Daily jaw muscle activity in freely moving rats measured with radio-telemetry

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The jaw muscle activity of rats has been investigated for specific tasks. However, the daily jaw muscle use remains unclear. The purpose of the present study was to examine daily jaw muscle activity, and its variability over time, in the rat ($n = 12$) by the use of radio-telemetry. A telemetric device was implanted for the continuous recording of masseter muscle and digastric muscle activity. Daily muscle use was characterized by calculating the total time that each muscle was active (duty time), the number of bursts, and the average length of bursts. All parameters were estimated for activities exceeding various levels (5–90%) of the day's peak activity. Daily muscle use remained constant for 4 wk. At the low-activity level, the duty time and burst number of the digastric muscle were significantly ($P < 0.01$) higher than those of the masseter muscle, whereas the opposite was true at the high-activity level ($P < 0.05$). No significant intermuscular correlation was observed between the number of bursts of the masseter and digastric muscles, but the interindividual variation of both muscles changed, depending on the level of activation. These findings suggest that the masseter muscle and the digastric muscle show a differential active pattern, depending on the activity level.

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Most skeletal muscles are activated during a wide variety of motor tasks, which can be examined by the recording of biopotentials [electromyograms (EMG)]. Radio-telemetry has enabled EMG recordings to be carried out in truly freely moving animals (1, 2), which ensures normal daily behavior. Recently, the transmitter has decreased sufficiently in size to be fully implantable into small animals, such as the rat.

Several studies have shown that various leg muscles are activated during clearly different time periods during the day, expressed as a duty time (2, 3). In the jaw muscles, remarkable differences in the duty time were also observed (4–8), which were closely related to the number of muscle activations (6). In general, these variations in duty time and burst numbers can be linked to the fiber type. Slow-type muscles show higher duty times and larger burst numbers than fast-type muscles (7, 9). The length of individual muscle activations was examined in a few studies (5, 6) and differed between the jaw muscles only when all activities were taken into account. Activities exceeding higher levels did not differ in length between the various jaw muscles. In rat, the daily duty times of the jaw muscles are still unknown, although these muscles have been studied only for specific motor tasks (10, 11), or during largely restrained behavior (12).

In the present study, the daily muscle activity was quantified in the masseter muscle (a jaw closer) and the digastric muscle (a jaw opener) of the rat by determining the total duration of muscle activity (duty time), the total number of bursts, and the average length of bursts, for various predefined levels of activity. In the digastric muscle, 5–8% of the fibers are of the slow type, and in the masseter muscle slow-type fibers are completely absent (13–15). Therefore, the digastric muscle will show a higher duty time than the masseter muscle. We would expect this to be represented by a larger number and longer duration of bursts. As the two muscles are antagonists both for vertical and antero-posterior directions, it can be hypothesized that the number of activation bursts in the two muscles will be similar at all activity levels.

Material and methods

Telemetric system

The current telemetric system has been used in previous studies (4, 5, 16), Briefly, implantable transmitters for bio-potential recording (F50-EEE, 45 mm × 17 mm × 10 mm, 14 g; Data Sciences International, St Paul, MN, USA) were

used to record muscle activity. The transmitter consists of an electronic module, a battery to supply energy for at least 2 months of continuous data transmission, and stainless steel wire electrodes (double helix, 0.45 mm diameter) with silicon tube (0.8 mm diameter). The distance between the two tips of the bipolar electrodes was 1 mm, and the effective electrode tip length was 7 mm. In the device, the biopotentials were filtered (first-order low-pass filter, 158 Hz), sampled (250 Hz) on the input of each channel, transmitted and then collected by a receiver (RPC-1; Data Sciences International) placed under the cage. The signals were stored onto a PC hard disk, using the Dataquest A.R.T. data acquisition system (Data Sciences International). Previously it has been shown that the EMG recorded with this system is a reliable reflection of the actual biopotentials and can be used for estimation of muscle use (16).

Muscle activity registration

Twelve, 14-wk-old Wistar strain male rats, weighing 410–450 g, were used in this study. The protocol of the experiment was approved by the Animal Care and Use Committee of Hiroshima University. Each animal was anesthetized with intra-abdominal injections of sodium pentobarbital (Nembutal; Dinabott, Osaka, Japan), at a dose of 50 mg kg^{-1} body weight. The transmitter was implanted in the shoulder area and the bipolar electrodes were subcutaneously led to an incision in the right submandibular region. From here, they were inserted into the center of the right superficial masseter and the anterior belly of the digastric muscles and sutured at the muscle surface to prevent them from dislodging. These procedures were performed under sterile conditions. An antibiotic, phosphomycin disodium salt (Sigma-Aldrich, St Louis, MO, USA), was administered for 3 d preceding, and for 2 d following, surgery. An analgesic, buprenorphine (Lepetan; Otsuka Pharmaceutical, Tokyo, Japan), was provided immediately after surgery.

Each animal was housed individually in a cage (45 cm × 22 cm × 18 cm) and fed with pellets and water *ad libitum*. The day/night rhythm was ensured by automatic dimmed lighting (08:00 h to 20:00 h). The animals were weighed twice weekly and their physical condition checked. Except for the daily care and regular physical examination, they were left undisturbed in order to minimize any external influence. Muscle activities were continuously recorded for 1 wk, starting 7 d after surgery. In six of the 12 animals, the EMG recordings were extended during an additional 3 wk to examine the consistency of the various daily muscle activity parameters over time. After the recording period, the animals were killed with an overdose of sodium pentobarbital for the verification of the electrode locations.

Analysis

The method of analysis was similar to that performed previously (5–7). Briefly, recordings of 24-h muscle activities were analyzed using SPIKE2 software (Cambridge Electronic Design, Cambridge, UK). After motion-artifacts had been removed (5 Hz high-pass filter), the signal was rectified and averaged (20 ms window, i.e. 5 samples). To eliminate possible artefacts, the 0.001% proportion of the samples (*c*. 43) with the largest amplitudes was excluded. The peak EMG was defined as the largest of the 99.999% remaining samples. Daily muscle use was characterized by means of the total duration of muscle activity (duty time), the total

burst number, and their average length. These parameters were determined for muscle activities exceeding 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90% of the day's peak activity. A burst was defined as a series of consecutive samples exceeding the aforementioned activity levels (5–7). Note that the duty time for activations exceeding a certain level includes the duty times for activations exceeding higher levels. The 5% of the peak EMG level was well beyond the noise level, attained after termination of the experiment. Duty time, burst number, and average length exceeding this 5% level was assumed to represent the overall muscle use, including all muscle activities. Muscle activity exceeding 80% of the peak EMG was considered representative for the most forceful muscle usage.

Analysis of variance (ANOVA) was used to examine intra-individual variation in daily duty times, burst numbers, and average burst lengths over 4 wk (*n* = 6). For this, the data of one day in each week were analyzed. For all animals (*n* = 12), the recordings on the seventh postsurgical day were used to assess intermuscular differences. Here, the daily duty times, burst numbers, and average burst lengths of the two muscles were compared (paired *t*-test) at each activity level. Hourly duty times for overall muscle use were compared over the course of the day to reveal any consistent day/night rhythm over time. Duty times of 4 h time periods were calculated and tested for significant differences (ANOVA). The Bonferroni/Dunn procedure was used as a post hoc test. To identify any intermuscular relationship between the amount of activity in both muscles, the correlation coefficients of burst numbers were calculated (regression analysis) for activations exceeding 5, 20, 50, and 80% levels.

Results

All animals showed normal feeding behavior and water intake except for the first 2 d after surgery. The weight of the animals remained constant. Representative daily duty times of the masseter and digastric muscles of one animal for four consecutive weeks are shown in Fig. 1. Over the 4 wk of recording no significant differences (*P* > 0.43) were found for any of the muscle activity parameters (duty time, burst number, average burst length). The duty times were highest for activities exceeding 5% of the peak EMG and declined rapidly with increasing activity level. This effect was stronger in the digastric muscle than in the masseter muscle.
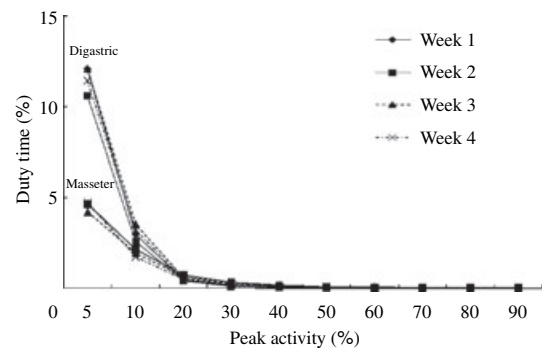

Fig. 1. The daily duty times, at various activity levels, of the masseter and digastric muscles in one animal during a 4-wk period. For clarity, one day of each week is shown.

For muscle activities exceeding 5% of the peak EMG (Fig. 2), the digastric muscle showed significantly higher duty times and burst numbers ($P < 0.01$) than the masseter muscle (respectively, 16% and 4%, and *c*. 125,000 and 54,000). For activities exceeding 20% of the peak EMG, this difference was only present for the number of bursts, whereas no differences were present for activities exceeding the 50% level. For activities exceeding the 80% level, the masseter muscle showed higher duty times and burst numbers ($P < 0.05$) than the digastric muscle [respectively, 0.0042% and 0.0008% ($t = 2.345$); and 160 and 114 ($t = 2.402$); Fig. 2]. The average burst length of the two muscles decreased with increasing activity level. Furthermore, the burst lengths of the masseter muscle were generally shorter than those of the digastric muscle [5%, $P < 0.05$; 20%, not significant; 50%, $P < 0.05$ ($t = 2.238$); 80%, $P < 0.01$ ($t = 3.726$); Fig. 2].

Hourly duty times showed a clear circadian rhythm in both muscles (Fig. 3), and duty times taken at 4-h time intervals indicated significantly higher duty times during the night than during the day in both muscles ($P < 0.01$; Fig. 3). The most consistent changes in the hourly duty time were observed around the times that the lighting was switched on or off.
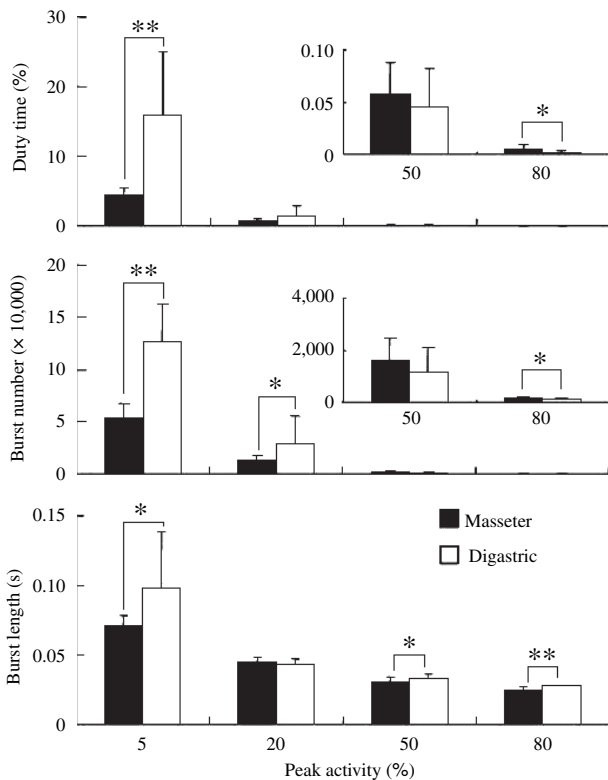

Fig. 2. Average duty times, burst numbers, and burst lengths and standard deviations (SD) ($n = 12$) of the masseter (solid) muscle and digastric (open) muscle for activities exceeding 5, 20, 50, and 80% of the peak electromyogram (EMG) value in the first week. For clarity, the duty times and burst numbers for activity levels exceeding 50 and 80% are shown enlarged. Significant differences between muscles are indicated by single ($P < 0.05$) or double ($P < 0.01$) asterisks.
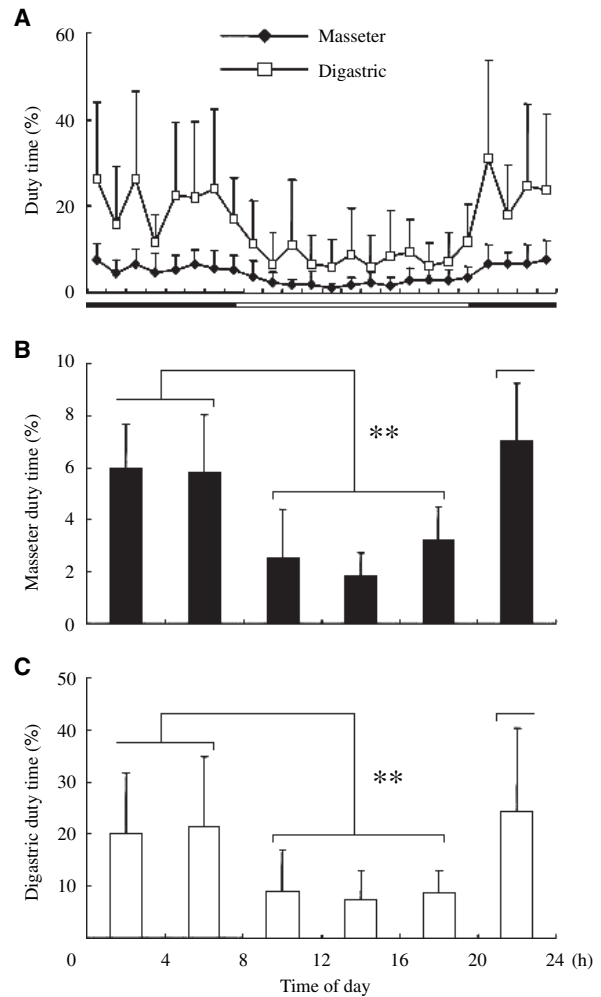

Fig. 3. (A) Average hourly duty times and standard deviations (SD) ($n = 12$) for activities exceeding 5% of the peak electromyogram (EMG) of the masseter (solid symbols) and digastric (open symbols) muscles. The bar below the timescale indicates the day (white) and night (black) periods. Average duty times ($\pm$ SD) of 4-h periods for activities exceeding 5% of the peak EMG of the masseter muscle (B) and digastric muscle (C). For both muscles ($n = 12$), the duty times of the three night periods were higher than those during the day. Significant differences between periods are indicated by double asterisks ($P < 0.01$).

No significant intermuscular correlation ($r < 0.33$) was observed between the number of bursts of the masseter muscle and the digastric muscle at any activity level (Fig. 4). However, for activities exceeding 5 and 20% levels, variation in activity of the digastric muscle was much larger than in the masseter muscle, whereas for the most powerful activities ($> 80\%$) this pattern was reversed.

Discussion

This is the first study to record the jaw muscle activity continuously in freely moving rats over a time-period of several weeks. Rats have previously been used to study
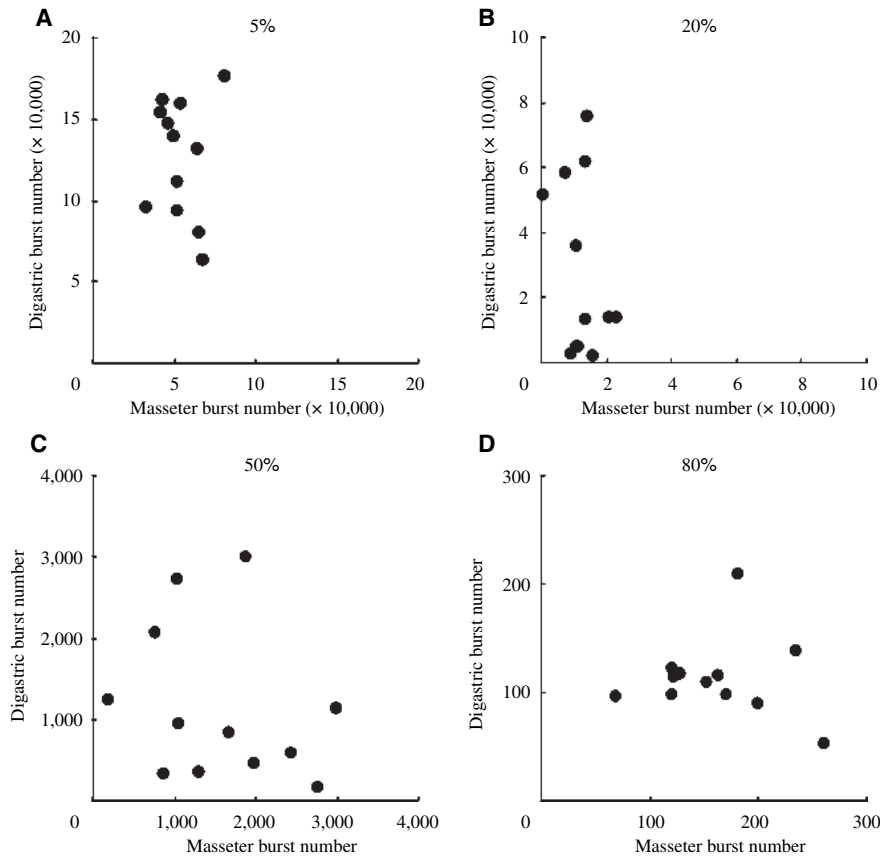
Fig. 4. Intermuscular correlation of burst numbers between the masseter and digastric muscles at activity levels exceeding 5% (A), 20% (B), 50% (C), and 80% (D).

masticatory behavior (10–12,17) and have served as an animal model for histological studies, including the jaw system (18). As an animal model, rats have the advantage of being small and easy to feed, and it is relatively simple to acquire inbred-strain rats, which standardize the experimentation. In addition, they grow fast, so that developmental studies can easily be undertaken. In the present study, the normal daily motor behavior of the rat's jaw muscles was investigated. We consider these findings important for future developmental and experimental studies in this animal model.

The rat's daily duty times were constant for 4 wk, indicating that the method used is reproducible. However, variations during the day were large and showed an apparent circadian rhythm. Various studies have been conducted in order to reveal the behavioral characteristics in relation to the circadian rhythm of the jaw system (8,12,19). In the rat, a higher nocturnal feeding activity was observed. ISHIZUKA & TANNE (12) examined the masseter muscle activity only for 4-h periods and showed that the activity of the masseter muscle was higher during the night. We calculated the hourly duty times of the masseter muscle and the digastric muscle, which allowed a more detailed examination of the circadian rhythm of rats. Both the masseter muscle and the digastric muscle showed significant intra-individual variations over 24 h. The activity of both muscles was higher during the night than during the day. It should be

noted that the muscle regions examined in this study, because of the bipolar electrodes, were small. Although the electrodes were inserted in predefined muscle locations (i.e. superficial masseter and anterior belly of the digastric), it remains undisclosed whether these activity characteristics are similar for the entire muscle.

By using bipolar electrodes indwelled into the muscles, we minimized the possibility of cross-talk. Moreover, we used the 5% of the peak EMG as the lowest level for the analysis because this level was well beyond the noise level. As any cross-talk signals would be very low in amplitude, this would further control the amount of cross-talk. Therefore, although it is possible that the cross-talk from the neighboring muscles was recorded (especially for the digastric muscle), we assume that the data for analysis was probably not affected by the neighboring muscle activities.

Functional heterogeneity between muscles is an important characteristic of the jaw system, especially during feeding (20). We revealed, in this study, that at lower activity levels, the digastric muscle is more active than the masseter muscle, while the opposite was true at higher levels of activity. This suggests that the digastric muscle is mainly engaged in low-power behavior, whereas the masseter muscle is used more frequently in high-power behavior. The masseter's high-power activities probably involve behaviors in which fast jaw motions or high occlusal forces are generated. It was

reported that in humans, almost all muscle activities exceeding the 50% level were present during mealtimes (21). The masseter's low-power activities are possibly related to (smaller) free jaw motion and the maintenance of the jaw's rest position. The low number of high-power digastric activities suggest that jaw motions do not need powerful contractions of this muscle. Jaw opening is indeed a jaw motion without too much resistance. The powerful digastric contractions are probably related to wide jaw opening or retrusive jaw motions. Muscle activity exceeding the 5% level was assumed to represent the overall muscle use, including all muscle activities, and muscle activity exceeding the 80% level was considered representative for the most forceful muscle usage.

Although the muscle-active pattern of the masseter and digastric muscles of rabbits, reported in previous studies (5, 6), were similar to our findings in rats, the total duration and number of bursts in rats were larger than those of rabbits, and the average burst length of rats was shorter than that of rabbits. Miyamoto *et al.* (21) reported the muscle activity of human masseter during the whole day. Although these data cannot be compared directly because they used surface electromyography, the muscle activity in humans was markedly lower than that of rats. The rapid and repetitive gnawing motions of rats are well known (20). This feeding pattern of rats might explain the difference of muscle activity between the species.

The behavioral differences in muscle use are related to the muscle's fiber type composition (2, 4, 7, 22, 23). The masseter muscle of rat contains fast-type fibers only (13, 14), whereas the digastric muscle contains 5–8% slow-type fibers (15). This difference in fiber type composition is in accordance with the results of the present study. The digastric muscle, with more slow-type fibers, shows a higher duty time and a larger number of daily bursts than the masseter muscle. VAN WESSEL *et al.* (7) revealed, for the rabbit jaw muscles, that there was a positive correlation between the duty time and the percentage of slow-type fibers for activities exceeding the 20% level. In the rat, however, slow-type muscle fibers are totally absent in the masseter muscle, but its duty time and burst numbers for the most powerful activations are significantly larger than in the digastric muscle. The relationship between muscle use and fiber type composition might be different from that in rabbits. For the rat, the speed of contraction might be of decisive importance at these high activity levels.

Because the two examined muscles are antagonists for vertical and antero-posterior directions, it can be assumed that for various behaviors the muscles will produce a similar number of activity bursts. However, the daily number of activities in these muscles showed no correlation. The relationship of the burst numbers between the two muscles seems to be dependent on the level of activation. For activities exceeding the 5 and 20% levels (Fig. 4), the interindividual variation in burst number is high for the digastric muscle, whereas the masseter muscle was activated at a relatively constant number in all individuals. In contrast, for activities exceeding the 80% level, the digastric muscles showed more consistency in number of bursts than the masseter muscle. This indicates that the control of these two jaw muscles changes, depending on the level of activation.

In conclusion, the masseter and digastric muscles differed in their duty time, burst number, and average burst length. This difference, however, was constant throughout the 4-wk experimental period. Including all activities, the digastric muscle was more active, but for the most powerful activities the masseter was the most active muscle. A consistent circadian rhythm was observed for both muscles, showing an increased nocturnal activity. The known fiber-type composition of the rat jaw muscles is largely reflected by the duty times, including all activity. This suggests that the fiber type composition of these muscles, and possibly other adaptive features of the jaw system, are influenced not only by the high muscle loads generated during behaviors such as chewing, but also by other behaviors generating lower levels of force. The data presented can serve as a reference for the possible effects of artificially altered function of the jaw system on daily muscle use.

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