Daily Jaw Muscle Activity in Freely Moving Rats Measured with Radio-telemetry

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Running title: Daily jaw muscle activity

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(4) Abstract

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 and digastric muscle activity. Daily muscle use was characterized by calculation of the total time 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 were higher than that of the masseter significantly ($p<0.01$), while 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 muscle but the inter-individual variation of both muscles changed depending on the level of activation. These findings suggested that the masseter and digastric muscle showed a differential active pattern depending on activity level.

5) Keywords: EMG, telemetry, rat, masseter, digastric

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Introduction

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 the EMG recordings in truly freely moving animals (1, 2) which ensures normal daily behavior. Recently, the size of transmitter has decreased enough to be fully implantable into small animals such as the rat.

Several studies showed that various leg muscles are activated during clearly different amounts of time per day, expressed as a duty time (2, 3). In the jaw muscles, remarkable differences in the duty time were also observed (4-8) 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 composition. 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, though 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 (a jaw closer) and the digastric (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 5-8% of the fibers are of the slow type, and in the masseter they are completely absent (13-15). Therefore, the digastric muscle will show a higher duty time than the masster muscle. We expect that this will be caused by a larger number and longer duration of the 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.

Materials and methods

Telemetric System

The current system has been used in previous studies (4, 5, 16), Briefly, implantable transmitters for biopotential recording (F50-EEE, 45 mm×17 mm×10 mm, 14 g, Data Sciences International (DSI), 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 two months of continuous data transmission, and stainless steel wire electrodes (double helix, diameter: 0.45 mm) with silicon tube (diameter: 0.8 mm). 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 (1st-order low-pass filter, 158 Hz), sampled (250 Hz) on the input of each channel, transmitted and then collected by a receiver (RPC-1, DSI) placed under the cage. The signals were stored onto a PC hard disk, using the Dataquest A.R.T. data acquisition system (DSI). 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 from 410 to 450 g, were used in this study. The protocol of the experiment was approved by the Animal Care and Use

Committee at Hiroshima University. Each animal was anesthetized with intra-abdominal injections of sodium pentobarbital (Nembutal; Dinabott, Osaka, Japan) at a dose of 50 mg/kg 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 done under sterile condition. An antibiotic, phosphomycin disodium salt (Sigma-Aldrich Co., St. Louis, MO, USA), was administered for 3 d preceding and for 2 d following surgery. An analgesic, buprenorphine (Lepetan; Otsuka Pharmaceutical Co., Ltd., 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*. Day-night rhythm was ensured by automatic dimmed lighting (8 am-8 pm). Twice a week the animals were weighed and checked for their physical condition. Except for the daily care and regular physical examination, they were left undisturbed to minimize any external influence. Muscle activities were continuously recorded during 1 wk, starting 7 d after surgery. In six of the twelve 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 (CED), 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% of the samples (ca. 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 activaties 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 was analyzed. For all animals (n=12), the recordings on the seventh post-surgical 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 hour-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 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 than in the masseter.

For muscle activities exceeding 5% of the peak-EMG (Fig. 2), the digastric showed significantly higher duty times and burst numbers ($p<0.01$) than the masseter (respectively, 16% and 4%, and ca. 125,000 and 54,000). For activities exceeding 20% of the peak-EMG, this difference was only present for the number of bursts, while no differences were present for activities exceeding 50% level. For activities exceeding 80% level, the masseter showed higher duty times and burst numbers ($p<0.05$) than the digastric (respectively, 0.0042% and 0.0008% ($t=2.345$), 160 and 114 ($t=2.402$); Fig. 2). The average burst length of the two muscles decreased with increasing activity level. Furthermore, burst length of the masseter were on average shorter than those of the digastric (5%: $p<0.05$; 20%: n.s.; 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 of every 4 hours indicated significantly higher duty times during night time than during day time in both muscles ($p<0.01$; Fig. 3). The most consistent changes in the hourly duty time were observed around the times the lighting was switched on or off.

No significant intermuscular correlation ($r<0.33$) was observed between the number of bursts of the masseter and the digastric at any activity level (Fig. 4). However, for activities exceeding 5 and 20% levels variation in the digastric activity was much larger than in the masseter, while 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 for several weeks. Rats have previously been used to study the 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 during 4 wk, indicating the used method 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 and Tanne (12) examined the masseter muscle activity only for four-hour periods and showed that activity of the masseter was higher during night. We calculated the hourly duty times of the masseter and the digastric, allowing a more detailed examination of the circadian rhythm of rats. Both the masseter and the digastric showed significant intra-individual variations over 24 h. During the night the activity of both muscles was higher than during day-hours. It has to 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 there is possibility that the cross-talk from the neighboring muscles was recorded especially for digastric, we assume that the data for analysis was likely not to be 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 is more active than the masseter while the opposite was true at higher levels of activity. This suggests that the digastric is mainly engaged in low power behavior, while the masseter 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 human study, almost all of muscle activities exceeding 50% level appeared in the mealtime (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 most likely related to wide jaw opening or retrusive jaw motions. Muscle activity exceeding 5% level was assumed to represent the overall muscle use including all muscle activities and muscle activity exceeding 80% level was considered representative for the most forceful muscle usage.

Although the muscle active pattern of the masseter and digastric 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 whole day. Although these data can not be compared directly because they used the surface electromyography, the muscle activity in human was quite less than that of rats. The rapid and repetitive gnawing motions of rats have been well-known (20). This feeding pattern of rats might cause the difference of muscle activity in species.

The behavioral differences in muscle use are related to the muscle's fiber type composition (2, 4, 7, 22, 23). The masseter of rat contains fast type fibers only (13, 14), while the digastric contains 5-8% slow type fibers (15). This difference in fiber type composition is in accordance with the present results. The digastric with more slow type fibers shows a higher duty time and a larger number of daily bursts than the masseter. 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 20% level. In the rat however, slow muscle fibers are totally absent in the masseter, but its duty time and burst numbers for the most powerful activations are significantly larger than in the digastric. The relation between the 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 bahviors the muscles will produce a similar amount 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 the activities exceeding 5 and 20% levels (Fig. 4), the inter-individual variation in burst number is high for the digastric, while the masseter was activated at a relatively constant number in all individuals. In contrast, for activities exceeding 80% level, the digastric muscles show 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 is 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 suggest 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 like chewing, but also by other behaviors generating lower levels of force. The presented data can serve as a reference for possible effects of artificially altered function of the jaw system on the daily muscle use.

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Figure legends

Figure 1

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

Figure 2

Averaged duty times, burst numbers, and burst lengths and standard deviations (n=12) of the masseter (solid) and digastric (open) muscles for activities exceeding 5, 20, 50, and 80% of the peak-EMG 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 asterisks ($p<0.01$).

Figure 3

(A) Averaged hourly duty times and standard deviations (n=12) for activities exceeding 5% of the peak-EMG of the masseter (solid symbols) and digastric (open symbols). The bar below the time scale indicates the day (open) and night (solid) periods. Averaged duty times (± SD) of 4 hour-periods for activities exceeding 5% of the peak-EMG of the masseter (B) and digastric (C). For both muscles (n=12), the duty times of the three night periods were higher than those during the day. Significant difference between periods are indicated by double asterisks ($p<0.01$).

Figure 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).

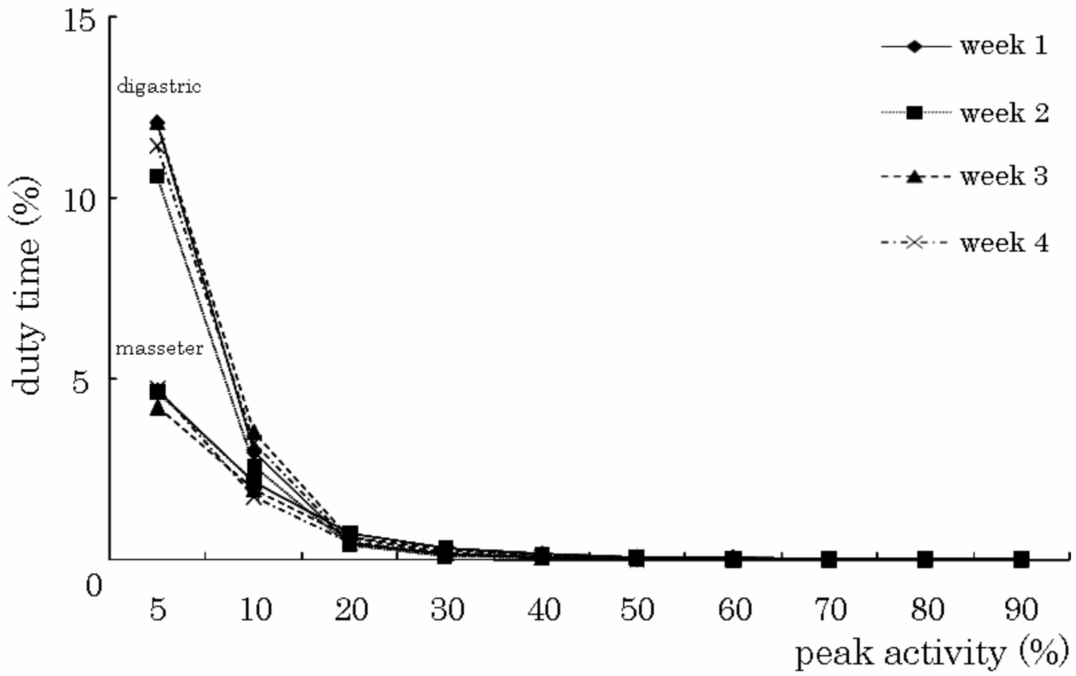

Figure 1

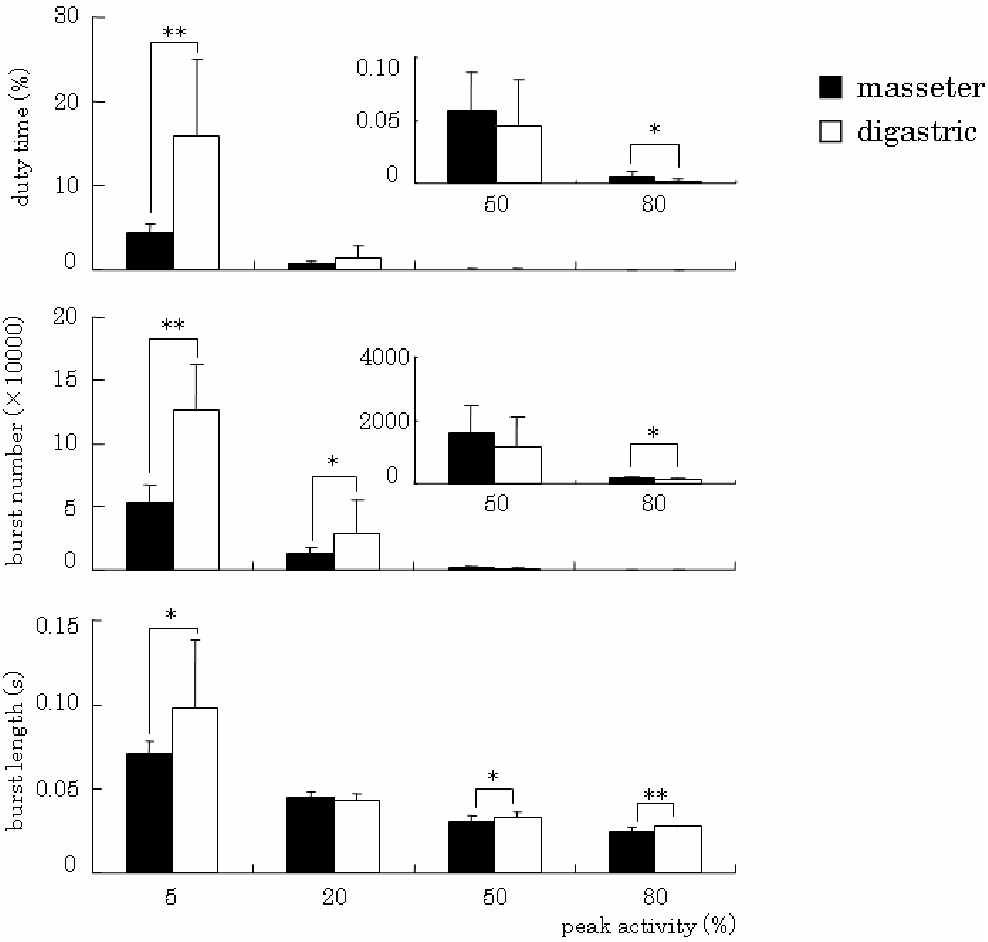

Figure 2

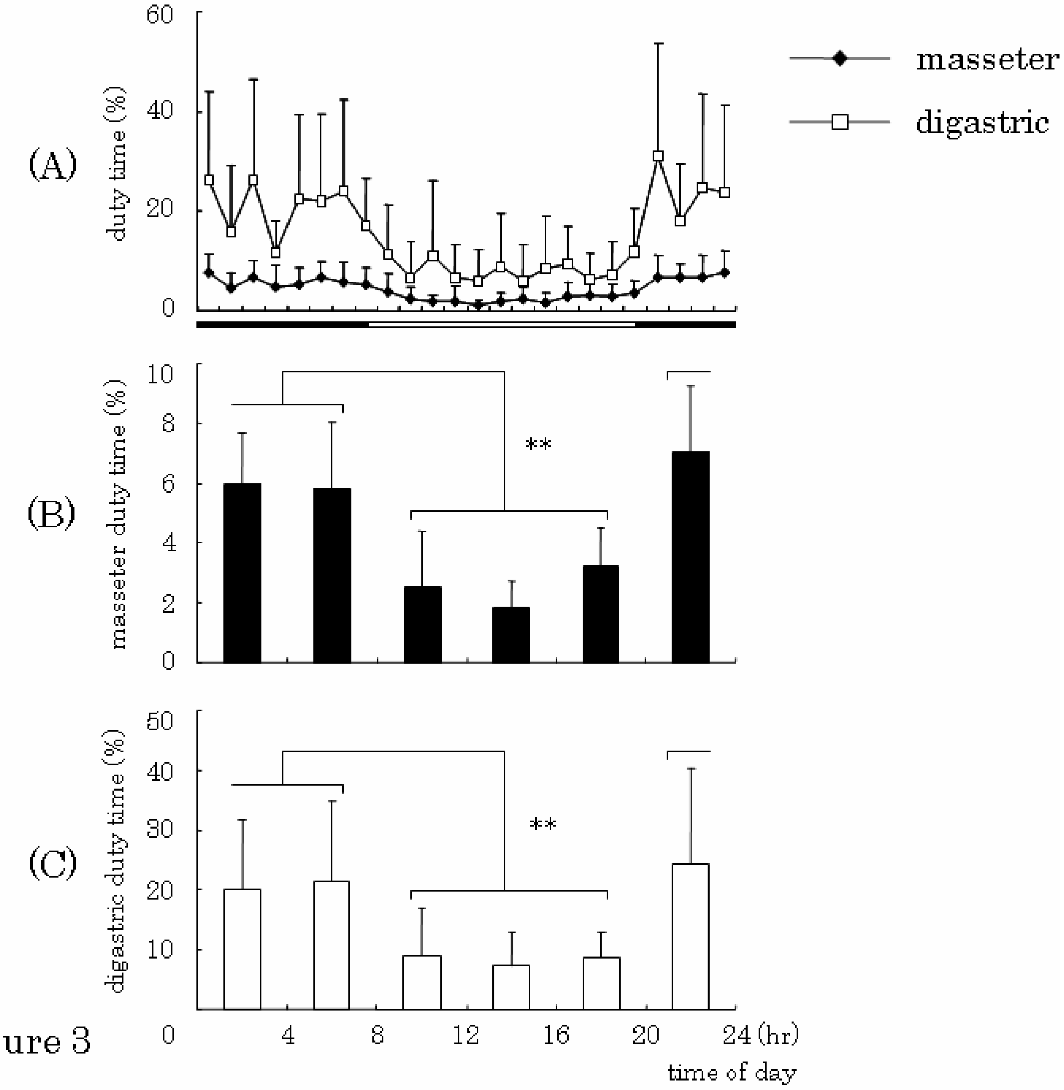
(A)
duty time (%)
60
40
20
0
masseter
digastric
(B)
masseter duty time (%)
10
8
6
4
2
0
**
(C)
digastric duty time (%)
50
40
30
20
10
**
0
4
8
12
16
20
24 (hr)
time of day

Figure 3

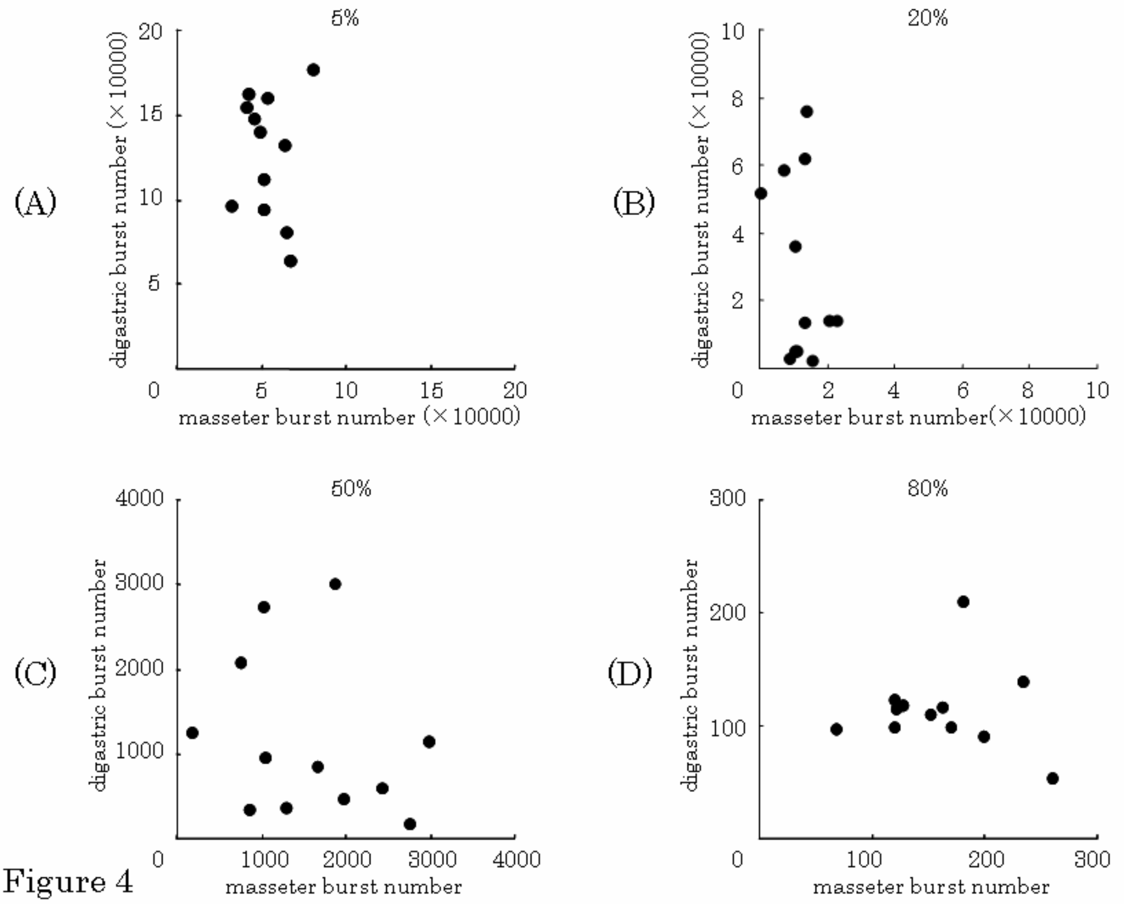
(A)
5%
digastric burst number (×10000)
20
15
10
5
0
5
10
15
20
masseter burst number (×10000)
(B)
20%
digastric burst number (×10000)
10
8
6
4
2
0
2
4
6
8
10
masseter burst number(×10000)
(C)
50%
digastric burst number
4000
3000
2000
1000
0
1000
2000
3000
4000
masseter burst number
(D)
80%
digastric burst number
300
200
100
0
100
200
300
masseter burst number

Figure 4