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Dark/light transition and vigilance states modulate jaw-closing muscle activity level in mice

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ABSTRACT

Bruxism is associated with an increase in the activity of the jaw-closing muscles during sleep and wakefulness. However, the changes in jaw-closing muscle activity across states of vigilance over a 24-h period are unclear. In this study, we investigated the effects of dark/light transition and sleep/wake state on EMG activity of the masseter (jaw-closing) muscle in comparison with the activity of the upper trapezius muscle (a neck muscle) over a 24-h period in mice. The activities of the masseter and neck muscles during wakefulness were much greater than during non-REM and REM sleep. In contrast, the activities of both muscles slightly, but significantly, decreased during the transition period from dark to light. Histograms of masseter activity during wakefulness and non-REM sleep showed bimodal distributions, whereas the neck muscle showed unimodal activation in all states. These results suggest that the activities of jaw-closing and neck muscles are modulated by both sleep/wake state and dark/light transition, with the latter being to a lesser degree. Furthermore, even during non-REM sleep, jaw-closing muscles display bimodal activation, which may contribute to the occurrence of exaggerated aberrant muscle activity, such as sleep bruxism.

1. Introduction

Bruxism is a risk factor for undesirable orofacial problems, such as tooth destruction, breakage of dental prostheses, and orofacial pain (Lavigne et al., 2003, 2008; Kato et al., 2013b). Compared with normal subjects, patients with sleep bruxism show increased activity of the masseter muscle (jaw-closing muscle) during non-REM sleep (Miyamoto et al., 1996; Lavigne et al., 2001, 2003; Kato et al., 2003, 2013B; Baba et al., 2005). Moreover, compared to subjects without awake bruxism, those with this condition have increased masseter muscle activity during wakefulness (Katase-Akiyama et al., 2009; Fujisawa et al., 2013). Thus, masseter activity levels over distinct vigilance states, probably in association with the circadian rhythm, are a promising physiological feature for studying the pathophysiology of bruxism in animal models (Lavigne et al., 2008; Kato et al., 2011). Several studies have investigated the vigilance state-dependent changes in masseter muscle activity in rats (Brooks and Peever, 2008; Burgess et al., 2008) and guinea pigs (Kato et al., 2007, 2010). However, these analyses were confined to a duration of 4–6 h in the inactive phase (light period for these animals). Some studies examined the activity of jaw-closing muscles over 24-h periods in rabbits (Langenbach et al., 2004; Grunheid et al., 2005). However, the effects of dark/light cycles on masseter muscle activity during a given vigilance state are unclear because vigilance states were not monitored in those studies.

The aim of the present study was to examine the effects of dark/light transition and sleep/wake state on the activity of masseter and upper trapezius (neck) muscles over a 24-h period. For this purpose, we chose mice as our experimental animal model because results obtained from this study can then be compared with results from genetically modified mouse studies, which may lead to significant advances in understanding the etiology of sleep bruxism.

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2. Materials and methods

All the experiments followed the Guide for the Care and Use of Laboratory Animals described by the National Institutes of Health (USA), and were approved by the International Animal Research Committee of Showa University in accordance with the Japanese Government Law No. 105 for the care and use of laboratory animals.

2.1. Animals

Mice (12–20 weeks old, C57BL/6 strain, $n = 19$) were used in this study. We made every effort to minimize the number of animals used. Mice were housed individually with 12-h light/dark periods (lights on from 08:00 to 20:00) and standard temperature conditions. Mice were given chow and water ad libitum.

2.2. Surgical implantation

Mice were prepared for long-term recording of neck and masseter muscles using electroencephalography (EEG), electrooculography (EOG), and electromyography (EMG). Surgery was performed under anesthesia using ketamine hydrochloride (100 mg/kg, i.p., KETALAR®, DAIICHI SANKYO, Tokyo, Japan) and xylazine hydrochloride (10 mg/kg, i.p., Sigma-Aldrich, St. Louis, MO). Three stainless steel screws (M1-2, Unique Medical, Tokyo, Japan) were implanted into the skull (two EEG screws were placed over the cortex and one as a ground). Urethane-coated stainless steel wires (diameter: 0.12 mm, Unique Medical) were soldered to the implanted screws before surgery. Two wire pairs were inserted in the neck and the left masseter muscles. Two EOG electrodes were positioned subcutaneously on each side of the orbit. The wires for EEG, EOG, and EMG were soldered to a multiple pin socket to be used as connectors (Unique Medical). The connectors were firmly fixed to the skull using screws and dental acrylic resin.

2.3. Recording procedures

Mice were individually housed in breeding racks during the recovery period, and for 1 week after the operation. During the training period, mice were transported from the vivarium to the testing room for three separate 24-h periods during which they were allowed to adapt to the recording cage and the connected recording cable (TY213-042, Unique Medical). Mice were given chow and water in the testing room. Recording sessions were started 1 day after the training sessions finished and lasted for 24 h. The recordings were amplified (FE135, AD Instruments, Colorado Springs, CO) to optimal bandwidths (EEG and EOG: 0.3–100 Hz; EMG 100–1000 Hz). Data from the EEG, EOG, and EMG were digitized at 400 Hz, 400 Hz, and 4 kHz, respectively, using PowerLab 8/35 (PL3508, AD Instruments) and stored on a personal computer with Chart 7 software (AD Instruments).

2.4. Scoring states of vigilance

In accordance with previous studies (Radulovacki et al., 1984; Tobler et al., 1997; Kato et al., 2007; Tsunematsu et al., 2011), we determined three states of vigilance (wakefulness, non-REM sleep, and REM sleep) in 10-s epochs on the basis of EEG, neck muscle EMG, and EOG activities. Wakefulness was defined as when neck muscle EMG activity was high and the EEG power density in the delta band was low. Non-REM sleep was defined as when neck muscle activity was lower than during wakefulness and power density in the delta band was relatively higher. REM sleep was defined as when neck EMG activity was lower than in non-REM sleep with low delta power. Neck muscle tone decreased significantly from wakefulness to non-REM sleep and further in REM sleep.

2.5. EMG analysis

EMG activities of the masseter and neck muscles were first rectified and integrated for every 10-s epoch using SleepSign software (KISSEI COMTEC, Nagano, Japan). To minimize the influence of the electrical baseline in the EMG data, the minimal integrated value was subtracted from all measurements (Lu et al., 2005; Kato et al., 2010). Next, EMG measurements were normalized using mean activity during total wakefulness over the 24-h period. To assess the variability of the normalized activity during each state of vigilance, standard deviations of normalized activity were calculated for each state (Kato et al., 2010).

2.6. Statistics

Values are presented as the mean ± the standard error of the mean (SEM). Differences within and between groups were analyzed using the Wilcoxon signed-rank test and Fisher's exact test. Correlations between EMG activity of the masseter and neck muscles during each vigilance state were determined using Pearson's correlation coefficient. The distribution of the EMG activity between neck and masseter muscles during three states of vigilance was analyzed using the Kolmogorov–Smirnov test. Statistical analyses were conducted with SPSS 22.0J (IBM Japan Inc., Tokyo, Japan) and Origin 9.1 (OriginLab Corp., Northampton, MA).

3. Results

3.1. Neck and masseter EMG activities were affected by dark/light transition and sleep/wake state

Fig. 1 shows a typical example of raw, filtered EEG, EOG, and EMG in a transition period of the sleep–wake cycle. Mice repeated ordinary sleep/wake cycles within a 24-h period in our experiment, similar to previous reports in rats (Lu et al., 2005; Burgess et al., 2008) and guinea pigs (Kato et al., 2007). Specifically, they first fell into non-REM sleep, then into REM sleep, and finally returned to wakefulness. As reported previously (Huber et al., 2000; Yasenkov and Deboer, 2012), the percentage of time between the three behavioral states differed with dark and light periods. In the dark period, animals were awake longer and slept for 32.0 ± 4.0% and 3.4 ± 0.5% of the total dark period in non-REM and REM sleep, respectively. In contrast, total non-REM sleep became longer (56.0 ± 4.0%) during the light period ($n = 19$, Fig. 1B).

The mean EMG activities of both masseter and neck muscles during wakefulness did not differ among 4-h periods in the dark (masseter: $p = 0.61$; neck: $p = 0.95$) or light periods (masseter: $p = 0.43$; neck: $p = 0.69$; Fig. 1C and D). However, the mean activities of the masseter and neck muscles during wakefulness decreased slightly, but significantly, during the transition period from the dark (4–7 h) to the light (8–11 h) period by 5.8 ± 11.1% and 14.4 ± 14.1%, respectively (masseter: $p < 0.05$; neck: $p < 0.001$; Fig. 1C and D). Similarly, the mean activities of the masseter and neck muscles during non-REM sleep decreased significantly during the transition period from the dark to the light period by 3.5 ± 3.2% and 0.3 ± 3.3%, respectively (masseter: $p < 0.001$; neck: $p < 0.05$).

Among vigilance states, the mean activities of both the masseter and neck muscles during wakefulness over any 4-h period were 4.7–19.9 times higher than the mean activities of both muscles during non-REM and REM sleep over the corresponding 4-h periods. The mean activities of both muscles during non-REM sleep