. Orthod. Dentofac. Orthop. 144, 357–367.
Masuda, K., Katsuda, Y., Niwa, Y., Sakurai, T., Hirano, A., 2023. Analysis of circadian rhythm components in EEG/EMG data of aged mice. Front. Neurosci. 17.
Opp, M.R., Toth, L.A., Tolley, E.A., 1997. EEG delta power and auditory arousal in rested and sleep-deprived rabbits. Am. J. Phys. Anthropol. 272, R648–R655.
Owen, A.B., 2007. A robust hybrid of lasso and ridge regression. Contemp. Math. 443, 59–72.
Park, S.-H., Weber, F., 2020. Neural and Homeostatic Regulation of REM sleep. Front. Psychol. 11.
Park, Y., Chung, W., 2019. Frequency-Optimized Local Region Common Spatial Pattern Approach for Motor Imagery Classification. IEEE Trans. Neural Syst. Rehabil. Eng. 27, 1378–1388.
Raymond, J.S., Everett, N.A., Gururajan, A., Bowen, M.T., 2023. Quiet wakefulness: the influence of intraperitoneal and intranasal oxytocin on sleep-wake behavior and neurophysiology in rats. Sleep 46.
Rothenberger, S.D., Krafty, R.T., Taylor, B.J., Cribbet, M.R., Thayer, J.F., Buysse, D.J., Kravitz, H.M., Buysse, E.D., Hall, M.H., 2015. Time-varying correlations between delta EEG power and heart rate variability in midlife women: the SWAN sleep Study. Psychophysiology 52, 572–584.
Ryabinina, A.Y., Bryk, A.A., Blagonravov, M.L., Goryachev, V.A., Mozhaev, A.A., Ovechkina, V.S., 2024. Circadian Rhythms of Body Temperature and Locomotor activity in Spontaneously Hypertensive Rats under frequent changes in Light Conditions. Pathophysiology 31, 127–146.
Saranya, S., Poonguzhali, S., 2024. Principal component analysis biplot visualization of electromyogram features for submaximal muscle strength grading. Comput. Biol. Med. 182, 109142.
Schott, A.L., Baik, J., Chung, S., Weber, F., 2023. A medullary hub for controlling REM sleep and pontine waves. Nat. Commun. 14, 3922.
Sei, H., Furuno, N., Morita, Y., 1997. Diurnal changes of blood pressure, heart rate and body temperature during sleep in the rat. J. Sleep Res. 6, 113–119.
Sheward, W.J., Naylor, E., Knowles-Barley, S., Armstrong, J.D., Brooker, G.A., Seckl, J. R., Turek, F.W., Holmes, M.C., Zee, P.C., Harmar, A.J., 2010. Circadian Control of Mouse Heart Rate and Blood pressure by the Suprachiasmatic Nuclei: Behavioral Effects are more significant than Direct Outputs. PLoS One 5, e9783.
Steinfels, G.F., Young, G.A., Khazan, N., 1980. Diurnal variations in REM and NREM sleep EEG power spectra in the rat. Brain Res. 181, 425–432.
Stephan, F.K., Zucker, I., 1972. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. PNAS 69, 1583–1586.
Stephenson, R., Lim, J., Famina, S., Caron, A.M., Dowse, H.B., 2012. Sleep-wake behavior in the rat: ultradian rhythms in a light-dark cycle and continuous bright light. J. Biol. Rhythms 27, 490–501.
Strubbe, J.H., Keyser, J., Dijkstra, T., Prins, A.J., 1986. Interaction between circadian and caloric control of feeding behavior in the rat. Physiol. Behav. 36, 489–493.
Thie, N.M., Kato, T., Bader, G., Montplaisir, J.Y., Lavigne, G.J., 2002. The significance of saliva during sleep and the relevance of oromotor movements. Sleep Med. Rev. 6, 213–227.
Thomas, R.J., Mietus, J.E., Peng, C.K., Guo, D., Gozal, D., Montgomery-Downs, H., Gottlieb, D.J., Wang, C.Y., Goldberger, A.L., 2014. Relationship between delta power and the electrocardiogram-derived cardiopulmonary spectrogram: possible implications for assessing the effectiveness of sleep. Sleep Med. 15, 125–131.
Toyota, R., Fukui, K.-I., Kamimura, M., Katagiri, A., Sato, H., Toyoda, H., Rompré, P., Ikebe, K., Kato, T., 2022. Sleep stage-dependent changes in tonic masseter and cortical activities in young subjects with primary sleep bruxism. Sleep 45.
Van Betteray, J.N., Vossen, J.M., Coenen, A.M., 1991. Behavioural characteristics of sleep in rats under different light/dark conditions. Physiol. Behav. 50, 79–82.
Vertes, R.P., 1981. An analysis of ascending brain stem systems involved in hippocampal synchronization and desynchronization. J. Neurophysiol. 46, 1140–1159.
Wendt, S.L., Welinder, P., Sorensen, H.B., Peppard, P.E., Jennum, P., Perona, P., Mignot, E., Warby, S.C., 2015. Inter-expert and intra-expert reliability in sleep spindle scoring. Clin. Neurophysiol. 126, 1548–1556.
Witting, W., Mirmiran, M., Bos, N.P., Swaab, D.F., 1993. Effect of light intensity on diurnal sleep-wake distribution in young and old rats. Brain Res. Bull. 30, 157–162.
Yasenkov, R., Deboer, T., 2010. Circadian regulation of sleep and the sleep EEG under constant sleep pressure in the rat. Sleep 33, 631–641.
Zhang, Z., Wang, H.-J., Wang, D.-R., Qu, W.-M., Huang, Z.-L., 2017. Red light at intensities above 10 lx alters sleep–wake behavior in mice. Light Sci. Appl. 6, e16231–e.