Analysis of novel oral motor training for elderly people

（高齢者に対する新規口腔リハビリテーションの検討）

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Ⅰ. Abstract

[Objective]

Final aim of this study was to establish and clarify the mechanisms underlying oral rehabilitation in elderly adults, standardized oral motor training using specific devices. The aims of the present study were twofold: first, to examine whether decreased temporal muscular activity after the removal of the temporalis muscle is related to the number of neurogenesis in hippocampal dentate gyrus (HDG) in rats, and second to investigate effect of bite position on masticatory muscle activity during tooth bite task using novel standardized bite device (BDs).

[Materials and methods]

Research 1 : Male Sprague Dawley rats (n = 18) were grown under three conditions: control group (n = 6), unilateral- and bilateral-cut groups (n = 6 each). At 5 weeks postnatally, six rats in control group were incised and sutured in the temporal region, whereas each of six rats in unilateral- and bilateral-cut groups underwent removal of the right and both temporalis muscles, respectively, and were sutured. The numbers of bromodeoxyuridine (BrdU) -labelled cells was calculated in all rats at 27 weeks postnatally.

Research 2 : Twenty-five healthy male volunteers were classified by age into two groups: young adults (YA) group (n = 12) and pre-elderly (PE) group (n = 13). Experimental design included three types of BDs (High-Force, Low-Force, and Dummy), two age groups (YA and PE), and two bite positions (premolar and molar). For training, participants performed biting task 50-times on six types of bite training (three types of BDs × two bite positions) in random order. During training, electromyographic (EMG) activities were recorded from both masseter and temporalis muscles. Working /

balancing side activity ratio (W/B ratio) of the masseter and temporalis muscles were calculated. The coefficient of variation (CV) from the relative ratio of the EMG root mean square amplitude during each bite task in each channel was calculated.

[Result]

Research 1： At 27 weeks postnatally, the numbers of BrdU-labelled cells in unilateral-cut and bilateral-cut groups were considerably lower than that in control group. The ratios of neurons to BrdU-labelled cells in unilateral-cut and bilateral-cut groups were considerably lower than that in control group.

Research 2： For all BDs, CV for EMG activities in both masseter and temporalis muscles were not significantly different by age or bite position. For High-Force and Low-Force BDs, W/B ratio of EMG activities in temporalis muscle were significantly higher than those in masseter muscle.

[Conclusion]

Our results suggest that decreased temporal muscle activity is associated with the presence of fewer neurogenesis in HDG. In addition, our results may suggest that our newly developed BD with built-in plate spring affected EMG activity in the temporalis muscle on the working side, but not in the masseter or temporalis muscles on the balancing side, regardless of age or bite position within the posterior teeth.

Ⅱ. Introduction

The human hippocampus is the temporal brain organization concerned in learning, emotions and memory [1] which retains the capacity for generating new neurons throughout the life [2]. Engaging in voluntary exercise significantly developed procedure of neurogenesis in hippocampal dentate gyrus (HDG) [3]. In addition, Praag

et al. showed neurogenesis might be improved in mice that engaged in voluntary exercise, and that these mice performed better on the reference memory task version of a Morris water maze [4]. Therefore, the voluntary exercise has been shown to raise plasticity in brain of rats on several levels [3].

Recently, several studies have suggested that mastication affects spatial learning and memory [5, 6]. Mitome et al. showed that mastication affects the viability of newly generated cells and may contribute to hippocampal function [7]. Previous studies have reported that the bite raising state impairs spatial learning in mice performing a Morris water maze [8 - 11]. Further, the decrease in masticatory function due to tooth loss has been shown to lead to learning deficits and decreased memory in mice via considerably decreased brain-derived neurotrophic factor in the hippocampus and cortex [12 - 16]. However, these learning deficits and decreased memory ability may be caused by the deterioration of muscle activity but not the malocclusion itself. In other words, whether changes in masticatory muscle activity affect the HDG remains unclear. Clarifying whether changes in masticatory muscle activity affect the HDG may help to prevent learning deficits and decreased memory ability. Therefore, it is essential to examine relationship between masticatory muscle activity and HDG.

On the other hand, previous animal studies indicated that the hippocampus may be damaged by stress [17, 18]. Mizoguchi et al. showed significant loss of hippocampal CA3 and CA4 neurons in rats stressed by restraint and water immersion [19]. Therefore, it is necessary to investigate chronic stress after decreasing temporal muscle activity by removing temporal muscle.

Due to the rapidly aging populations in many countries, the World Health Organization has identified the oral health of elderly adults as one of its key concerns

[20]. In recent years, observational studies have reported that oral frailty and hypofunction are associated with physical function and nutritional status in community-dwelling elderly adults [21 - 24]. Oral health problems in elderly adults represent possible exposure risk factors for so-called frailty syndrome [25 - 27]. Given this background, it is important for elderly adults to maintain good oral health and function to promote a healthy and independent life. Epidemiological research has suggested that a low number of teeth is associated with low masticatory ability in both males and females [28], so maintaining occlusal support and the largest number of teeth is necessary to ensure good oral health. In Japan, the mean ± standard deviation (SD) numbers of remaining posterior teeth in elderly adults aged 60 - 69, 70 - 79, and 80 - 89 years have been reported as 11.12 ± 7.13, 8.02 ± 9.47, and 5.08 ± 9.43, respectively [29]. Therefore, to help elderly adults maintain good oral function, oral rehabilitation that considers missing posterior teeth needs to be established.

A number of studies have demonstrated that shortened dental arches comprising the anterior and premolar regions can meet the requirements of functional dentition [30, 31]. Functional magnetic resonance imaging studies have suggested that shortened dental arches affect human brain activity during gum chewing [32]. Some multicenter studies have also suggested that prosthetic restoration for shortened dental arches benefit oral health-related quality of life and objective masticatory performance [33, 34]. These findings indicate that occlusal support is important for preserving masticatory performance and oral health in elderly adults. On the other hand, although previous studies have reported that mastication training using chewing gum can help maintain or improve oral function in elderly adults [35 - 37], they have not considered the occlusal condition. Therefore, the target of novel oral rehabilitation is elderly people with

shortened as opposed to full dental arches. To the best of our knowledge, no studies have proposed novel oral rehabilitation considering occlusal support.

Regarding bite force, Kikuchi et al. [38] suggested that the occlusal force at the second molar increased most under increasing teeth-clenching conditions. Horie et al. [39] suggested that occlusal contact and near contact areas of the second molar were strongly correlated with mixing ability in dentate adults. In a comparison of the occlusal contact area between teeth, our previous study found that the occlusal contact area tended to differ between anterior, premolar, and molar teeth [40]. Based on the average number of remaining posterior teeth in Japan [29], it is essential to investigate the effect of bite position on oral function (e.g. bite force, masticatory muscle activity).

Given this background, final aim of this study was to establish and clarify the mechanisms underlying oral rehabilitation in elderly adults, standardized oral motor training using specific devices. The aims of the present study were twofold: first, to examine whether decreased temporal muscular activity after the removal of the temporalis muscle is related to the number of neurogenesis in HDG in rats, and second to investigate effect of bite position on masticatory muscle activity during tooth bite task using novel standardized bite device (BDs).

Ⅲ. Materials and methods

Research 1 : Influence of Temporal Muscular Activity on Neurogenesis in Rat Hippocampal Dentate Gyrus

Male Sprague Dawley rats (n = 18) were grown in our animal quarters under constant environmental conditions (temperature, 23 ± 2 °C; humidity, 60% ± 5%; lights on from 7:00 to 19:00). At 5 weeks postnatally, rats in control group (n = 6) were incised

and sutured in the temporal region, whereas rats in unilateral- and bilateral-cut groups (n = 6 each) underwent removal of the right and both temporalis muscles, respectively, and were sutured (Fig. 2). Body weight and daily food intake were measured every week from 6 to 27 weeks postnatally at the Nihon University School of Dentistry at Matsudo. The experiments were carried out according to the Guide for Care and Use of Laboratory Animals, and were approved by the ethics committee of animal experiments of Nihon University School of Dentistry at Matsudo (AP12-MD019).

The temporalis muscles were resected under anesthesia (sodium pentobarbital, 50 mg/kg, intraperitoneally [i.p.]) between 10:00 and 10:30. For acute resection of the temporalis muscles, the animals were placed in the prone position on an apparatus originally designed in our laboratory.

The time schedule for this experiment is shown in Figure 1. At 4 weeks postnatally, the animals were placed in standard cages (one animal per cage). The temporalis muscles were excised under anesthesia at 5 weeks postnatally. After the procedure, they were given ad libitum access to pellet chow and water. At 23 weeks postnatally, the rats were treated with bromodeoxyuridine (BrdU) (70 mg/kg, i.p.; Sigma-Aldrich, Burlington, MA, USA) once per day for 3 consecutive days. At 27 weeks, the rats in each group were given an overdose of anesthetic and perfused transcardially with cold 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS). The rats were killed the next day for BrdU staining.

For immunohistochemistry, rats were anesthetized with thiamylal sodium and transcardially perfused with 0.01 M PBS followed by 4% PFA (pH 7.4) in PBS. The brains were removed and cryostat sections (50-μm thick coronal sections) were prepared using the standard protocols. The brains were post-fixed in the same fixative for 24 h at 4 °C

and then placed in PBS containing 10% sucrose for 24 h. Next, they were resected to obtain transverse sections using a microtome (DTK-1000W, Dosaka, Kyoto, Japan). These free-floating 50-μm transverse sections were used for the various staining procedures. For immunohistochemical detection of BrdU, the sections were pretreated with methanol containing 1% H_2O_2 for 10 minutes. DNA was denatured in 2N HCl (37 °C for 60 min), rinsed in 1 × Tris-buffered saline (TBS), and blocked in 10% normal goat serum (Vector Laboratories, Burlingame, CA, USA) and 0.1% Triton X-100 (Sigma-Aldrich) in TBS for 1 hour, followed by incubation with anti-BrdU monoclonal antibody (1:20, Oxford Biotechnology, Oxfordshire, UK) at 4 °C overnight. All staining was performed on free-floating 50-μm coronal sections. The antibodies used were mouse anti-NeuN (1:1000, Chemicon International, Temecula, CA, USA) and rat anti-BrdU (1:20, Oxford Biotechnology Ltd., UK). Anti-mouse Alexa 488 and anti-rat Alexa 594 (1:200 for both; Invitrogen, Waltham, MA, USA) were used as secondary antibodies for double immunofluorescent staining. A total of 60 slices of 50-μm coronal sections were originally stored in 5 separated tubes containing 12 slices in each tube. Sections from every 200-μm across the whole hippocampus were selected (12 sections from anterior to posterior). Labelled cells were counted visually in 12 sections (every fifth section of 50-μm serial sections) with a light microscope (TE2000-U, Nikon, Tokyo, Japan) at 20 × objective magnification, starting from the most rostral section by a blinded observer. The total numbers of labelled cells in the HDG of each group were determined.

To measure basal corticosterone levels, the rats were decapitated between 09:00 and 10:00. Blood from the left ventricle was collected in 2.0 mL heparinized tubes containing an anticoagulant (heparin sodium; Sigma-Aldrich). The blood samples were immediately centrifuged at 2000 rpm for 10 minutes at 4 °C, and the serum was stored

at -80 °C until the assay was performed. Serum corticosterone concentration was determined by immunoassay according to the manufacturer's instructions (AssayMax Corticosterone ELISA Kit; AssayPro, St. Charles, MO, USA). To evaluate the stress response, serum was prepared at 27 weeks postnatally.

The percentage of neurons (NeuN-labelled cells) in BrdU-labelled cells was calculated. All data on body weight, daily food intake, corticosterone levels, number of BrdU-labelled cells, and percentage of neurons in BrdU-labelled cells were analyzed by one-way analysis of variance. Post hoc comparisons were conducted using Tukey's honestly significant difference test. Values of $P < 0.05$ were considered statistically significant.

Research 2 : Effect of Bite Position on Masticatory Muscle Activity during Tooth Bite task Using Novel Standardized Bite Device

In this study, 25 healthy male volunteers who had four occlusal support zones based on Eichner classification were classified by age into two groups: a young adults (YA) group (n = 12; mean age ± SD, 28.1 ± 2.3 years) and a pre-elderly (PE) group (n = 13; mean age ± SD, 60.5 ± 3.5 years). The exclusion criteria were: a medical, physical, or psychological condition; scheduled dental treatment as of the time of the study; use of medication (analgesics, antidepressants, or hypnotics) within 48 hours of the investigation. All participants were recruited from staff members who were currently working at our dental school and provided informed consent before the study began. This study was approved by the Ethics Committee of the Nihon University School of Dentistry at Matsudo (EC20-034) and conducted according to the guidelines of the Declaration of Helsinki.

BD used in this study (dimensions: 22 × 16 × 8 mm) was constructed of silicon rubber and included a built-in plate spring that made a distinct sound during biting at a specific force level. The BD was available in three types: a high-force plate spring (High-Force: 41.7 ± 0.3 N of compressive stiffness), a low-force plate spring (Low-Force: 22.7 ± 0.2 N of compressive stiffness), and no plate spring (Dummy: 15.9 ± 0.1 N of compressive stiffness). The High-Force and Low-Force BDs were bitten until the plate spring sounded, at which time, it was counted as one tooth-bite task.

The experimental design included three types of BDs (High-Force, Low-Force, and Dummy) (Fig. 3A and B), two age groups (YA and PE), and two bite positions (premolar and molar). For training, the participants performed each jaw motor task using the three BDs while sitting upright and relaxed in a dental chair with their head supported by a headrest. All participants were allowed to familiarize themselves with the measurement procedures and instruments before the actual data collection. Before the training, the participants performed maximum voluntary teeth clenching for 3 seconds to determine the 100% maximal voluntary contraction (MVC) at the intercuspal position without the BD. The MVC measurement was repeated three times. In the single training task, the participants performed a bite task 50 times using three types of the BDs in the right premolar and molar regions with visual feedback on a PC screen at a rate of once/second. The participants performed six types of bite training (three types of BDs × two bite positions) in random order. To avoid masticatory muscle fatigue, a 30-second rest period was allowed between each training session (Fig. 4). During each bite training session, masticatory muscle fatigue was assessed based on self-reported scores on a numerical rating scale (NRS; range, 0 - 10) at the following intervals: 0, 10, 20, 30, 40, and 50 times.

During training, electromyographic (EMG) activities were recorded from the left masseter (LM), right masseter (RM), left temporalis (LT), and right temporalis (RT). Disposable bipolar surface electrodes (NM31; Nihon Kohden, Tokyo, Japan) were placed 10 mm apart along the central part of the muscle, midway between the anterior and posterior and the inferior and superior borders of the LM and RM, and along the anterior part of the LT and RT approximately 2 cm lateral to the eyebrow after the skin over the EMG recording positions had been cleaned with alcohol. The EMG signals were amplified 2,000 times (PL3508 Power Lab 8/35; Bio Research Center, Nagoya, Japan), filtered in the bandwidth of 10 Hz to 1 kHz, sampled at 2 kHz, and stored for offline analysis.

For the EMG data analysis, EMG activities during each bite task were quantified by calculating the EMG root mean square (RMS) amplitude at 0.5 seconds before and after each peak amplitude from each EMG channel in all participants. Next, to normalize the masticatory muscle activity during each task to diminish possible variations within participants, the relative ratio of the averaged EMG RMS amplitude during 50 times of the single bite task to 100% MVC was calculated from each channel in each task in all participants. Finally, to evaluate the influence of muscle activity on the working and balancing sides during each bite task with unilateral occlusal support, the working / balancing side activity ratio (W/B ratio) of the masseter and temporalis muscles were calculated from the relative ratio of EMG activities during each bite task in each channel. In addition, to evaluate the precision of each bite task, the variability of each bite position in each bite task was calculated as the coefficient of variation (CV) from the relative ratio of the EMG RMS amplitude during each bite task in each channel.

All data are presented as means and SDs. The relative ratio of EMG activities

in each channel during each bite task using three types of BDs were analyzed using three-way analysis of variance (ANOVA) with age groups, bite positions and device levels as factors. The CV values in each channel during each bite task using three types of BDs were analyzed using two-way ANOVA with age groups and bite positions as factors. The W/B ratios from the relative ratio of EMG activities from both the masseter and temporalis muscles during the bite tasks were analyzed using two-way ANOVA with age groups and muscles as factors. NRS scores during the bite task were analyzed using three-way ANOVA with age groups, BD levels and times as factors. When appropriate, the ANOVAs were followed by post hoc Tukey tests to compensate for multiple comparisons. P values < 0.05 were considered statistically significant. All analyses were performed using the SigmaPlot 14.5 package (Systat Software, San Jose, CA, USA).

Ⅳ. Results

Research 1：Influence of Temporal Muscular Activity on Neurogenesis in Rat Hippocampal Dentate Gyrus

1. Body weight and daily food intake

No significant difference in body weight or daily food intake was found between control, unilateral-, and bilateral-cut groups (P > 0.05) (Fig. 5 and 6).

2. BrdU and NeuN

As shown in Figure 7, the numbers of BrdU-labelled cells in unilateral-cut (42.6 ± 2.0) and bilateral-cut groups (30.7 ± 3.8) were considerably lower than that in control group (57.9 ± 6.7; P < 0.05) (Fig. 7). As shown in Figure 8, the ratios of neurons to BrdU-labelled cells in unilateral-cut (85.3% ± 2.2%) and bilateral-cut groups (83.3% ± 2.9%) were considerably lower than that in control group (89.4% ± 1.6%; P < 0.05) (Fig. 8).

Figure 9 shows BrdU-stained dentate sections in control, unilateral-cut, and bilateral-cut groups (Fig. 9).

3. Plasma corticosterone level

As shown in Figure 10, no significant differences in blood corticosterone levels at 27 weeks postnatally were found among control (38.3 ± 10.6 ng/ml), unilateral-cut (46.9 ± 12.1 ng/ml), and bilateral-cut groups (45.1 ± 7.9 ng/ml) ($P > 0.05$) (Fig. 10).

Research 2 : Effect of Bite Position on Masticatory Muscle Activity during Tooth Bite task Using Novel Standardized Bite Device

1. EMG activity

The relative ratios of the EMG activities in RM, LM and LT were not significantly dependent on age or bite position or device level. The relative ratios of the EMG activities in RT were significantly dependent on device level ($F_{2,137}$=16.185, P<0.001), but not on age ($F_{1,137}$ = 0.109, P = 0.742) or bite position ($F_{1,137}$ = 0.222, P = 0.639). The relative ratios of the EMG activities in RT during the bite tasks at each bite position using the using High-Force and Low-Force BDs was significantly higher than that using the Dummy BD in both age groups (all $P < 0.05$). The CVs of the EMG RMS amplitudes during the standardized bite task on masseter and temporalis muscles were 14.4% - 19.4% and 14.5% - 20.5%, respectively. For all BDs, the CVs calculated from the relative ratio of the EMG RMS amplitudes for all muscles were not significantly dependent on age or bite position. During the bite tasks at each bite position using the High-Force and Low-Force BDs, the W/B ratio of the EMG activity was significantly dependent on muscle (premolar-High-Force: $F_{1,46}$ = 15.41, P < 0.001, premolar-Low-Force: $F_{1,46}$ = 22.05, P < 0.001, molar-High-Force: $F_{1,46}$ = 14.83, P < 0.001, molar-Low-Force: $F_{1,46}$ = 17.09, P <

0.001), but not on age. Regarding the High-Force and Low-Force BDs, the W/B ratio of the EMG activity during the bite tasks on premolars and molars in the temporalis muscle was significantly higher than that in the masseter muscle in both age groups (both $P < 0.05$) (Fig. 11A, B, C and D). During the bite tasks at each bite position using the Dummy BD, the W/B ratio of the EMG activity was not significantly dependent on muscle (premolar: $F_{1,46} = 0.266$, $P = 0.609$, molar: $F_{1,46} = 0.164$, $P = 0.687$) or age (premolar: $F_{1,46} = 2.925$, $P = 0.094$, molar: $F_{1,46} = 2.152$, $P = 0.149$) (Fig. 11E and F).

2. NRS score

A comparison of masticatory muscle fatigue based on self-reported NRS scores during the bite tasks at each bite position for each age group is shown in Figure 12. The NRS scores for each BD were significantly dependent on times (High-Force: $F_{5,276} = 21.32$, $P < 0.001$, Low-Force: $F_{5,276} = 25.61$, $P < 0.001$, Dummy: $F_{5,276} = 26.66$, $P < 0.001$), but not on bite position (High-Force: $F_{1,276} = 0.542$, $P = 0.470$, Low-Force: $F_{1,276} = 1.262$, $P = 0.262$, Dummy: $F_{1,276} = 0,010$, $P < 0.001$) or age (High-Force: $F_{1,276} = 0.384$, $P = 0.051$, Low-Force: $F_{1,276} = 3.568$, $P = 0.060$, Dummy: $F_{1,276} = 3.724$, $P = 0.055$). For each age group, NRS scores at intervals of 40 and 50 times for each BD were significantly higher than 0 time (all $P < 0.05$) (Fig. 12A, B and C).

Ⅴ. Discussion

Research 1：Influence of Temporal Muscular Activity on Neurogenesis in Rat Hippocampal Dentate Gyrus

In the present study, no relevant differences in body weight and daily food intake were observed between the control, unilateral-cut, and bilateral-cut groups at 5 - 27 weeks postnatally. These results suggest that despite its involvement in mastication,

removal of the temporalis muscle does not affect body weight or daily food intake. Moreover, no significant differences in blood corticosterone levels at 27 weeks postnatally were found between control, unilateral-cut, and bilateral-cut groups, which suggests the absence of chronic stress in unilateral-cut and bilateral-cut groups. Miyake et al. [41] reported that malocclusion induced by the bite raising state, might be a risk factor for hypersensitiveness to novel stress. Furthermore, Kubo et al. [42] indicated that the morphologic loss induced by the molarless state in mice increased plasma corticosterone levels. Accordingly, our results suggest that the chronic stress associated with mastication may be more related to occlusal disharmony than to the deterioration of muscle activity caused by the removal of the temporalis muscle. Therefore, executing optimal occlusion may not cause stress even in cases of decreased masticatory muscle activity due to aging.

The number of BrdU-labelled cells and the ratios of neurons to BrdU-labelled cells were considerably lower in unilateral-cut and bilateral-cut groups than in control group, which suggests that the decreased muscle activity after removal of the temporalis muscle leads to fewer neurogenesis in the HDG. Moreover, the number of BrdU-labelled cells and the percentage of neurons in BrdU-labelled cells were showed a tendency to be lower in bilateral-cut than in unilateral-cut groups, which suggests a relation to temporal muscle activity. The decrease in masticatory function due to tooth loss has been shown to lead to learning deficits and decreased memory in mice via considerably decreased brain-derived neurotrophic factors in the hippocampus and cortex [12 - 16]. The present study suggested that these learning deficits and decreased memory ability may be caused by the deterioration of muscle activity not the malocclusion itself. Therefore, the decreased blood flow caused by the removal of the temporalis muscle

may result in fewer BrdU-labelled cells and a smaller percentage of neurons in BrdU-labelled cells. Further research is needed to evaluate the effects of removing the temporalis muscle on hippocampal blood flow. Moreover, the decreased masticatory muscle activity due to aging may reduce the presence of fewer neurogenesis in the HDG. Further research is needed to investigate a relationship between the masticatory muscle activity due to aging and neurogenesis in the HDG.

Research 2 : Effect of Bite Position on Masticatory Muscle Activity during Tooth Bite task Using Novel Standardized Bite Device

This study compared masticatory muscle activity in groups of YA and PE males during a standardized bite task at the right premolar and molar. The main findings in this study were as follows: first, for the RT, the relative ratios of the EMG activities in each bite position using High-Force and Low-Force BDs was significantly higher than that using the Dummy BD in both age groups; second, for all BDs, the CVs for the EMG RMS amplitudes in both the masseter and temporalis muscles were not significantly different by age or bite position; third, for the High-Force and Low-Force BDs, the W/B ratios of the EMG activities in the temporalis muscle were significantly higher than those in the masseter muscle; fourth, for all BDs, masticatory muscle fatigue was not significantly different by age or bite position.

Our previous EMG study indicated that the CVs of the EMG RMS amplitudes during tooth clenching with and without visual feedback were 15% - 20% and 30% - 40%, respectively [43]. The results of the present study indicate that the CVs of the EMG RMS amplitudes during a standardized bite task on the masseter and temporalis muscles were both around 15% - 20%. The variability of a standardized bite task on the masseter and

temporalis muscles was similar to that during tooth clenching with visual feedback; therefore, the present results suggest that a bite task using a BD as a novel oral rehabilitation method can help pre-elderly adults perform bite tasks steadily. In addition, the present study demonstrated that the CV values calculated from the EMG RMS amplitudes for all muscles did not significantly depend on bite position in all BDs, which suggests that the bite position within the posterior teeth does not affect the variability of standardized bite tasks.

Bilt et al. [44] investigated EMG activity during maximum unilateral tooth clenching in which participants performed tooth clenching on a force transducer located between the occlusal surfaces in the first molar region. Their results indicated that although EMG activity was not significantly different between the working and balancing sides in the masseter muscles, those in the temporalis muscle were significantly higher on the working than on the balancing side. In addition, Wang et al. [45] compared EMG activity between the masseter and temporalis muscles during unilateral tooth clenching in which participants performed tooth clenching on a cotton roll. Their results showed that normalized EMG activity was higher on the working than on the balancing side in both the masseter and anterior temporalis muscles during unilateral clenching on molar support, but only in the anterior temporalis muscle during unilateral clenching on premolar support. The present study also found that the W/B ratio of the EMG activities during the bite task using High-Force and Low-Force BDs were significantly higher in the temporalis than in the masseter muscle. In addition, our present result showed that the relative ratios of the EMG activities in RT during the bite task in each biting position using High-Force and Low-Force BDs was significantly higher than that using the Dummy BD in both age groups. Although the masseter muscles play a relatively large role in

generating side-to-side (i.e., transverse) jaw movements during chewing, and transversely-oriented forces during the power stroke of mastication, the temporalis muscles produce vertical force and jaw movements [46]. The results of the present study suggest that the temporalis muscle activities on the working side is higher than that on the balancing side, regardless of bite position.

Regarding masticatory muscle fatigue, Farella et al. [47] investigated the effects of a 40-minute gum chewing task at a constant rate of 80 cycles/minute on fatigue in the jaw muscles. They found that the visual analogue scale (VAS) scores for jaw muscle fatigue increased significantly during hard gum chewing. Svensson et al. [48] investigated the effects of jaw clenching at 10% of MVC for 60 minutes on a bite force meter, and found that the perception of fatigue increased during the jaw motor task. The present study applied 50 times of bite force using each BD on the premolars or molars and found that self-reported NRS scores on muscle fatigue increased with time. However, these scores were not affected by bite position or age, which suggests that considering masticatory muscle fatigue, the present standardized bite task using all BDs is useful for elderly adults regardless of the number of molars.

This study has limitations. To avoid the influence of gender differences on the experimental data, only male participants were included. Torisu et al. [49] compared the effects of jaw muscle fatigue evoked by low-level tooth clenching between females and males and found that visual analog scale scores for fatigue were significantly higher in males than in females. Alhilou et al. [50] also suggested gender differences in the characteristics of the masseter muscles. To establish a more effective method for oral motor training, future studies should investigate the effects of gender on the improvement of oral function.

Ⅵ. Conclusion

Our results suggest that decreased temporal muscle activity is associated with the presence of fewer neurogenesis in HDG. In addition, our results may suggest that our newly developed BD with built-in plate spring affected EMG activity in the temporalis muscle on the working side, but not in the masseter or temporalis muscles on the balancing side, regardless of age or bite position within the posterior teeth.

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Ⅷ. Table and Figures

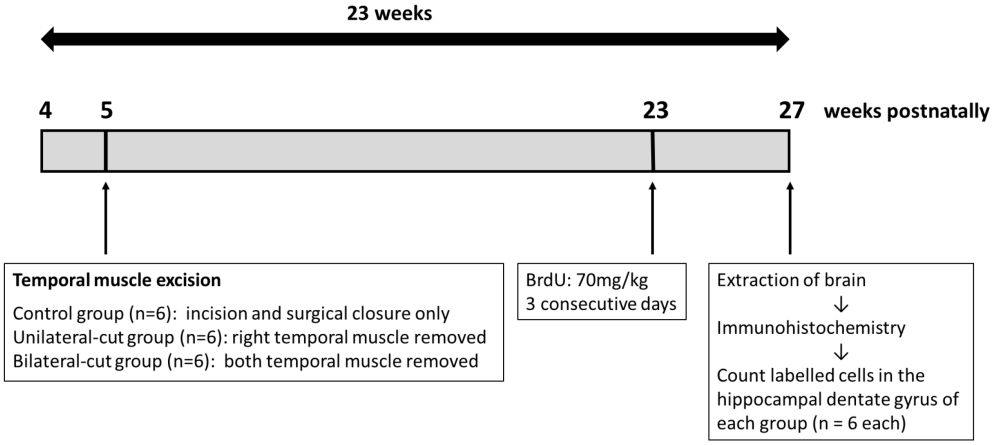

Figure 1. Study design for 23 weeks of the study period. The temporalis muscle was resected at 5 weeks postnatally. The animals were treated with 70 mg/kg bromodeoxyuridine (BrdU) intraperitoneally at 23 weeks postnatally.

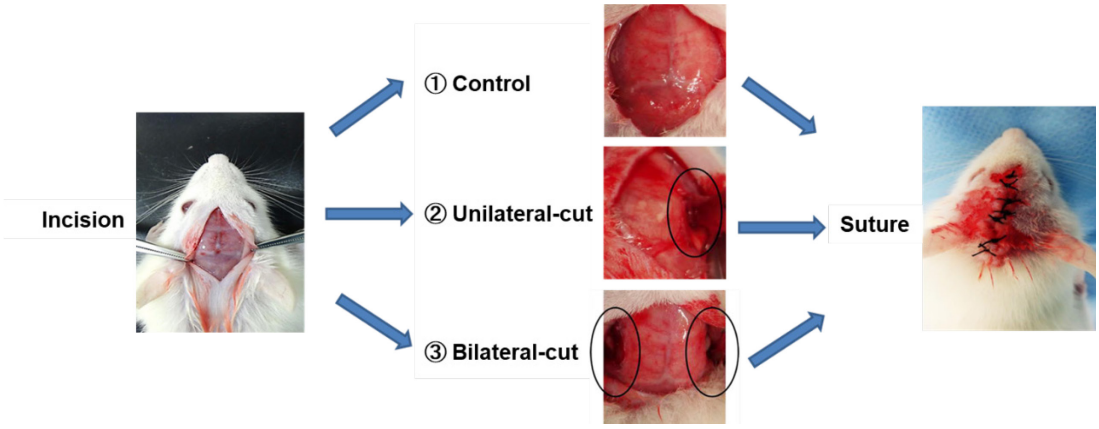

Figure 2. Surgeries for temporal muscle excision in each group (i.e., control group, unilateral-cut temporalis muscle group, and bilateral-cut temporalis muscle group).

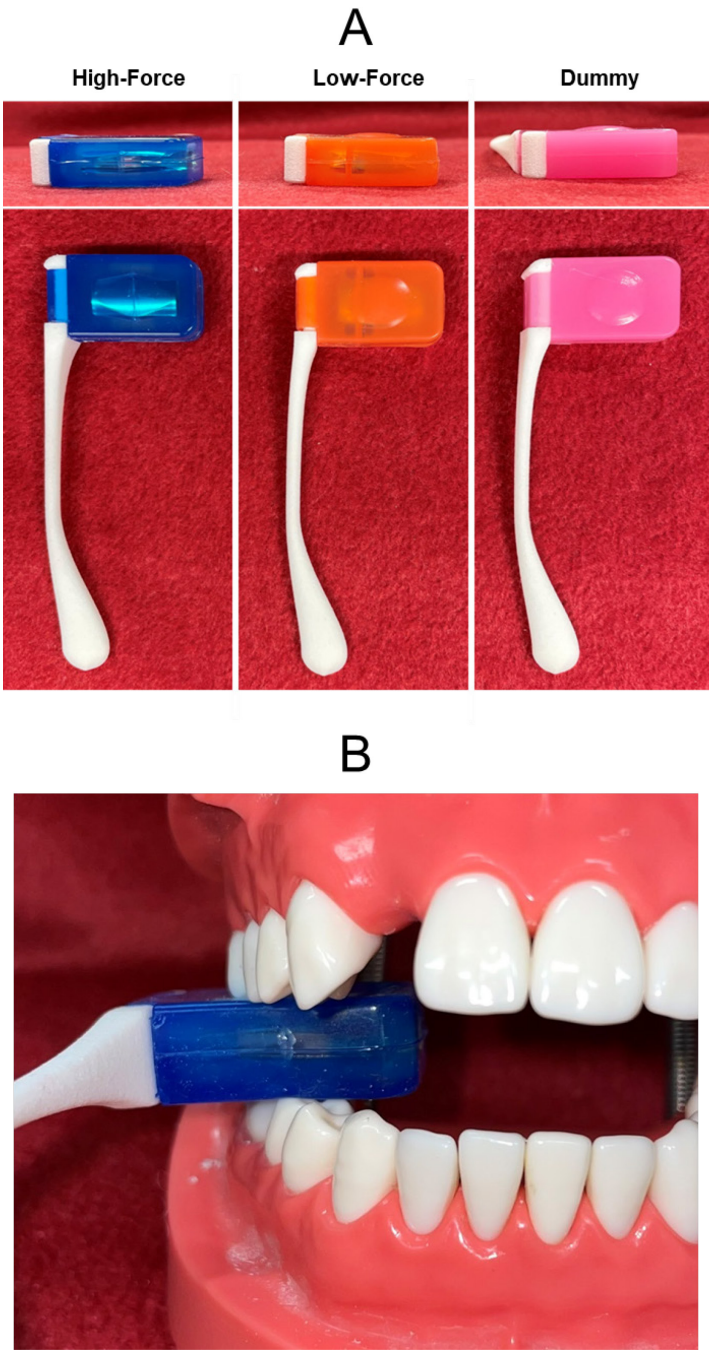

Figure 3. (A) Three types of standardized oral motor training bite devices (BD). BDs with three standardized hardness types (high-force plate spring (High-Force), low-force plate spring (Low-Force), and no plate spring (Dummy)). (B) Bite training using the BD.

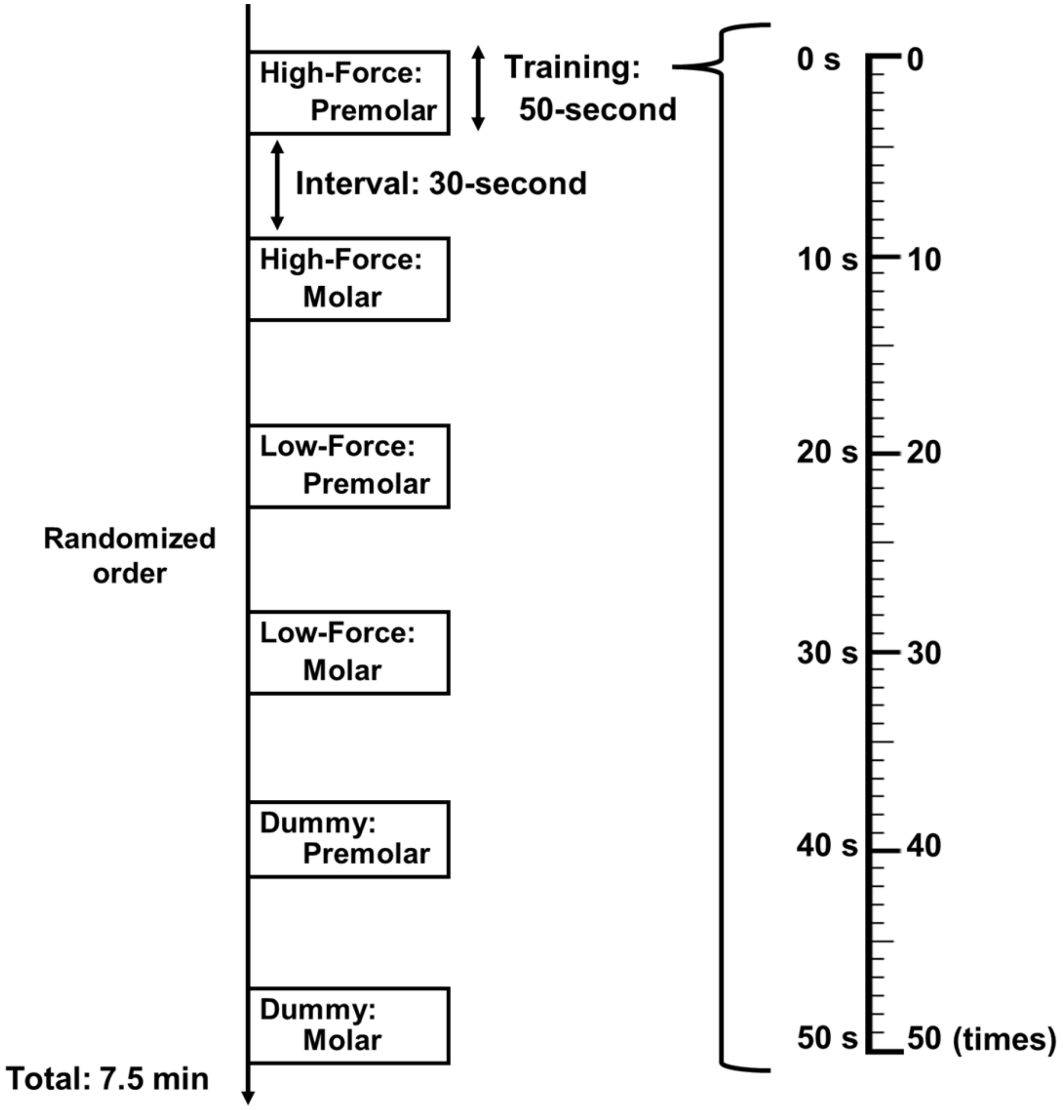

Figure 4. Overview of the study design.

Abbreviations: High-Force, high-force plate spring bite device; Low-Force, low-force plate spring bite device; Dummy, no plate spring bite device.

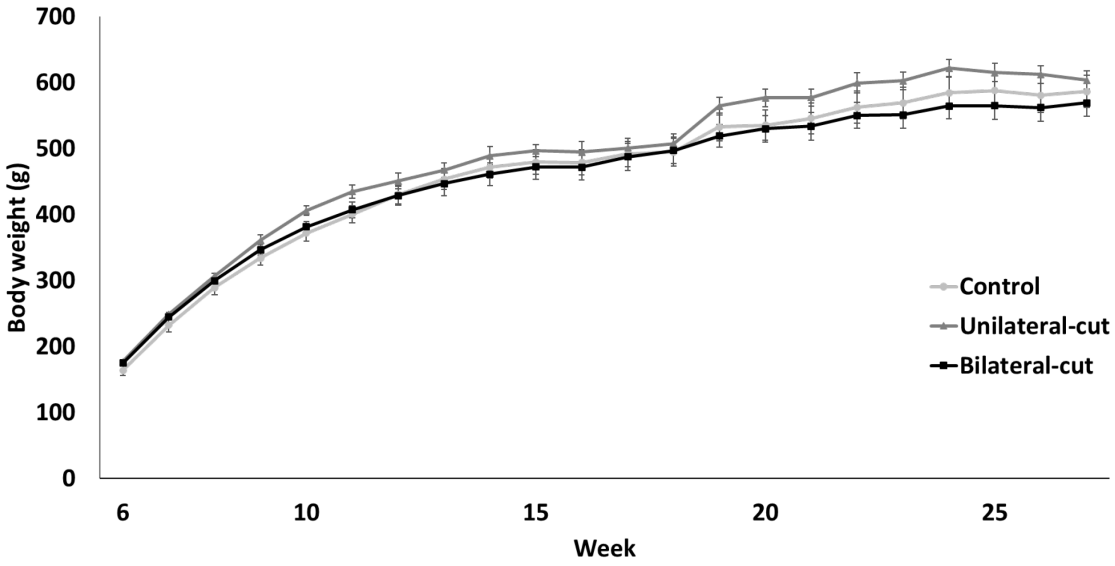

Figure 5. Body weight for each of the 27 weeks in each group (i.e., control group, unilateral-cut temporalis muscle group, and bilateral-cut temporalis muscle group) (n = 6 each).

Error bars indicate the standard error of the mean.

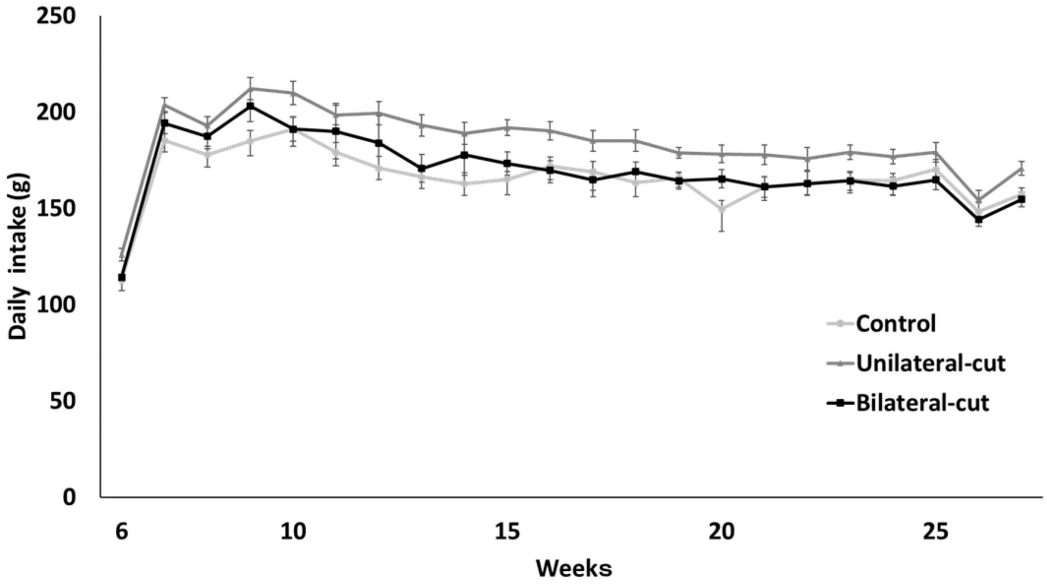

Figure 6. Daily food intake for each of the 27 weeks in each group (i.e., control group, unilateral-cut temporalis muscle group, and bilateral-cut temporalis muscle group) (n = 6 each).

Error bars indicate the standard error of the mean.

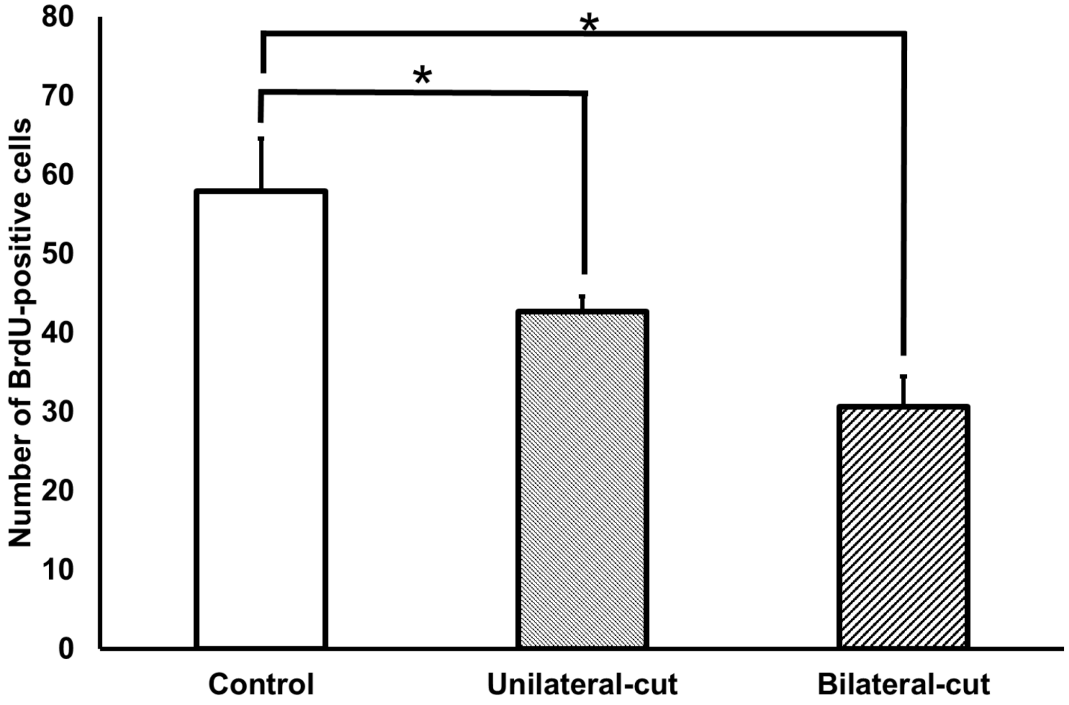

Figure 7. Comparison of the numbers of bromodeoxyuridine (BrdU)-labelled cells between control, unilateral-cut, and bilateral-cut groups.

Error bars indicate the standard error of the mean.

Asterisks indicate statistically significant differences ($P < 0.05$).

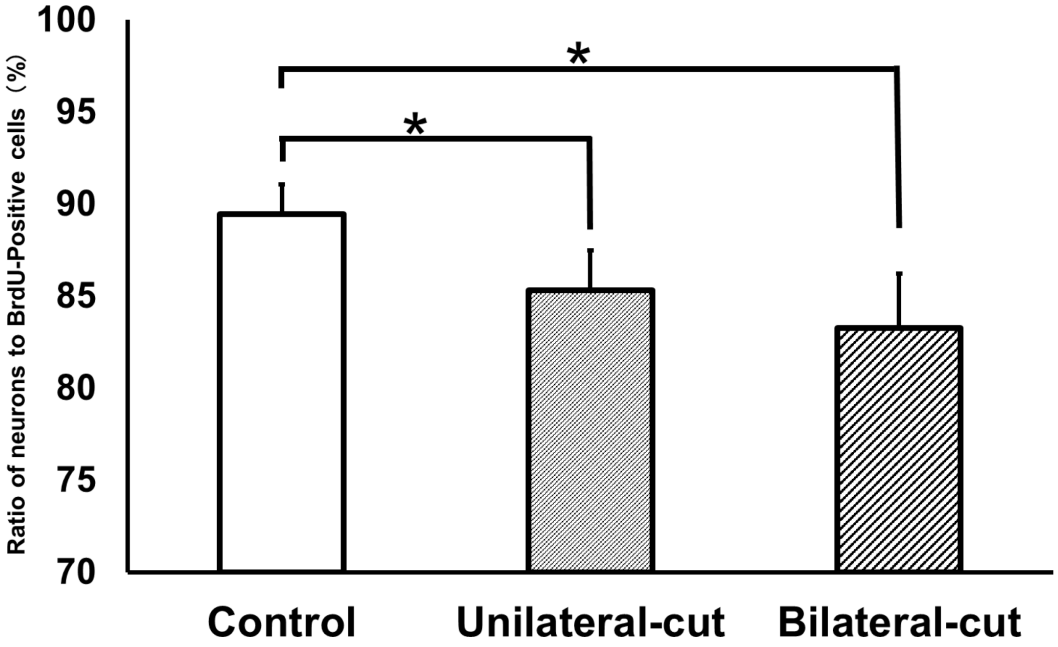

Figure 8. Comparison of the percentage of neurons in bromodeoxyuridine (BrdU)-labelled cells between control, unilateral-cut, and bilateral-cut group.

Error bars indicate the standard error of the mean.

Asterisks indicate statistically significant differences ($P < 0.05$).

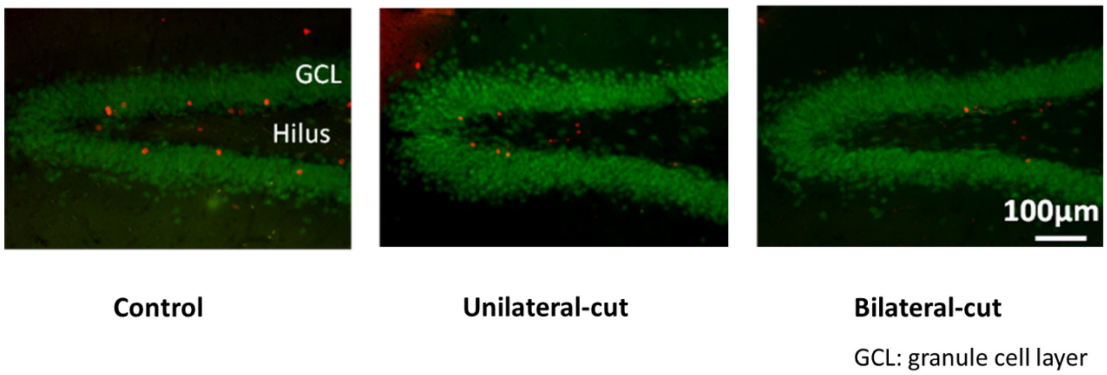

Figure 9. Representative images of bromodeoxyuridine (BrdU)-stained dentate sections from control, unilateral-cut, and bilateral-cut group.

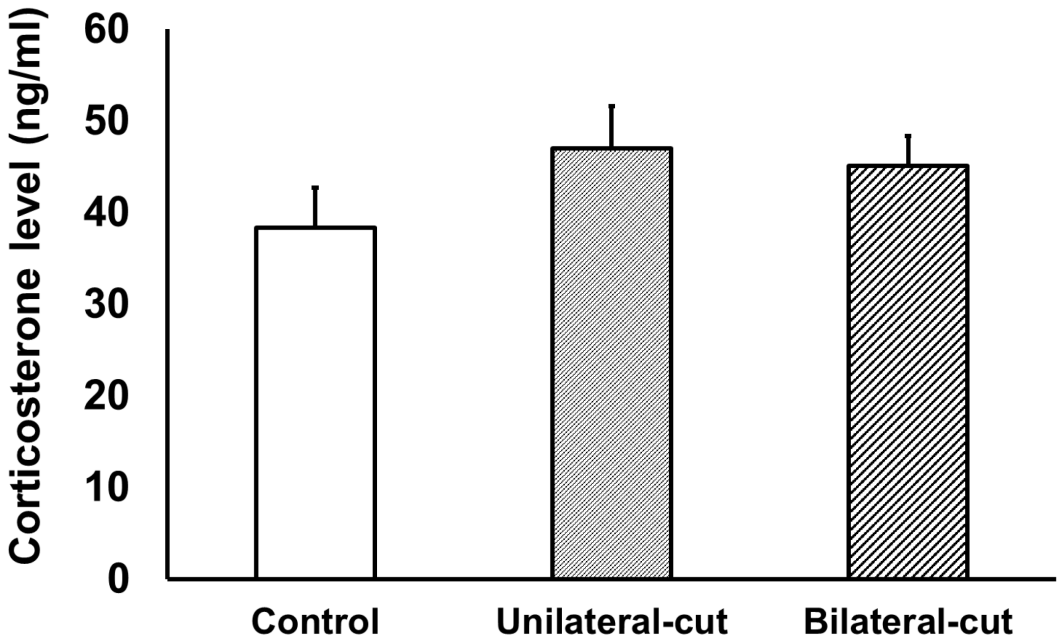

Figure 10. Comparison of corticosterone levels between control, unilateral-cut, and bilateral-cut group.

Error bars indicate the standard error of the mean.

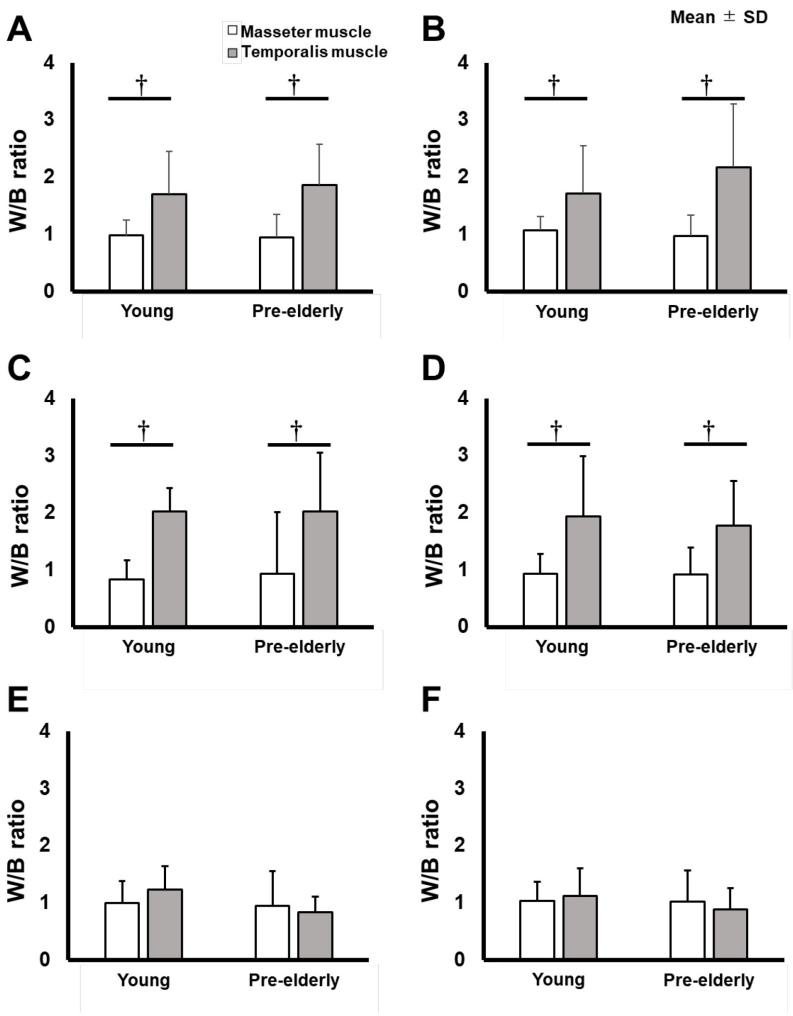

Figure 11. Comparison of the working / balancing side activity ratio from the relative ratio of the electromyographic (EMG) root mean square (RMS) amplitude during the bite task at the premolars using the High-Force bite device (BD) (A), Low-Force BD (C), and Dummy BD (E) for each age group and muscle, and at the molars using the High-Force BD (B), Low-Force BD (D), and Dummy BD (F) for each age group and muscle.

† Significantly higher than masseter muscle ($P < 0.05$).

Abbreviations: W/B ratio; the working / balancing side activity ratio, High-Force, high-force plate spring BD; Low-Force, low-force plate spring BD; Dummy, no plate spring BD.

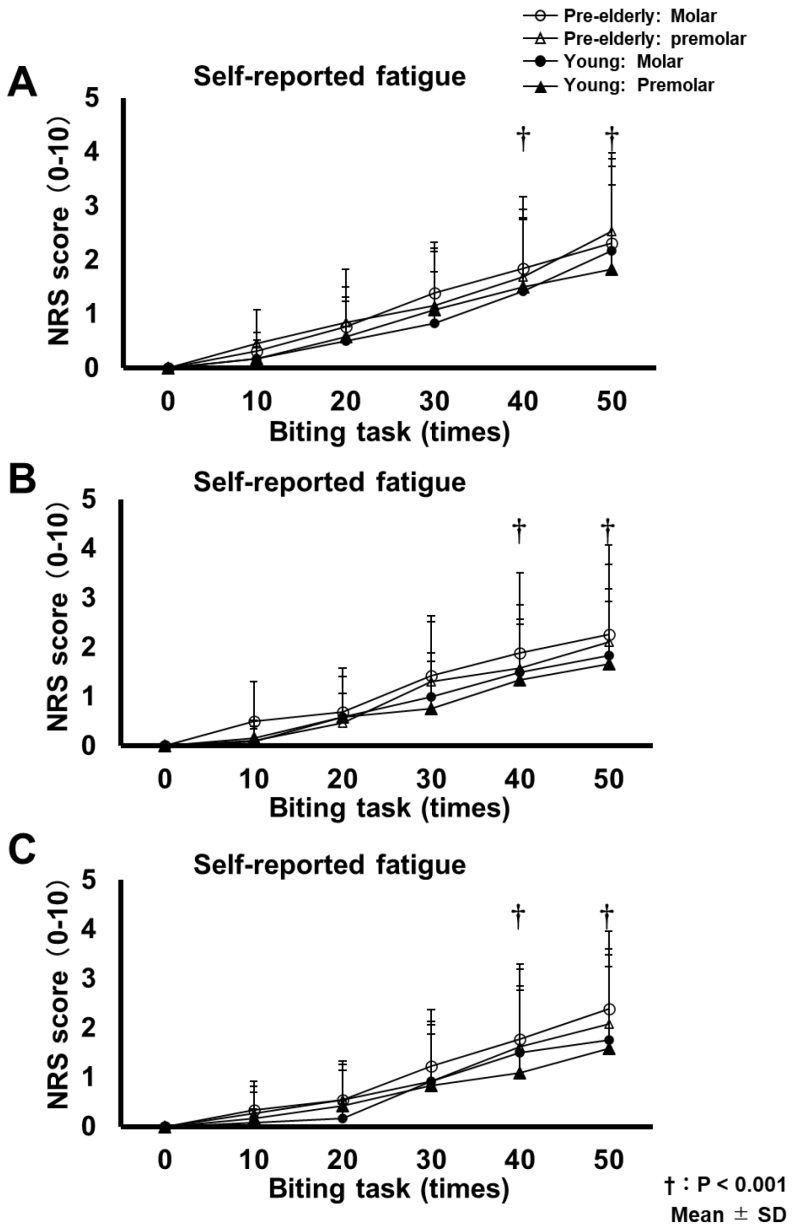

Figure 12. Comparison of self-reported numerical rating scale (NRS) scores on masticatory muscle fatigue during the bite task using the High-Force bite device (BD) (A), Low-Force BD (B), and Dummy BD (C) at intervals of 0, 10, 20, 30, 40, and 50 times for each bite position and age group.

† Significantly higher than 0 time ($P < 0.001$).

Abbreviations: High-Force, high-force plate spring BD; Low-Force, low-force plate spring BD; Dummy, no plate spring BD.